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Bacille Calmette-Guérin Vaccination Policy Change and Childhood Mycobacterial Infections in Finland

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To Astrid and Alvar

TB, or not TB, that is the question…

Abstract

The World Health Organization declared tuberculosis (TB) a global emergency over 25 years ago, yet TB remains a significant public health concern and a leading infectious killer of our time. Young children are especially vulnerable to rapid and debilitating TB disease, and infected children should be identified and therapy initiated rapidly. Nontuberculous mycobacteria (NTM) infections have also emerged in Western countries. Childhood NTM infections predominantly manifest as prolonged cervical lymphadenitis, which is a diagnostic challenge for the clinician due to the limitations of NTM cultures. Bacille Calmette-Guérin (BCG) vaccine effectively prevents severe TB disease forms in young children. Some studies have further suggested that BCG might also offer protection against childhood NTM infections.

In Finland, BCG coverage of infants was very high until the vaccination policy changed in 2006 to a risk group-based approach. Subsequently, for the first time since the 1940s, a generation of children has grown in Finland without the protection of BCG against mycobacterial diseases. Furthermore, the healthcare and national surveillance registries allowing retrospective evaluation of TB and NTM cases in Finland are exceptional and provide a rare look into paediatric TB and NTM epidemiology with or without universal BCG vaccinations. In addition, a novel in-house diagnostic test developed in the Hospital District of Helsinki and Uusimaa (HUS) laboratory has shown potential in childhood NTM lymphadenitis diagnostics but has not been evaluated.

In the first study, we evaluated the performance of the novel modified T.SPOT.TB test in children under five years of age with culture-confirmed NTM lymphadenitis and compared the results to a control group of healthy children. The estimated sensitivity and specificity of the modified T-SPOT.TB test were 1.00 and 0.81, respectively. The modified T.SPOT.TB was a promising noninvasive diagnostic test for childhood NTM lymphadenitis.

In the second study, we identified native-born children aged 0–4 years infected with NTM between 1995 and 2016 from the Finnish National Infectious Diseases Register (NIDR) and estimated the NTM incidence rate change between birth cohorts born during universal or selective BCG vaccination policy. We identified 97 native-born children infected with NTM under the age of five. The estimated incidence rates of NTM in universal-BCG and selective-BCG cohorts were 0.2 and 3.9 per 100,000 person-years, respectively. The incidence rate ratio (IRR) of selective-BCG cohorts compared to universal-BCG cohorts was 19.03 (95% confidence interval [CI], 8.82–41.07). Childhood NTM infections increased drastically after the infant BCG coverage decreased, suggesting that BCG offers protection against childhood NTM lymphadenitis.

In the third study, we identified all newly diagnosed active TB cases under 15 years of age in Finland 1995–2015 by linking data from the NIDR, Finnish Care Register for Health Care, medical patient records, and Finnish Population Information System. We compared the under-five TB incidence rate ratio of birth cohorts with universal and selective BCG vaccinations. We identified a total of 139 paediatric TB cases. The under-five TB rate of birth cohorts with selective-BCG compared to birth cohorts with universal-BCG remained stable (IRR 1.3; 95% CI, 0.7–2.3). Paediatric TB in Finland was concentrated in families with an immigrant background from high TB incidence countries. The native under-five TB morbidity did not increase after the BCG vaccination policy change in Finland, suggesting that well-implemented selective vaccinations can prevent TB in the most vulnerable age group effectively in low-incidence settings.

In the fourth study, we retrospectively reviewed paediatric TB contact tracing results from 2012 to 2016 in the HUS area. The yield for TB disease or infection was 4.6% and 12.8% for household con-

tacts, 0.5% and 0% for contacts exposed in a congregate setting, and 1.4% and 5.0% for other contacts, respectively. Contact tracing in the HUS area identified exposed young children quickly: most of the TB infections among the children under five years of age were found before progression to disease, and none had severe disease forms. The maximum delay until the first contact investigation visit among the household contacts under five years of age with either TB disease or infection was seven days from the index case diagnosis. Contacts born in a TB endemic country (adjusted odds ratio [aOR] 3.07; 95% CI, 1.10–8.57), with household exposure (aOR 2.96; 95% CI, 1.33–6.58), or a sputum smear-positive index case (aOR 3.96; 95% CI, 1.20–13.03) were more likely to have TB disease or infection. The yield for TB disease or infection of large-scale investigations after exposure in a congregate setting was very low, and investigations in such events should be cautiously targeted.

In summary, the epidemiological landscape of childhood mycobacterial infections in Finland has changed. The BCG vaccination policy change in 2006 resulted in an increase in childhood NTM infections, but childhood TB infections did not increase, and restarting universal BCG vaccinations seems unwarranted. Childhood TB, however, remains an essential public health issue, and future surveillance is vital. The focus of childhood TB prevention in Finland should be further targeted to those with an immigrant background from high TB burden countries.

Tiivistelmä

Maailman terveysjärjestö WHO julisti tuberkuloosin kansainväliseksi terveydelliseksi hätätilanteeksi jo yli 25 vuotta sitten, mutta tuberkuloosi on edelleen yksi merkittävimmistä infektiotaudeista ja kansanterveydellisistä haasteista maailmassa. Pienet lapset ovat erityisen alttiita nopealle ja vakavalle tuberkuloositaudille. Tästä syystä infektion saaneet lapset tulisi löytää nopeasti ja heidän hoitonsa aloittaa viipymättä. Ympäristömykobakteeri-infektiot ovat yleistyneet länsimaissa. Lapsilla ympäristömykobakteeri-infektiot ilmenevät yleensä kaulan tai kasvojen alueen imusolmuketulehduksina, joiden diagnostiikka bakteeriviljelyn avulla on haasteellista. Tuberkuloosi- eli BCG-rokotukset ehkäisevät tehokkaasti pienten lasten vakavia tuberkuloositautimuotoja, ja ne saattavat myös ehkäistä lapsuuden ympäristömykobakteeri-infektiota.

Suomessa BCG-rokotuskattavuus oli erittäin hyvä rokotusohjelman muutokseen saakka: vuonna 2006 siirryttiin rokottamaan vain korkean tuberkuloositartunnan riskiryhmiin kuuluvia lapsia. Muutoksen seurauksena Suomessa on kasvanut uusi BCG-rokottamattomien lasten sukupolvi ensimmäistä kertaa sitten 1950-luvun. Suomalaiset terveydenhuoltorekisterit mahdollistavat lasten mykobakteeri-infektioiden ilmaantuvuuden tarkastelun yleisten BCG-rokotusten aikana ja näiden jälkeen. Helsingin ja Uudenmaan sairaanhoitopiirin (HUS) laboratorio on myös kehittänyt uuden testin, jota voidaan hyödyntää lasten ympäristömykobakteerien aiheuttamien imusolmuketulehdusten diagnostiikassa, mutta testin herkkyyttä tai tarkkuutta ei ole arvioitu.

Ensimmäisessä tutkimuksessa tarkasteltiin muunnellun T.SPOT.TB testin tuloksia alle 5-vuotiailla lapsilla, joilla oli todettu viljelyvarmennettu ympäristömykobakteerin aiheuttama imusolmuketulehdus, ja testituloksia verrattiin terveeseen verrokkiryhmään. Testin arvioitu herkkyys (1.00) ja tarkkuus (0.81) olivat lupaavia ympäristömykobakteerien aiheuttamien lasten imusolmuketulehduksen diagnostiikassa.

Toisessa tutkimuksessa valtakunnallisesta tartuntatautirekisteristä haettiin kaikki vuosina 1995–2016 ilmoitetut alle 5-vuotiaiden lasten ympäristömykobakteeri-infektiot. BCG-rokotusohjelman muutosta ennen ja tämän jälkeen syntyneiden syntymäkohorttien ympäristömykobakteeri-infektioiden ilmaantuvuutta verrattiin keskenään. Suomessa syntyneillä lapsilla todettiin yhteensä 97 tapausta viiden vuoden ikään mennessä. Ympäristömykobakteeri-infektioiden arvioitu ilmaantuvuus ennen BCG-rokotusohjelman muutosta syntyneillä lapsilla oli 0.2/100,000 henkilövuotta ja tämän jälkeen syntyneillä lapsilla 3.9/100,000 henkilövuotta. Ilmaantuvuustiheyksien suhde oli 19.03 (95% luottamusväli, 8.82–41.07). Lasten ympäristömykobakteeri-infektiot lisääntyivät BCG-rokotusohjelman muutoksen jälkeen, mikä viittaa siihen, että BCG-rokotus ehkäisee ympäristömykobakteereiden aiheuttamia imusolmuketulehduksia lapsilla.

Kolmannessa tutkimuksessa valtakunnallisen tartuntatautirekisterin, terveydenhuollon hoitoilmoitusrekisterin ja potilasasiakirjarekisterin tietoja yhdistämällä tunnistettiin Suomessa vuosina 1995–2015 alle 15-vuotiailla todetut tuberkuloositapaukset. Ennen BCG-rokotusohjelman muutosta ja tämän jälkeen syntyneiden syntymäkohorttien tuberkuloosi-ilmaantuvuutta verrattiin keskenään. Kaiken kaikkiaan alle 15-vuotiaiden tuberkuloositapauksia oli 139. Alle 5-vuotiaiden lasten tuberkuloosi-ilmaantuvuus, ennen BCG-rokotusohjelman muutosta ja tämän jälkeen syntyneillä, pysyi ennallaan (ilmaantuvuustiheyksien suhde 1.3; 95% luottamusväli, 0.7–2.3). Lasten tuberkuloositapaukset keskittyivät pääosin korkean ilmaantuvuuden maista Suomeen muuttaneisiin perheisiin. Suomessa syntyneiden alle 5-vuotiaiden tuberkuloosisairastuvuus ei lisääntynyt BCG-rokotusohjelman muutoksen jälkeen, mikä viittaa siihen, että riskiryhmiin suunnatut rokotukset ovat onnistuneet hyvin.

Neljännessä tutkimuksessa käytiin läpi kaikki vuosina 2012–2016 HUS-alueen tuberkuloosin tartunnanjäljityksissä tutkitut lapset. Tuberkuloosin ja tuberkuloosi-infektion prosenttiosuudet olivat perhepiirissä altistuneilla lapsilla 4.6% ja 12.8%, joukkoaltistuksissa altistuneilla lapsilla 0.5% ja 0%, ja muilla altistuneilla lapsilla 1.4% ja 5.0%. Tartunnanjäljitys tunnisti altistuneet lapset nopeasti: valtaosa alle 5-vuotiaiden lasten infektioista löydettiin ennen taudin kehittymistä, eikä kenelläkään sairastuneista todettu vakavaa tuberkuloositautia. Tuberkuloosi-infektion saaneiden alle 5-vuotiaiden lasten viive ensimmäiseen tartunnanjäljitystutkimukseen oli enintään seitsemän vuorokautta indeksitapauksen diagnoosista. Tuberkuloosia tai infektioita todettiin erityisesti korkean tuberkuloosi-ilmaantuvuuden maassa syntyneillä (aOR 3.07; 95% CI, 1.10–8.57), samassa taloudessa altistuneilla (aOR 2.96; 95% CI, 1.33–6.58) ja yskösvärjäyspositiiviselle tuberkuloosille altistuneilla (aOR 3.96; 95% CI, 1.20–13.03). Joukkoaltistustilanteiden johdosta tutkituilla lapsilla todettiin hyvin vähän tuberkuloosi-infektioita, joten joukkoaltistumisen jälkeiset tutkimukset tulisi suunnata entistä tarkemmin.

Lasten mykobakteeri-infektioiden epidemiologia on Suomessa muuttunut. BCG-rokotusohjelman muutoksen jälkeen pienten lasten ympäristömykobakteeri-infektioiden määrä on kasvanut, mutta tuberkuloosisairastuvuus ei ole lisääntynyt, joten kaikkien lasten BCG-rokotusten uudelleen aloittamiselle ei ole perusteita. Lapsuuden tuberkuloosin ennaltaehkäiseviä toimia on syytä suunnata entistä enemmän maahanmuuttajiin, jotka tulevat korkean tuberkuloosi-ilmaantuvuuden maista.

Contents

List of Original Publications

This thesis is based on the following publications:

I Kontturi A, Tuuminen T, Karttunen R, Salo E.
Elispot IGRA with PPD stimulation for diagnosing nontuberculous mycobacterial cervical lymphadenitis.
Pediatr Infect Dis J. 2016;35(3):349-51.

II Kontturi A, Soini H, Ollgren J, Salo E.
Increase in childhood nontuberculous mycobacterial infections after BCG coverage drop – a retrospective population-based study, Finland, 1995 to 2016.
Clin Infect Dis. 2018;67(8):1256-1261.

III Kontturi A, Kekomäki S, Soini H, Ollgren J, Salo E.
Paediatric tuberculosis during universal and selective Bacille Calmette-Guérin vaccination policy: a nationwide population-based retrospective study, Finland, 1995–2015.
Euro Surveill. 2021;26(11):1900711.

IV Kontturi A, Kekomäki S, Ruotsalainen E, Salo E.
Tuberculosis contact investigation results among paediatric contacts in low-incidence settings in Finland.
Eur J Pediatr. 2021;180(7):2185-2192.

The publications are referred to in the text by their roman numerals. The articles have been reprinted with the permission of the copyright holders.

Abbreviations

AFB	acid-fast bacillus
aOR	adjusted odds ratio
BCG	Bacille Calmette-Guérin vaccine
CFP-10	10 kDa culture filtrate protein
CI	confidence interval
CT	computed tomography
DOT	directly observed therapy
ESAT-6	6 kDa early secretory antigenic target protein
QFT-GIT	QuantiFERON®-TB Gold In-Tube
Hilmo	Finnish Care Register for Health Care
HIV	human immunodeficiency virus
HUS	Hospital District of Helsinki and Uusimaa
HUSLAB	laboratory of the Hospital District of Helsinki and Uusimaa
ICD	International Classification of Diseases
IFNγ	interferon-gamma
IGRA	interferon-gamma release assay
IL-12	interleukin-12
INH	isoniazid
IQR	interquartile range
IRR	incidence rate ratio
LAM	lipoarabinomannan
LOWESS	locally weighted scatterplot smoothing
LTBI	latent tuberculosis infection
MAC	Mycobacterium avium complex
ManLAM	mannose-capped lipoarabinomannan
MDR-TB	multidrug-resistant tuberculosis
MHC	major histocompatibility complex
MSMD	mendelian susceptibility to mycobacterial disease
NAAT	nucleic acid amplification test
NIDR	Finnish National Infectious Diseases Register
NTM	nontuberculous mycobacteria
OR	odds ratio
PAMPs	pathogen-associated molecular patterns
PIC	personal identity code
PPD	purified protein derivative
PTB	pulmonary tuberculosis
RD1	region of difference 1
TB	tuberculosis
TBI	tuberculosis infection
THL	National Institute for Health and Welfare
TLRs	toll-like receptors
TNFα	tumor necrosis factor-alpha
VRK	the Population Register Centre of Finland

1 Introduction

Tuberculosis (TB) is one of the leading infectious killers in humanity's history and regrettably has remained so. The Mycobacterium genus originated over 150 million years ago, and the common ancestor of modern *Mycobacterium tuberculosis* likely appeared approximately 20,000 years ago. (Kapur 1994, Barberis 2017) Deformities caused by the disease have been found in Egyptian mummies dating back to 2400 BCE, and the first descriptions of the disease are recorded in Biblical books in Ancient Hebrew. (Morse 1964, Daniel 1999) The Greek physician Hippocrates carefully described the adult disease manifestations, and TB is still falsely regarded by many as a primarily adult pulmonary disease. (Barberis 2017)

Until the discovery of streptomycin approximately 70 years ago, there was no effective treatment for TB and approximately 40% of those in whom it occurred died. (Marais 2004, Starke 2016) In the pre-TB medication era, TB was commonly known as "consumption". (Barberis 2017) Although the term appropriately describes the slow disease progression in adults, it fails to agree with the distinct nature of childhood disease. For successful public health policy, it is fundamental to understand the natural progression of childhood TB: especially young children are vulnerable to infection and subsequent rapid, severe, and lethal disease. Without preventive treatment, after primary infection, as many as 20% of children under the age of one will develop severe, miliary or meningeal, TB disease compared to less than 0.5% of adults. (Marais 2004, Starke 2016)

The global burden of childhood TB is extensive, and it is a significant cause of child mortality, especially in countries affected by poverty. (World Health Organization 2020) Even in low-incidence countries, TB remains a significant public health concern that has severe social implications. Due to triumphant anti-TB work in Finland, TB incidence and paediatric morbidity drastically decreased for decades and is presently considered very low. The influence of the anti-TB movement on Finnish society is still visible, e.g., in school meals provided free of charge and the social welfare system for the ill. TB was also the first disease to be collected in Finland to a national health care register. (Sund 2012)

A crucial component of paediatric TB prevention in Finland used to be, and still globally is, universal and comprehensive Bacille Calmette-Guérin (BCG) vaccinations at infancy. (Salo 2006) BCG vaccination effectively prevented severe childhood disease and likely prevented numerous untimely TB deaths among young children. (Roy 2014) However, as the overall risk of TB exposure in Finland became low, and the BCG vaccine itself can cause serious adverse events among the vaccinees, in September 2006, the BCG vaccination policy in Finland changed to a risk group-based approach. (Kilpi 2006, Salo 2006) Since then, only children deemed to have a high risk of TB exposure have been eligible for vaccination. As a result, a novel BCG-unvaccinated and vulnerable generation of children has grown in Finland for the first time since the 1940s.

Another essential component of TB prevention is contact tracing. Although children are rarely infectious, an essential part of contact tracing is to identify TB infected children and start therapy quickly before progression to disease. (Erkens 2010) Due to the recent generation of BCG-naïve young children, the need for rapid and effective TB contact tracing in Finland has become even more critical.

In recent years, awareness of infections caused by nontuberculous mycobacteria (NTM) has risen in the global community of paediatricians. (Lopez-Varela 2015) Although childhood morbidity caused by NTM is much lower and milder compared to TB, it has been recognized as a major infectious cause of prolonged childhood lymphadenitis. Currently, the epidemiology of childhood NTM infections is poorly understood due to a lack of nationwide epidemi-

ological studies. (Lopez-Varela 2015) Previous experience from countries discontinuing universal BCG vaccinations suggests that the BCG vaccine might provide protection also against NTM infections. (Romanus 1995) However, due to the lack of recent publications, the issue has remained debated.

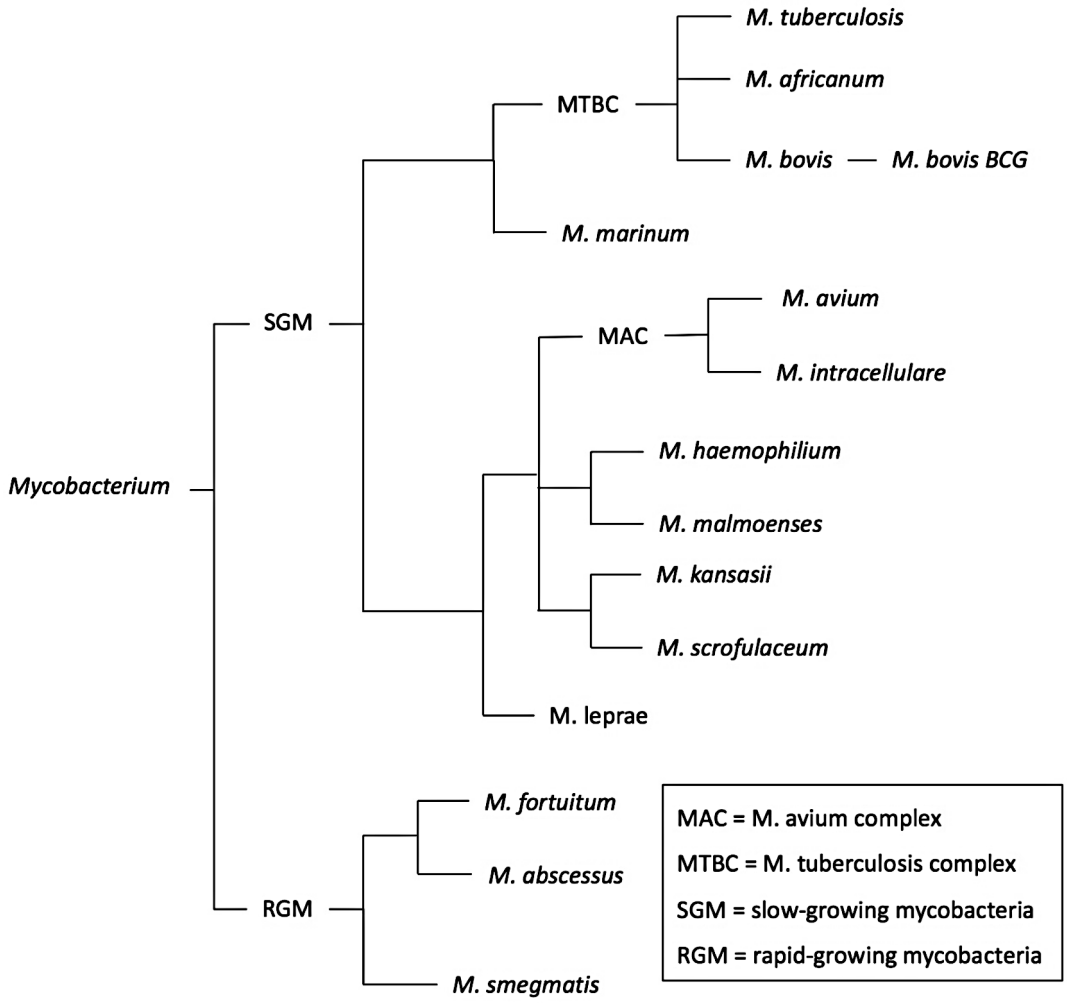
Current diagnostic methods for NTM lymphadenitis are limited due to poor sensitivity and the need for invasive interventions. (Zimmermann 2017) During universal BCG vaccinations, in-house modifications were made in the laboratory of the Hospital District of Helsinki and Uusimaa (HUSLAB) to a commercial immunological test for TB. The modifications were originally designed to increase the test's reliability and investigate immunological memory caused by BCG vaccination. After the BCG vaccination policy in Finland changed, the utility of the modified test in childhood NTM lymphadenitis diagnostics was noticed.

Effects of the BCG vaccination policy change on childhood mycobacterial infections in Finland have remained to a large extent unexamined. The potential of the modified immunological test for NTM lymphadenitis diagnostics and effectiveness of TB contact tracing among children have not been studied after the BCG vaccination policy change.

2 Review of the Literature

2.1 MYCOBACTERIA

The genus *Mycobacterium* belongs to the family of *Mycobacteriaceae* from the order of *Actinomycetales*. (Goren 1978) The taxonomy and phylogenetic tree of the genus are constantly evolving. Currently, the *Mycobacterium genus* includes >190 different species, and more species are almost certainly yet to be discovered. The genus is commonly divided into three subgroups *Mycobacterium tuberculosis complex*, NTM, and *M. leprae*. (Lopez-Varela 2015)



Graphic 1 *Phylogenetic tree of the genus Mycobacterium. Modified from Biet 2005 and Zhang 2013.*

2.1.1 General Characteristics

Mycobacteria are approximately 0.2 to 0.6 micrometers broad and 1.0 to 10 micrometers long, slightly curved or straight, rod-shaped bacteria (i.e., bacillus). The bacilli are immobile, aerobic, and gram-positive. Due to the high-lipid structure, the mycobacterial cell wall resists staining with routine dyes such as Gram stain. After staining with specific methods such as Ziehl-Neelsen staining, the cell wall also resists decolorizing by acid alcohol. Thus, mycobacteria are characterised as alcohol- and acid-fast bacillus (AFB) as a distinguishing feature from other bacteria. (Goren 1978)

2.1.1.1 **Cell Envelope**

The cell envelope is comprised of the capsule, cell wall, and cytoplasmic membrane. The capsule is the outmost layer of the cell envelope. The primary compounds of bacterial capsules are polysaccharides, and the capsule of *M. tuberculosis* is mostly α-glucan. The mycobacterial capsule also consists of lower amounts of other polysaccharides, arabinomannan and mannan, as well as various proteins and lipids. (Chiaradia 2017, Kalscheuer 2019)

A remarkable feature of mycobacteria is the complex cell wall structure. The outer cell wall is very waxy as it is abundant with mycolic acid, long-chain cross-linked fatty acids, and various cell-wall lipids. These tightly packed cell-wall lipids form a lipid layer called mycomembrane or outer membrane that functions as a hydrophobic barrier. (Barry 2001, Bansal-Mutalik 2014) The mycolic acids of the outer membrane are covalently linked via arabinogalactan to the underlying peptidoglycan structure, and the peptidoglycan-arabinogalactan complex composes the structure of the inner cell wall. (Chiaradia 2017, Kalscheuer 2019) Lipoarabinomannan (LAM) is a polysaccharide skeleton that anchors capsule polysaccharides to the cell wall. It is also one of the most essential antigenic polysaccharides on the cell surface of *M. tuberculosis*. (Sani 2010, Li 2020) Mannose-capped lipoarabinomannan (ManLAM) is a lipoglycan present in more pathogenic Mycobacterium species. The location of ManLAM is still somewhat unresolved, and it is proposed to have a non-permanent transit location through the cell envelope. (Turner 2018)

The inmost layer of the cell envelope is the cytoplasmic plasma membrane or the so-called inner membrane. (Kalscheuer 2019) In contrast to the other cell envelope structures, the mycobacterial inner membrane is more similar to other bacteria. (Daffe 1998) It is a conventional phospholipid bilayer containing proteins that perform all the membrane-associated functions within bacteria, such as energy production and lipid biosynthesis. (Silhavy 2010, Chiaradia 2017) Proteins within the inner membrane are also involved in protein secretion, such as the vital ESX protein secretion system. (Bosserman 2017)

2.1.1.2 **Genetic Structure**

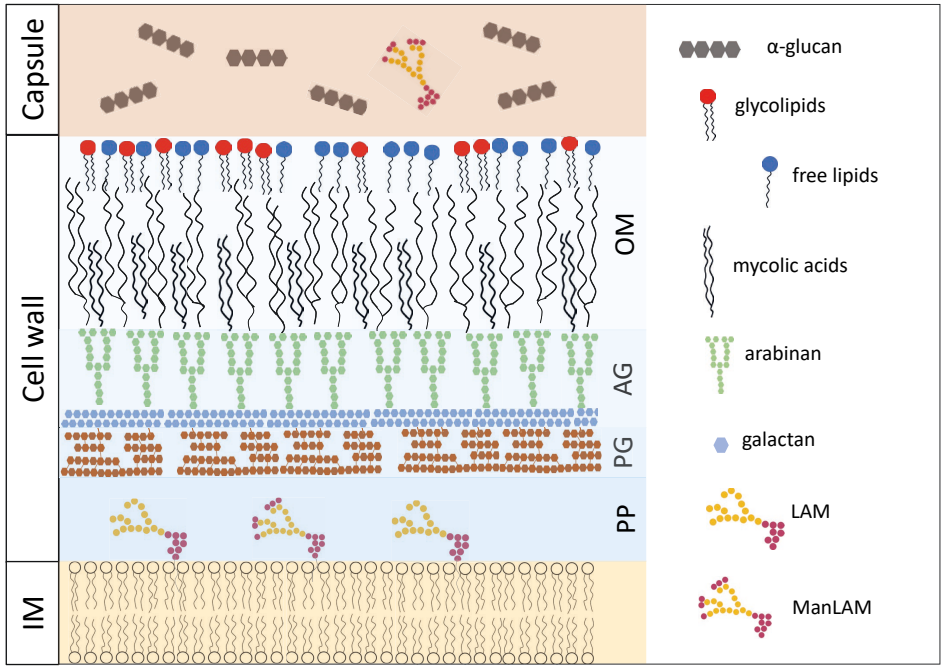
The mycobacterial genome is approximately 4.4 million base pairs long and encodes for approximately 4000 genes. (Cole 1998) The genome has a high content of guanine plus cytosine. Different mycobacterial species can rapidly be identified by sequencing the 16S RNA, RNA polymerase, or hsp 65 genes. The genotypic classification also translates to the phenotypic in vitro classification of cultured strains into rapid-growing or slow-growing species. (Rogall 1990, Kim 1999, Ringuet 1999)

The region of difference 1 (RD1), which is absent in most NTM species, includes genes that encode ESX-1: the protein secretion system of 6 kDa early secretory antigenic target (ESAT-6) and 10 kDa culture filtrate protein (CFP-10) which are significant contributors to the virulence of *M. tuberculosis*. (Romagnoli 2012, Houben 2012, Hermansen 2014)

2.1.2 Mycobacterium Tuberculosis Complex

The *Mycobacterium tuberculosis complex* includes multiple species, mainly *M. tuberculosis, M. bovis, and M. africanum.* All of them are known to cause disease in humans, but clinically the most important *Mycobacterium tuberculosis complex* species are *M. tuberculosis* and *M. bovis*. *M. tuberculosis*, the "tubercle bacillus", was first described in 1882 by Robert Koch, who later

Graphic 2 *Schematic representation of the mycobacterial cell wall. Modified from Hett 2008 and Kieser 2014.*



AG=arabinogalactan, IM=inner membrane, LAM=lipoarabinomannan, ManLAM=mannose-capped lipoarabinomannan, OM=outer membrane, PG=peptidoglycan, PP=periplasm

received the Nobel Prize in Physiology or Medicine for his discovery. (Barberis 2017)

This "Koch's bacillus" is an exceptionally successful pathogen causing most TB morbidity in humans. The success of *M. tuberculosis* is mainly related to the ability to infect macrophages and ultimately persist and replicate within them. (Houben 2012, van der Wel 2007, Lerner 2017) *M. tuberculosis* grows slowly with a doubling time of approximately 15–20 hours, and, therefore, it can take two to six weeks for *M. tuberculosis* to grow on standard cultures. *M. tuberculosis* is also capable of infecting other animals, but it has never been identified in environmental specimens. (Casanova 2002) This suggests that it is an obligate parasite, and the reservoirs of *M. tuberculosis*, thus the source of infections, are other humans. *M. bovis* causes bovine TB. However, it can also cause disease in humans. An attenuated strain of *M. bovis* is used as a live vaccine against TB: the vaccine is commonly known as the BCG vaccine. *M. bovis* BCG is further addressed in a separate chapter.

2.1.3 Nontuberculous Mycobacteria

NTM represents the largest fraction of the *Mycobacterium genus*. (Lopez-Varela 2015) They are primarily non-pathogenic, free-living environmental saprophytes. (Falkinham 1996) Hence, they are also sometimes referred to as environmental mycobacteria or atypical mycobacteria. Some NTM species can, however, cause disease in humans. NTM are ubiquitous in the environment and have widely been found in soil and drinking water systems in Finland. (Collins 1984, Covert 1999, Iivanainen 1993, Iivanainen 1997, Torvinen 2004) An important group within NTM is the *Mycobacterium avium complex* (MAC) which includes multiple pathogenic species (i.e., *M.*

avium, M. intracellulare). Other important pathogenic NTM species are *M. malmoense, M. lentiflavum, M. scrofulaceum, M. heamophilum, M. kansasii, M. abcessus, M. fortuitum,* and *M. marinum*. Similar to *M. tuberculosis*, it can take several weeks for NTM to grow on conventional cultures. However, some NTM species (i.e., *M. fortuitum, M. abscessus, M. smegmatis*) belong to the rapid-growing mycobacteria group, previously identified based on growth in less than seven days. (Brown-Elliott 2017). NTM can form and survive on biofilms in pipelines and other surfaces such as catheters. Some NTM species (i.e., MAC) are also thermotolerant and can survive even in hot water. (du Moulin 1988)

2.2 IMMUNOLOGICAL RESPONSE TO MYCOBACTERIA

Mycobacterial exposure of humans is constant, and the subsequent immune response complex. Both the innate and adaptive immune responses play a crucial role in the host immune response against TB and other mycobacterial infections. Much of the outcome is determined soon after the immune system first encounters the mycobacteria, and multiple mycobacterial virulence factors aim to evade or tolerate the antimicrobial mechanisms of the immune system. In most cases, the host immune response results in the complete elimination of the bacteria or control of the infection without progression to disease. Nevertheless, the range of clinical outcomes is puzzlingly various between individuals. Many identified factors affecting both innate and adaptive immunity, such as young age and immune deficiencies, increase mycobacterial disease susceptibility. Many questions concerning the transition from infection to disease and immunity against mycobacteria remain unanswered. Understanding the fundamental aspects of the immune response is crucial for clinicians and for the development of novel diagnostic tests, vaccines, and treatments against mycobacterial diseases.

2.2.1 Innate Immune Response

Important cell types involved in the innate immune response include airway epithelial cells, macrophages, dendritic cells, neutrophils, and natural killer cells. (Lerner 2015, Sia 2015) Pathogen-associated molecular patterns (PAMPs), such as carbohydrate, glucolipid, and lipoprotein surface components, enable the recognition of mycobacteria by the immune cells. Various pattern recognition receptors facilitate the recognition of PAMPs. Important receptors believed to be involved in the recognition of mycobacteria include Toll-like receptors (TLRs), nucleotide oligomerization domain-like receptors, Dectin-1, and C-type lectin receptors. (Li 2012, Killick 2013, Lerner 2015) In addition to phagocytosis, the pattern recognition receptors also facilitate a complex signaling cascade, including the synthesis of multiple inflammatory cytokines, promoting autophagy, apoptosis, and inflammasome activation. (Lerner 2015). One of the multiple inflammatory cytokines is tumor necrosis factor-alpha (TNFα) that plays a crucial role in the host immune response against intracellular pathogens such as mycobacteria. (Hedman 2011, Solovic 2010) TNFα is a proinflammatory cytokine that activates macrophages and recruits other immune cells to the infection site. (Pfeffer 2003, Parameswaran 2011)

2.2.1.1 **Airway Epithelial Cells**

Airway epithelial cells provide a physical barrier against various pathogens, including mycobacteria, entering the respiratory tract. Furthermore, airway epithelial cells express specific pattern recognition receptors that can recognize mycobacterial PAMPs and modulate the mucus composition to increase its antimicrobial capacity. (Li 2012)

2.2.1.2 **Macrophages**

Macrophages located in the airways (i.e., alveolar macrophages located in the alveoli) enact as sentinel cells; they are the first "professional" immune cells to encounter the bacteria. (Lerner 2015) They play an important role in the removal of microbes and other foreign particles through phagocytosis: the process of engulfing and enclosing the particle in an internal vesicle called the phagosome that fuses with

an intracellular lysosome to form a phagolysosome. The primary receptors thought to mediate phagocytosis of *M. tuberculosis* in human macrophages are the mannose receptors and complement receptor 3. (Schlesinger 1993, Kang 2005) Structural components on the *M. tuberculosis* cell capsule functioning as ligands for nonopsonic phagocytosis are LAM and mannan for mannose receptors, and α-glucan for complement receptor 3. (Schlesinger 1993, Cywes 1996) Macrophages also express multiple additional pattern recognition receptors that play an essential role during mycobacterial infections: macrophage TLRs, for instance, induce the synthesis of TNFα that in turn activates macrophages. (Parameswaran 2011) After activation, macrophages demonstrate multiple mechanisms for bacterial elimination, such as the production of oxygen and nitrogen component, phagosome acidification, and autophagy of intracellular pathogens. (Liu 2017) *M. tuberculosis* is, in some instances, able to modulate and evade the normal microbicidal functions of macrophages and phagosomes, enabling it to persist and replicate within macrophages. (Houben 2012, van der Wel 2007, Lerner 2017)

2.2.1.3 **Dendritic Cells**

Immature or resting dendritic cells are also involved in the first line of defence. They are highly efficient phagocytes and enact as a link between the innate and adaptive immune responses. (Henderson 1997) In addition to mannose receptors, dendritic cells express dendritic cell-specific intercellular adhesion molecule grabbing nonintegrin, which recognises certain mycobacterial PAMPs. (Liu 2017) Mycobacterial ligands for nonopsonic phagocytosis mediated by this receptor are LAM and mannan. (Tailleux 2003) After phagocytosis of the bacteria, maturation of the dendritic cells is initiated and continues in the lymphoid tissue. (Marino 2004) The migration via lymphatic vessels to the draining lymph node and further maturation of dendritic cells is promoted by local inflammatory cytokines. (Rescigno 2001) In the lymph node, mature dendritic cells express high levels of Major Histocompatibility Complex (MHC) class I and II molecules that facilitate the presentation of mycobacterial antigens to T cells. (Marino 2004) Mature dendritic cells also produce cytokines such as chemokines attracting naïve T cells to the lymph node and interleukin-2 that promotes T-helper- (Th-) 1-type adaptive immune response. (Adema 1997)

2.2.1.4 **Neutrophils And Natural Killer Cells**

As a result of the initial innate immune response, neutrophils and natural killer cells are also recruited to the infection site. In TB disease, neutrophils are the predominant immune cell type at the site of infection. (Eum et al. 2010) Neutrophils are phagocytic cells that aim to kill phagocytosed pathogens through multiple mechanisms, including lytic enzymes, antimicrobial peptides, and reactive oxygen species. (Lerner 2015) Neutrophils have also demonstrated an extracellular role during *M. tuberculosis* infection: through the formation of neutrophil extracellular traps, they can capture but not eliminate *M. tuberculosis*. (Ramos-Kichik 2009) Furthermore, neutrophils secrete cytokines and release extracellular vesicles that recruit and activate other immune cells and modulate the functions of dendritic cells and macrophages. (Riedel 1997, Alvarez-Jiménez 2018) Natural killer cells recognise infected macrophages and lyse them. (Vankayalapati 2002, Vankayalapati 2005) Natural killer cells also secrete multiple inflammatory cytokines. (Lerner 2015)

2.2.2 Adaptive Immune Response

The two main classes of the adaptive immune response are antibody and cell-mediated immune response. Lymphocytes carry out adaptive immune response; T lymphocytes account for the cell-mediated immune response and B lymphocytes for the antibody response.

2.2.2.1 **T Lymphocytes**

Distinguished by the presence of either molecule on their surface, the main two subsets of T lymphocytes are CD_4 cells, also commonly referred to as

Th-cells, and CD8 cells. After antigen presentation via MHC, naïve CD4 cells differentiate into effector cells that produce multiple cytokines. (Spellberg 2001, Kaufmann 2001) The activation of CD4 at the infection site is characterised by the formation of granuloma, which is essential in the containment of *M. tuberculosis*. The effector CD4 cells can be further divided into specific subtypes by the types of cytokines they produce. The two classic subtypes are Th1 lymphocytes that tend to promote inflammation and Th2 lymphocytes that produce a counteracting anti-inflammatory response. (Spellberg 2001, Berger 2000) The Th1-type cytokines, particularly interferon-gamma (IFNγ), facilitate the critical microbicidal response steered to kill intracellular pathogens such as mycobacteria. CD8 cells have a less essential role in mycobacterial infection. However, CD8 knockout murine models have demonstrated that CD8 cells are also involved in mycobacterial control through the production of cytotoxic perforins and granulysin that can kill mycobacteria. (Tena-Coki 2010, Semple 2011)

2.2.3 Virulence Factors of Mycobacteria

M. tuberculosis is an exceptionally successful pathogen that has evolved various strategies counteracting the immune response. The success of *M. tuberculosis* is mainly related to its abilities as an intracellular pathogen: *M. tuberculosis* can persist in the intracellular phagosome, escape into cytosol, and replicates within the infected macrophage. (Houben 2012, van der Wel 2007, Lerner 2017) Several virulence factors targeted against macrophage and other immune cell functions have been identified among *M. tuberculosis* and other mycobacterial species.

2.2.3.1 **Cell Wall Structure**

The cell wall structure of mycobacteria provides resistance to chemical injury and certain antibiotics. (Daffé 1998, Barry 2001) The cell wall intrinsically exhibits low permeability that is also partly explained by the organisation of the whole-cell envelope. (Kalscheuer et al. 2019) Furthermore, LAM inhibits the fusion of phagosome with lysosome and activation of IFNγ. (Hmama et al. 2004, Welin 2008, Chan 1991)

2.2.3.2 **ESX-1 Secretion System**

Translocation of certain mycobacteria species from the phagolysosome into the cytosol has been suggested as one of the key features of pathogenicity. (Houben 2012) Thus, an essential virulence factor of *M. tuberculosis* is the ESX-1 system that involves the secretion of ESAT-6 and CFP-10 into the host cell. (Jonge 2007) In an acidic environment such as a phagolysosome, ESAT-6 dissociates from the chaperone protein CFP-10 and interacts with the phagolysosome biomembranes. (Jonge 2007) It demonstrates pore-forming activity allowing entry of the bacilli from the phagolysosome into the cytosol. (van der Wel 2007) ESX-1 secretion system, particularly ESAT-6, also inhibits the production of IFNγ, induces host cell death, and impairs the autophagic functions of the immune response. (Wang 2009, Welin 2011, Romagnoli 2012)

2.2.4 Susceptibility to Mycobacterial Disease

2.2.4.1 **Inherent Susceptibility**

Certain inherent defects involving the immune responses contribute to vulnerability for mycobacterial disease, especially those involving the important IFNγ/interleukin-12 (IL12) pathway that plays an essential role in the immune response to mycobacterial infection. (Blackwell 1994, Altare 1998, Casanova 2002) Inherent traits affecting this signaling pathway, encompassing multiple different mutations, are collectively referred to as Mendelian susceptibility to mycobacterial disease (MSMD). (Rosain 2018) The mutations within IFNγ or IL12 genes result in defects of the IFNγ or IL12 receptors. Subsequently, the associated signaling pathways are affected, leading to impaired granuloma formation and, thus, containment of mycobacteria. (Casanova 2002) As a result, the affected individuals are vulnerable to se-

vere disseminated mycobacterial infections. Therefore, investigations of the IFNγ/IL12 pathway are essential especially among patients with unusually severe disseminated infection caused by less virulent mycobacterial species (i.e., BCG or NTM). (Casanova 1995) However, the required immunological investigations are advanced and rarely available outside developed countries, and the prevalence of MSMD remains largely unknown. Additionally, congenital immune cell deficiencies such as severe combined immunodeficiency affect the number and function of T- and B-lymphocytes debilitating the immune system severely. This leads to a severe vulnerability to mycobacterial disease among other infections. (Starke 2016)

2.2.4.2 **Acquired Susceptibility**

Several acquired immune deficiencies increase vulnerability to mycobacterial infections. The h*uman immunodeficiency virus* (HIV) targets CD4 cells and leads to a T cell deficiency. Due to the crucial role of CD4 cells in effective anti-mycobacterial immune response, the HIV pandemic is a significant factor that increases global TB morbidity. (World Health Organization 2020) HIV-infected patients are also more prone to disseminated and extrapulmonary TB disease. (Gilks 1990) Furthermore, HIV-infected children are at risk of disseminated BCG *M. bovis* infections after BCG vaccinations as well as respiratory and disseminated NTM infections. (Hesseling 2006, Borand 2019)

Anti-TNFα medication, sometimes used in the treatment of rheumatic and inflammatory bowel diseases, blocks the normal functions of TNFα and results in an acquired susceptibility to mycobacterial diseases. Therefore, investigations and treatment of TB infection are recommended before anti-TNFα medication is initiated. Some anti-TNFα molecules can also pass through the placenta into the fetus and affect the immune defence of the newborn. (van der Woude 2015, Esteve-Solé 2017) Anti-TNFα molecules with a monoclonal immunoglobulin G structure (i.e., infliximab, adalimumab, golimumab) are transported through the placenta via neonatal Fc receptors that also function as the transporter of maternal immunoglobulin G. (Kane 2009, Djokanovic 2011) Transportation of maternal immunoglobulin G is highest during the second and third trimester. (Simister 2003) Thus, high anti-TNFα concentrations have been observed in newborns exposed during the second and third trimesters. (Mahadevan 2013, Julsgaard 2016, Esteve-Solé 2017) Attenuated responses to BCG vaccinations and disseminated *M. bovis* BCG infections have been described in newborns exposed to anti-TNFα during pregnancy. (Esteve-Solé 2017, Cheent 2010) Therefore, several national health agencies recommend stopping anti-TNFα medication during pregnancy or postponed vaccinations with live-attenuated vaccines. (Public Health Agency of Canada 2016, Centers for Disease Control and Prevention 2014)

2.2.4.3 **Susceptibility of Young Children**

Young age has been identified as an important factor contributing to the development of infection, progression to disease, and severity. (Perez-Velez 2012) The full array of different immunological factors affecting the susceptibility of young children is not fully understood. Nevertheless, both innate and adaptive immunity undergoes multiple changes during the early years of life. (Shey 2014) The recruitment and regulation of immune cells in infants is distinct from adults and sometimes also referred to as immature. (Starke 2016) Macrophage phagocytosis and dendritic cells antigen presentation are also less sufficient in young children. (Smith 1997) Other factors, including maturation of TLRs and signaling pathways involved in proinflammatory responses, likely explains a part of the age-dependent vulnerability to mycobacterial disease. (Burl 2011, Shey 2014)

2.3 CHILDHOOD TUBERCULOSIS

2.3.1 Pathogenesis

2.3.1.1 **Transmission**

Excluding laboratory settings, exposure to the pathogen through contact with a person with TB disease is required for the transmission of tuberculosis infection (TBI). (Perez-Velez 2012) The risk of TBI after exposure is highly associated with the infectivity of the source case and closeness of contact. (Grzybowski 1975, Perez-Velez 2012) The infection is usually transmitted via the respiratory tract. A person with active pulmonary TB (PTB) produces droplets containing aerosolised bacilli while coughing or through other respiratory movements. After aerosolisation, the infectious droplets can remain in the air for several hours, but the aerosol survival rate of *M. tuberculosis* over an hour is poor. (Loudon 1969, Lever 2000) Inhalation of these droplets allows the bacilli to enter alveoli, where it is ingested by alveolar macrophages. (Lerner 2015) Other possible transmission routes include aerosolisation of the bacilli from infectious tissue (e.g., during autopsy or other medical procedures) or direct inoculation of the bacilli (e.g., into a wound). (Flavin 2007, Franco-Paredes 2018) TB is, however, not transmitted through surface contact. (Starke 2016) Congenital TB is transmitted from an infected mother to the child through transplacental transmission during the pregnancy or ingestion of the bacilli during the delivery. (Samedi 2017, Chang 2018)

2.3.1.2 **Primary Infection**

Depending on the infectivity of the source case, eventually 30–80% of children living in the same household are infected. (Grzybowski 1975, Martinez 2020) Primary infection typically begins in the lungs, characterised as primary PTB, resulting in localised granulomatous parenchymal inflammation referred to as a primary Ghon focus. (Perez-Velez 2012) Bacilli from this primary focus drain through the lymphatic system into the regional lymph nodes and onwards into the systemic circulation causing occult spread to different organs. After the bacilli are phagocytosed, they are either eradicated completely or survive within macrophages in the target organs.

2.3.1.3 **Infection Without Disease**

Usually, the primary infection is contained by the immune system without signs of active disease. (Davies 1961, Starke 2016) However, live bacilli can survive and lie dormant within the host for years. At any point during the lifetime of the infected person, the dormant infection can progress into TB disease. (Getahun 2015) The risk of progression to disease is highest within the first two years after the primary infection, and in 90% of paediatric TB cases the primary infection originated within the previous year (Perez-Velez 2012). In adults, this dormant infection without signs of active disease is commonly referred to as a latent tuberculosis infection (LTBI). (Getahun 2015) In children, however, an infection without signs of active disease after recent exposure is also referred to as a tuberculosis infection (TBI) to highlight the different nature and risk of disease progression compared to an adult LTBI.

2.3.1.4 **Disease**

Children under the age of five have the highest risk of developing TB disease after primary infection. Data from the pre-chemotherapy era indicates that the risk of developing the disease in children under one year of age is as high as 50% without preventive treatment. (Marais 2004) The risk falls in the older age groups but is still approximately 20-30% in children aged 1–2 years and 5% in children age 2–5 years. (Marais 2004) However, recent data suggest that the risk might be slightly lower, approximately 20% in children under one year of age and approximately 18% in children age 2–5 years. (Martinez 2020) In contrast, children aged 5–10 years seem to have less risk of developing the disease (2%) than adolescents and young adults (10–20%). (Marais 2004)

Childhood TB is typically paucibacillary: the bacterial load of *M. tuberculosis* is low. TB disease most commonly manifests as a PTB. (Starke 2016)

In PTB, the pulmonary infection caused by *M. tuberculosis* is accompanied by clinical symptoms or radiological signs indicating failed containment of the bacilli. There are several different PTB manifestations characterised primarily by radiological findings. (Perez-Velez 2012) Intrathoracic lymph node disease, where enlarged regional lymph nodes are observed, is the dominant manifestation in under-five children. (Starke 2016) After ten years of age, adult-type disease with apical involvement of the upper lobes of lungs and cavity formation becomes the dominant PTB manifestation. (Davies 1961, Starke 2016)

Young children have a remarkably higher risk for developing severe disease manifestations: meningeal or miliary (disseminated) disease affects 10–20% of those infected under the age of one and 2-5% of those aged 1–2 years. (Marais 2004) Among older children and adults, only approximately 0.5% develop a meningeal or miliary disease. (Marais 2004) In miliary infection, each focus results in local granuloma with central necrosis. (Kwong 1996) Due to haematogenous spread during primary infection, TB disease can also manifest solely in any organ throughout the human body, such as bone, spleen, or kidneys.

In the pre-chemotherapy era, the estimated fatality rate of TB was 40%. (Marais 2004) However, without treatment, the disease essentially killed every young child whom it touched. Although prompt treatment reduces mortality, young children are still vulnerable due to more severe disease manifestations: even with current anti-TB regimens, the mortality for tuberculous meningitis is approximately 15–19%. (Christensen 2011, Rohlwink 2019)

Symptoms commonly associated with childhood PTB are summarised in Table 1. (Marais 2005) However, approximately half of children with newly diagnosed TB do not report any symptoms. (Marais 2005) In general, TB symptoms such as cough are characterised as persistent and unremitting in nature. In endemic settings, the combination of unremitting cough lasting over two weeks, weight loss within the preceding three months, and reported fatigue are highly suggestive for TB in children over three years of age (Marais 2006)

Symptom	Prevalence
Cough[1]	**44%**
Weight loss[2]	**28%**
Fever	**22%**
Night sweats	**17%**
Fatigue	**17%**
Dyspnoea	**5%**
Haemoptysis	**0%**
None	**50%**

[1] persistent and unremitting

[2] i.e., failure to thrive

Table 1 *Symptoms commonly associated with paediatric PTB. (Marais 2005)*

2.3.2 Epidemiology

TB is one of the most important infectious diseases globally, both historically and presently. At the beginning of the 19th century, TB mortality in Europe ranged from 200 to 300 per 100,000. TB burden is highly associated with poverty and limited resources. (World Health Organization 2019) Most of the current global TB burden is concentrated in the 22 high burden countries, mainly in Southeast Asia and Africa. Overall, an estimated one-third of the global population is infected with *M. tuberculosis,* and annually 10 million people fall ill with TB. (Houben 2016, Dye 1999, Getahun 2015, World Health Organization 2019) Males are thought to be more at risk as they are overrepresented in many TB risk groups. (World Health Organization 2020 European Centre for Disease Prevention 2020) However, several factors may affect reporting of women with TB and cause bias in the reported data. (Thorson 2001) The gender differences in notifications seem to be more distinct among older adults and less so in children. (European Centre for Disease Prevention 2020)

Childhood TB indicates recent transmission. (Perez-Velez 2012) Therefore, it is a sentinel event indicating ongoing transmission and reflecting the success, or failure, of TB prevention within the com-

munity. Adults infected while young are also an important reservoir for future TB disease due to later LTBI reactivations and subsequent transmission to others. (Erkens 2010) In 2017, there were an estimated 1.3 million children under the age of five living in the same household with an infectious PTB case, but only roughly 23% received preventive treatment. (Hamada 2019) An estimated one million children develop TB each year, accounting for approximately 10% of the global burden. (World Health Organization 2019) However, poor reporting of paediatric TB cases makes it difficult to accurately estimate the disease burden among children. Underestimation of childhood TB is an acknowledged problem of the global TB data (Perez-Velez 2012). In endemic countries, TB is also a significant cause of childhood mortality, although TB is likely underrepresented in reported causes of deaths as many cases are designated as HIV infection or pneumonia alone. Autopsy studies of African children who died of pneumonia found that *M. tuberculosis* was found in the lungs in approximately 10–20% of the cases. (Chintu 2002, Bates 2016) An estimated 239,000 children under 15 years of age died from TB in 2015: >70% in southeast Asia or Africa, and >90% did not receive any TB treatment. (Dodd 2017)

The HIV pandemic, also highly associated with poverty and limited resources, further increases the burden and clinical challenges of TB. TB incidence is estimated to be twenty-fold higher among HIV infected population and also very high among HIV-infected children in high TB burden countries. (Gutman 1994, Madhi 2000) In children with TB, HIV co-infection is present in <5% of cases in high-income countries, in contrast to >50% in some high-burden countries. (Graham 2001, Nelson 2004) The outcome of children with an HIV co-infection is also poorer and mortality higher. (Palme 2002)

In European countries, the proportion of under-15 children among newly diagnosed cases ranges from <1% to 15% and accounts for approximately 4% of all new TB cases. (European Centre for Disease Prevention 2020) In low incidence counties, migration from high TB burden countries is shifting epidemiological trends. Most of the new TB cases occur in a population with foreign backgrounds, and the epicenter of TB has moved from older natives to young immigrants. (European Centre for Disease Prevention 2015). However, in Finland, native adults ≥65 years of age still account for more than a third of new TB cases. (European Centre for Disease Prevention 2020) Multidrug-resistant tuberculosis (MDR-TB), resistance to more than one drug and at least to isoniazid (INH) and rifampicin, is also an increasing challenge for TB prevention in Europe: approximately 18% of all new TB cases in 2018 were MDR-TB. (European Centre for Disease Prevention 2020)

2.3.3 Diagnosis

It is essential to separate TB disease and infection without the disease. In adults, microbiological confirmation (i.e., proof of actively multiplying *M. tuberculosis*) is commonly required to diagnose TB disease. However, due to the paucibacillary nature of childhood disease and children's poor ability to produce sputum samples, achieving microbiological confirmation is challenging, especially for young children. (Perez-Velez 2012, Starke 2016) Therefore, childhood TB disease is commonly diagnosed through a combination of a positive immunological test result with clinical symptoms or radiological findings consistent with TB disease.

2.3.3.1 **Microbiological Investigations**

The gold standard for PTB is sputum smear microscopy and mycobacterial cultures. A positive sputum smear result suggests that the number of bacilli within the sample is at least 5,000 to 10,000 per millilitre. In contrast, a positive culture is possible with a sample containing as little as 10 to 100 bacilli per millilitre. (Rasool 2019) Positive cultures also enable *in vitro* sensitivity testing to detect drug resistance of the *M. tuberculosis* strain.

Smear microscopy and mycobacterial cultures require an adequate sample: a tissue sample attained straight from the infection focus in extrapulmonary

TB or secretions from lower airways produced as sputum in PTB. Young children do not produce sufficient sputum samples easily and, thus, alternative methods for sputum collection are commonly needed. Induced sputum collection includes inhalation of aerosolised isotonic or hypertonic saline solution administered by nebulisation. The saline inhalation increases the production of mucus and induces a cough reflex. (Pizzichini 2002) Because young children also tend to swallow sputum, alternative methods for sample collection include gastric lavage and string test. (Zar 2005, Nansumba 2016) Gastric lavage is routinely performed early morning, and the sample is collected into a tube containing sodium carbonate. Multiple gastric lavage samples, for example collected on three consecutive days, are recommended to attain better yield. In the string test, a gel capsule attached to a string is swallowed and left in the stomach for four hours to dissolve while the string is coated with secretions that are retrieved for investigations. The string test is, however, not suitable for young children. (Chow 2006, Tafur 2018) Induced sputum seems to result in a better yield compared to gastric lavage; a single induced sputum sample is equivalent to three gastric lavage samples collected with the aforementioned protocol. (Zar 2005, Nansumba 2016) Nevertheless, only approximately 15–20% of new TB cases in children are identified by the presence of AFB in sputum smear samples, and bacterial confirmation through cultures is usually achieved in only 30–40% of cases. (Starke 2003, Zar 2005, Newton 2008)

2.3.3.2 **Nucleic Acid Amplification Tests**

Nucleic acid sequences specific for *M. tuberculosis* can be detected with a nucleic acid amplification test (NAAT). NAATs can be performed directly from a clinical specimen, and the results are usually ready within 24 to 48 hours. (Walter 2012) Certain line probe assay NAATs can also provide information on the drug sensitivity of the *M. tuberculosis* strain. (Rasool 2019) The commercially available Xpert MTB/RIF test (Cepheid, Sunnyvale, California) is a rapid automated PCR test that amplifies specific sequences within the *rpoB* gene that indicates resistance to rifampicin. Because resistance to rifampicin usually co-exists with INH resistance, the test can rapidly detect a potential MDR-TB case. The specificity of the Xpert MTB/RIF test is generally almost 100%, and the estimated sensitivity among adults with culture-positive PTB is 98% in smear-positive and 67% in smear-negative cases. However, the test's sensitivity is much lower in childhood paucibacillary disease: among children, the overall sensitivity of the Xpert MTB/RIF has ranged from 54 to 63%, and sensitivity among smear-negative children has been as low as 28%. (Sabi 2018, Nicol 2018) An updated version of the Xpert MTB/RIF test, designated Xpert Ultra, has demonstrated slightly higher sensitivity among children, with an overall sensitivity ranging from 64 to 74% and 44% among smear-negative cases. (Sabi 2018, Nicol 2018)

2.3.3.3 **Immunological Tests**

Tuberculin skin test (TST) and Interferon-Gamma Release Assays (IGRAs) measure *M. tuberculosis*-specific T cell responses suggestive of a TB infection. However, because the tests measure an immunological response, they cannot differentiate between infection and disease alone. It can also take several months for the tests to convert to positive after primary TB infection, and IGRAs may take longer to convert positive than TST. (Bennet 2019) Therefore, repeated testing after two to three months from suspected TB infection is recommended. Furthermore, immunocompromised patients can lack a sufficient number of effector T cells for a positive response. (Starke 2016)

The first TST was developed by an Austrian paediatrician Von Pirquet as a TB test for children. (Von Pirquet 1907). In TST, a purified protein derivative (PPD) (i.e., tuberculin) preparation acts as the antigen stimulant. PPD was initially derived from culture filtrates of *M. tuberculosis* and contains a mixture of over one hundred heterogeneous proteins. Several TST methods have been developed, and currently,

the most widely used is the Mantoux method. In the Mantoux method, PPD is intradermally injected into the forearm and causes delayed-type hypersensitivity response: a cell-mediated immune response that causes a local skin induration. (Sokal 1975, Lange 2010) The test result is reported as the diameter of the induration in millimetres measured 48 to 72 hours after the injection. (Sokal 1975) Reliable administration and interpretation of the test require specific training. Commonly, induration ≥5 mm is considered positive, and induration over 10 or 15 mm strongly positive. However, the test result should be interpreted in accordance with other factors such as BCG vaccination status and risk of TB exposure. (Kröger 1992) For TB disease, the estimated overall sensitivity of TST is 77%. (Pai 2008) In children with culture-confirmed TB, the estimated sensitivity of TST is 83% with a cut-off of ≥15 mm and 88% with a cut-off of ≥10 mm. (Kampmann 2009) The sensitivity of TST is, however, much lower in young children. (Kampmann 2009) NTM infection, BCG vaccination, or repeated TST testing can also cause a positive reaction to PPD and lead to a false-positive test result. (Powel 2000, von Rey 2001)

In IGRAs, the T cell responses are measured from a blood sample. Certain *M. tuberculosis*-specific antigens are used to stimulate effector T cells, which may release IFNγ if sensitised to the antigen. The commercially available IGRAs are QuantiFERON®-TB Gold In-Tube (QFT-GIT) and T-SPOT®. TB test. Both tests include synthetic peptides representing ESAT-6 and CFP-10 stimulatory antigens, and QFT-GIT also includes a third anti-mycobacterium tuberculosis antigen. QFT-GIT measures IFNγ concentration in a whole blood sample stimulated with the antigen mixtures. In T-SPOT.TB, peripheral blood mononuclear cells are separated from a whole blood sample and stimulated in separate wells, and the results are reported as spots (i.e., number of IFNγ producing cells) for each antigen. The overall estimated sensitivity of QFT-GIT is approximately 80% and T-SPOT.TB is approximately 60% for TB disease in children. (Kampmann 2009, Lange 2010). However, the sensitivity of QFT-GIT is approximately 57–77% and T-SPOT.TB is approximately 63–75% for LTBI in children. (Connell 2008, Kampmann 2009) The sensitivity of IGRAs in young children is even more questionable. (Lange 2010) In childhood primary TB, the number of *M. tuberculosis*-specific IFNγ-producing T cells and subsequent IFNγ response is likely less than in adults. (Upham 2006, Kampmann 2009) The sensitivity is likely also lower in extrapulmonary TB due to stronger containment of the immune response and a limited number of circulating *M. tuberculosis*-specific effector T cells. (Wilkinson 2005, Kampmann 2009)

2.3.3.4 **Radiological Appearance**

None of the radiological features seen in PTB are pathognomonic for TB. (Lange 2010) However, certain findings are typical with PTB. (Jeong 2008) TB in children under five years of age usually manifests as primary PTB and is characterised by enlarged regional lymph nodes. Thus, the most common abnormality observed in 90–95% of children is an enlarged lymph node, usually located unilaterally in the hilum or paratracheal region on chest radiography. (Weber 1968, Leung 1992) Parenchymal granulomatous inflammation during primary PTB in children may also result in unilateral consolidation on chest radiography which is seen in approximately 70% of cases. (Leung 1992) If pleural effusion is present, it is usually located on the same side with the primary PTB focus. (Jeong 2008) Computed tomography (CT) detects and characterises parenchymal abnormalities and mediastinal lymphadenopathy more accurately. (Kim 1997) In CT, the caseous necrosis of lymph nodes is exhibited by central low attenuation and granulomatous inflammation by peripheral rim enhancement. (Im 1987, Pombo 1992) Common CT abnormalities associated with the parenchymal consolidation of primary PTB are dense and homogeneous and sometimes depicted as patchy, linear, or nodular. (Leung 1999) In disseminated miliary TB, numerous randomly distributed nodules ranging from one to three mm in diame-

ter are seen in both lungs on chest radiography or CT. (Kwong 1996, Jeong 2008) Interlobular septal thickening and fine intralobular networks may also be present. (Im 1993)

2.3.4 Treatment

The treatment regimen depends on whether the infection is considered active (i.e., disease stage) or not active (i.e., primary infection or latent stage). TB treatment in children is in most parts similar to adult TB treatment; however, the dosages for children are calculated in proportion to body weight. Due to high metabolism, the proportional doses per kg are usually higher than in adults. Treatment should also include careful monitoring of possible side effects, regular follow-up, and a combination of vitamins (i.e., vitamin D and B6).

2.3.4.1 **Preventive Treatment**

Preventive therapy, i.e., treatment of TB infection without signs of active disease, encompasses recent primary infection and LTBI. After infection, preventive treatment prevents childhood disease in >90% of cases. (Martinez 2020) The first preventive therapy regimen that is still commonly used is daily INH for six or nine months. (International Union Against Tuberculosis Committee on Prophylaxis 1982, Nahid 2016) Current alternative regimens include daily rifampicin for four months or daily INH and rifampicin for three months. (Spyridis 2007, Assefa 2018) Children over the age of two can also be treated with high-dose INH-rifapentine administered once weekly for 12 weeks. (Sterling 2011, CDC 2012) Significant advantages of the shorter course regimens are better compliance, adherence, and completion rate. (Spyridis 2007, Sterling 2011, Assefa 2018)

2.3.4.2 **Treatment for the Disease**

Each anti-TB medicine demonstrates different effects on *M. tuberculosis* (i.e., bactericidal or bacteriostatic) and, therefore, active TB treatment includes a combination regimen of several agents. The World Health Organization recommends that the first-line regimen for PTB or peripheral lymphadenopathy is INH, rifampicin, pyrazinamide, and ethambutol for two months followed by a continuation phase regimen of INH and rifampicin for four months. (World Health Organization 2014) In children with meningeal or osteoarticular disease, the continuation phase regimen of INH and rifampicin should be prolonged to ten months. If drug resistance is suspected or confirmed, the regimen should be amended appropriately. (World Health Organization 2014)

Incomplete TB treatment may result in the development of relapse or drug resistance. (Moonan 2011, Hirpa 2013) Thus, the World Health Organization endorsed strategy is the Directly Observed Therapy (DOT) protocol. In DOT, the taking of each dose is observed and recorded by a health care worker or another person. The main goal of DOT is to ensure full adherence and completion of the designed treatment regimen. (World Health Organization 1999) In early studies, DOT was shown to be a cost-effective strategy that increased cure and coverage rates. (World Health Organization 1999) However, more recent studies have not shown increased cure or treatment completion rates in all TB patients treated with the DOT strategy. (Karumbi 2015)

2.3.5 Contact Tracing

TB prevention is a crucial part of the end TB strategy set by the World Health Organization. (World Health Organization 2019) A fundamental part of TB prevention, especially in low-incidence settings, is contact tracing. From a public health perspective, the main concern is that the progression of the infection into an infectious pulmonary disease will lead to further TB exposure in the community. Young children are, however, rarely infectious but more likely to develop severe disease rapidly. (Perez-Velez 2012) Therefore, the main goal of contact tracing regarding young children is to avoid severe disease with timely preventive treatment, and young children should be considered high-priority contacts. (Erkens 2010)

2.3.5.1 **Factors Affecting the Degree of Exposure**

The infectivity of the index case can vary substantially and is especially high in sputum smear-positive or cavitary PTB. Other clinical characteristics associated with increased infectivity of the index case are infection focus, cough, drainage, or treatment failure. (Erkens 2010)

Environmental factors affecting the intensity of exposure, such as proximity and duration, also increase the risk of infection. The risk is highest for contacts living in the same household (i.e., household contacts) or occupying the same small enclosed space (i.e., car or room) during the period of infectivity. (Fox 2013) Other environmental factors identified to increase the risk for infection are sharing a bed with the index case and poor ventilation. (Acuña-Villaorduña 2018)

2.3.5.2 **Recommendations**

Contact tracing and preventive treatment are widely practised across Europe. (Bothamley 2008) Contact tracing is initiated after an infectious case of new or recurrent TB is initially identified (i.e., index case). An index case with sputum smear-positive or cavitary PTB is typically considered highly infectious, and culture-positive and sputum smear-negative PTB or extrapulmonary TB index cases are considered less infectious. (Terveyden ja hyvinvoinnin laitos 2011, Terveyden ja hyvinvoinnin laitos 2017) The infectiousness of smear- and culture-negative PTB patients, particularly young children, is considered very low or non-existent. (Starke 2016) The retrospective period of infectiousness is usually deduced from the duration of symptoms such as cough. However, in the absence of cough, a general guideline is three months in sputum smear-positive or cavitary PTB and one month in only culture-positive PTB. (Erkens 2010) A contact case is any person who may have been exposed to TB of the index case. (World Health Organization 2012) The European consensus is that contacts should be informed of the contact investigations within a week after identification of the index case. (Erkens 2010) In Finland, the national TB program published by the Finnish Institute for Health and Welfare (THL) includes national contact tracing guidelines. The current guideline was issued in 2017 and the previous in 2011. (Terveyden ja hyvinvoinnin laitos 2011, Terveyden ja hyvinvoinnin laitos 2017) The Finnish guidelines are briefly summarised in Table 2.

Table 2 *The Finnish tuberculosis contact investigation guidelines published in 2011 and 2017. (Terveyden ja hyvinvoinnin laitos 2011, Terveyden ja hyvinvoinnin laitos 2017)*

Guideline	Index Case	Exposure Type	Contacts[2]
2011	Smear-positive or cavitary PTB	household	all
		cumulative >8 hours[1]	all
		cumulative <8 hours[1]	children <5 years
	Smear-negative and noncavitary PTB	household	children <5 years
		cumulative >8 hours[1]	children <5 years
2017	Smear-positive or cavitary PTB	household	all
		cumulative >40 hours[1]	all
		cumulative >8 hours[1]	children <7 years
	Smear-negative and noncavitary PTB	household	all
		cumulative >40 hours[1]	children <7 years

[1] repeated exposure in an enclosed space

[2] recommended for TB investigations

2.4 CHILDHOOD NONTUBERCULOUS LYMPHADENITIS

NTM infection can manifest in various organs, including lymph nodes, skin and soft tissue, lungs, ear, and bone. NTM lymphadenitis represents the vast majority of childhood NTM infections, and other disease manifestations are much rarer. (Tebruegge 2016) The epidemiology, diagnosis, and treatment for infections outside lymph nodes differ remarkably from that of NTM lymphadenitis. Therefore, the following chapter focuses especially on NTM lymphadenitis.

2.4.1 PATHOGENESIS

2.4.1.1 **Transmission**

In contrast to *M. tuberculosis*, NTM reservoirs are ubiquitous and vast in the environment. (van der Werf 2014) NTM have been widely found in environmental samples also in Finland. (Iivanainen 1993, Iivanainen 1997, Torvinen 2004) The specific transmission mechanisms are poorly understood. High exposure to soil has been suggested to increase infection risk, and certain MAC isolates from patients have been linked to their household water systems. (Maekawa 2011, Falkinham 2011) The infection is, therefore, likely acquired from environmental exposure to NTM. (Marshall 2011) Because most childhood NTM infections present as cervicofacial lymphadenitis, the pathogen is thought to enter through the mucosa of the oral or nasal cavity, and teething has been suggested to provide an entry point for the infection. (Haverkamp 2010)

2.4.1.2 **Disease**

The disease usually manifests unilaterally in the cervicofacial region. (Loeffler 2004, Hatzenbuehler 2014) The infection usually affects submandibular, submaxillary, cervical, or preauricular lymph nodes. (Griffith 2007) However, lymph nodes outside the cervicofacial region, such as mediastinal lymph nodes, can also be affected. (Wolinsky 1995)

A typical clinical picture is a nontender mass in a child with no apparent other symptoms. (Griffith 2007) The lymphadenitis develops slowly, and during the course of the disease, vivid color changes of the overlying skin may appear. Ultimately a fistula with drainage might develop, but some cases heal without fistulisation or drainage. (Lyly 2020) Full resolution occurs spontaneously within six months in approximately 70% of cases and within a year in all. (Zeharia 2008)

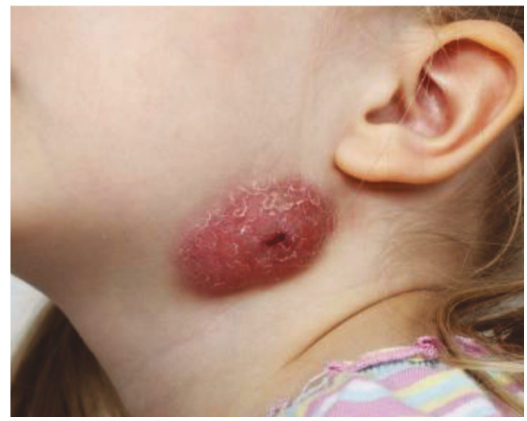


Figure 1 *Typical appearance of late-stage NTM lymphadenitis. (Photo courtesy of HUS)*

2.4.2 Epidemiology

2.4.2.1 **Incidence**

Virtually all humans are exposed to air- and water-borne NTM at some point in their life, and approximately 30% of asymptomatic children and 40% of adults exhibit a positive reaction in skin tests with *M. avium* sensitin. (Larsson 1993, von Reyn 2001) Skin test studies also suggest that exposure occurs similarly in both developed and developing countries. (von Reyn 1993) However, national surveillance studies concerning the incidence of childhood NTM lymphadenitis are scarce. The reported annual incidence of NTM lymphadenitis or infection among children under five years of age varies from

0.06 to 5.7 per 100,000, and seasonal variability in the incidence has been suggested. (Romanus 1995, Haverkamp 2004, Hermansen 2017, Thegerstrom 2008, Tebruegge 2016) In Finland, the annual incidence of cervical NTM lymphadenitis among children aged 1–5 years from 1977 to 1986 was 0.6 per 100,000. (Katila 1987)

2.4.2.2 **Patient Characteristics**

Childhood NTM lymphadenitis typically presents at the age of one to five years (Haverkamp 2004, Zimmermann 2015). A slight female predominance in children with any NTM infection, in addition to adults with pulmonary NTM disease, has been observed in some studies. (Romanus 1995, Trnka 1994, Pham-Huy 2010, Reuss 2009, Wolinsky 1995, Cassidy 2009)

Impaired immunity or chronic lung disease are recognized risk factors for severe or pulmonary NTM disease. (Lopez-Varela 2015) As in TB, HIV infection, low CD4 cell count, and MSMD also increase susceptibility to severe NTM disease. (Horsburgh 1991, Gona 2006) However, most children who develop NTM lymphadenitis are healthy and immunocompetent. (Haverkamp 2004, Zimmermann 2015)

2.4.3 Diagnosis

Due to the limitations of diagnostics investigations, the diagnosis of NTM lymphadenitis is usually based on a typical clinical picture and exclusion of other likely aetiological causes.

2.4.3.1 **Microbiological investigations**

Mycobacterial cultures are still considered the diagnostic gold standard, and specimens obtained from the lymph node are typically cultured on solid media (i.e., Löwenstein-Jensen) and broth media (i.e., BACTEC). However, NTM cultures are limited by a slow growth rate and poor yield. The cultures usually take two to three weeks, but some NTM species might require as long as 12 weeks of incubation (Hatzenbuehler 2014). The yield of NTM cultures varies depending on the inclusion criteria of cases, and the reported yields range from 40%–80% (Haverkamp 2004, Pham-Huy 2009, Romanus 1995, Katila 1987, Tebruegge 2016). After successful cultures, the most common NTM species can rapidly be identified with molecular probes, and other NTM species can be identified with DNA sequencing.

2.4.3.2 **Histopathology**

Histopathological examination of a lymph node specimen can reveal features suggestive of an NTM disease such as microabscesses or noncaseating granulomas. (Kraus 1999) Other compatible cytopathology includes degenerating granulocytes, lymphocytes, and epithelioid histiocytes. (Griffith 2007)

2.4.3.3 **Immunological Tests**

Skin tests with PPD, NTM sensitins, or a combination of both have been suggested as diagnostic tests (von Reyn 1994, Haimi-Cohen 2001, Lindeboom 2006). Approximately 50% of children with NTM lymphadenitis exhibit an induration of ≥15 mm, but as many as 25% an induration of <10 mm in TST. (Haimi-Cohen 2001) The induration depends on the specific causative NTM species (Haimi-Cohen 2001). TST does not, however, differentiate between NTM and TB infection. (Wang 2016)

NTM sensitins are more specific and seem more sensitive: patients with culture-confirmed MAC infections demonstrate higher indurations to *M. avium* sensitin than PPD (von Reyn 1994). However, in some studies, 6–32% of asymptomatic children have demonstrated high reactivity to NTM sensitins in skin tests suggesting that false-positive results may be common (Larsson 1992, Hansen 1989, Lind 1991). NTM sensitins are also currently not commercially available. (Willemse 2018)

The commercial IGRAs are negative in most NTM infections. Thus, a positive TST combined with a negative IGRA has been suggested as a non-invasive diagnostic method for low TB incidence settings. (Hermansen 2014, Detjen 2007, Staufner 2012) However, cross-reaction in IGRAs is possible in infections caused by some NTM species. (Caruso 2009) In-house enzyme-linked immunospot assays

with NTM antigens have also been developed and showed promising results. (Della Bella 2019)

2.4.3.4 **Radiological Appearance**

Sonographic appearance of the affected lymph nodes includes decreased echogenicity and, depending on the stage of the disease, liquefaction, intranodal cystic necrosis, nodal matting, and surrounding soft-tissue oedema. The size of the affected lymph node ranges from 1.9–4.4 cm. In later stages, multiple intranodal calcifications can be seen on sonography. (Lindeboom 2006)

Contrast-enhanced CT usually exhibits asymmetric adenitis with ring-enhancing masses with surrounding fat and skin involvement and minimal inflammatory stranding in the subcutaneous fat. (Hazra 1999, Griffith 2007)

2.4.4 Treatment

Currently, there is no consensus over the treatment protocol for NTM lymphadenitis. This is due to the lack of well-performed prospective studies comparing different treatment protocols. Treatment protocols include surgical interventions, antimicrobials, or a combination of both, and a wait-and-see approach. (Zimmermann 2015)

2.4.4.1 **Antibiotic Treatment**

Anti-mycobacterial antibiotics, in combination with surgery or alone, are a commonly used treatment approach. (Luong 2005, Pilkington 2010) The mean cure rate with anti-mycobacterial is estimated to be 73%. (Zimmermann 2015) However, the heterogeneity in regard to the treatment regimens and duration is substantial. Furthermore, in a prospective study comparing observational approach and anti-mycobacterial antibiotics, there was no significant difference in time to resolution between the treatment arms. (Lindeboom 2011)

2.4.4.2 **Surgical Treatment**

Surgical treatment involves complete excision of the affected lymph node, incomplete excision, and curettage, or incision and drainage. Complete excision is associated with the highest cure rate compared to the others: the mean cure rate for complete excision is estimated to be 98%. (Zimmermann 2015) Complete surgical excision is also associated with faster resolution than curettage. (Lindeboom 2012) In one of the few prospective studies, surgery seemed more effective than antimicrobials (cure rate 96% vs. 66%). In a follow-up study, the aesthetic outcome was also deemed better in the surgery group. (Lindeboom 2007, Lindeboom 2009)

A major complication of surgery is facial nerve damage that can result in transient or permanent facial nerve palsy. (Lindeboom 2007) For complete excision, the risk for facial nerve palsy is approximately 10% and permanent palsy 2%. (Zimmermann 2015) The risk for facial palsy depends highly on the proximity of the affected lymph node to the facial nerve. (Zimmermann 2015) Delayed diagnosis also increases the risk of nerve damage and poor cosmetic outcome. (Loeffler 2004, Scott 2012)

2.4.4.3 **Wait-and-see Approach**

Because NTM lymphadenitis is a naturally limiting and noninvasive disease, specific treatment may not be necessary. In retrospective cohorts, observation alone has been a safe alternative without complications to surgery. (Zeharia 2008, Lyly 2020) Thus, observation alone seems sufficient, especially in cases with high risk for surgical complications.

2.5 BCG VACCINE

Since the discovery of *M. tuberculosis,* scientists have attempted to develop a vaccine against TB. BCG vaccine is used in TB prevention, and it is one of the most widely used vaccines in the world.

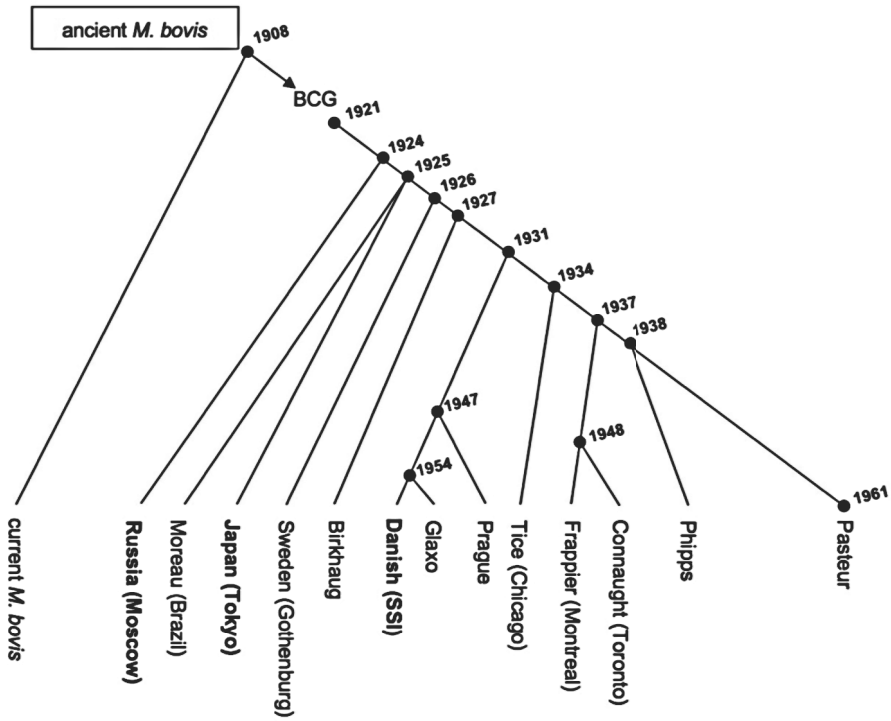
2.5.1 Past and Present

Over a century ago, two French scientists Albert Calmette and Camille Guérin demonstrated that weakened *M. bovis* had a protective effect against bovine TB in cattle. They started working on a vaccine

against TB for humans by cultivating a virulent *M. bovis* strain in a bile media and discovered that the bacillus was weakened after repeated culturing. After repeated passage from 1905 to 1921, the virulence of the culture strain decreased to a level believed to produce immunity without the disease. (Calmette 1931) The attenuated strain of *M. bovis* was referred to as Bacille Calmette-Guérin, and the first BCG vaccinations of humans started in Paris in 1921. (Calmette 1931)

The original BCG vaccine strain was distributed across the globe to other laboratories for vaccine preparation, and since the vaccine has become one of the most commonly used vaccines globally. Over the years, the strains in different laboratories evolved into separate BCG vaccine strains (Graphics 3). (Behr 1999, Behr 2002) The development of lyophilisation (i.e., freeze-drying) in the 1960s finally stopped the divergence of the BCG vaccine strains. The most commonly used BCG vaccine strains are the Danish (Copenhagen 1331), Japan (Tokyo 172-1), and Russia (BCG-I). (Behr 1999, Behr 2002)

Currently, approximately 90% of the global paediatric population is BCG vaccinated, and each year an estimated 120 million children will receive BCG. (United Nations Children's Fund 2014, Ritz 2008) The BCG vaccination policies in different countries vary: universal vaccinations are common in TB endemic countries, selective vaccinations are the preferred policy in countries where the incidence of TB is low, and some countries do not offer BCG vaccinations at all. (World Health Organization: Reported estimates of BCG coverage, Dierig 2015)



NOTE. **Bold indicates strains commonly used globally.**

Graphic 3 *Phylogenetic tree of the BCG vaccine strains. Modified from Behr 2002.*

2.5.2 BCG vaccinations in Finland

In Finland, BCG vaccinations started in the 1940s and universal vaccinations of infants in the 1950s. (Kontturi 2016) The first BCG vaccine used in Finland was produced in Gothenburg. In the 1970s the vaccine production was moved from Gothenburg to Copenhagen. (Kröger 1994, Korppi 2020) It was soon discovered that the new BCG vaccine produced in Copenhagen caused more adverse events such as BCG osteitis in Finland and Sweden. Therefore, the vaccine was changed to that produced by Glaxo from the BCG Glaxo-Evans strain. However, Glaxo stopped production of the BCG vaccine in 2002, and the BCG vaccine supplier changed back to Copenhagen to the Staten Serum Institut (SSI, Danish strain). The subsequent increase in adverse vaccine reactions expedited the discussions regarding the future of universal BCG vaccinations in Finland. Due to the decreasing TB incidence and increasing adverse events, the BCG vaccination policy changed in 2006 to a risk group-based approach. (Kilpi 2006, Salo 2006)

Since September 2006, BCG vaccinations in Finland have been only recommended for children under the age of seven deemed to have a higher TB exposure risk. (Salo 2006) Previously the vaccine was administered to the left thigh, and in 2006, the injection site was also changed to the left upper arm. (Kansanterveyslaitoksen rokotussuositus 2006) BCG vaccinations in Finland usually take place in the maternity hospital during early infancy. During the universal BCG vaccination policy, over 98% of infants were BCG vaccinated, and the coverage among birth cohorts born after the BCG vaccination policy changed is estimated at 6–10%. (Hersh 2003, Salo 2006) The risk groups are defined in a national guideline published by the THL. The current risk groups eligible for BCG vaccination are summarised in the following box. In addition, children born or living with a person born in Estonia are also recommended for BCG vaccination due to the higher MDR-TB incidence in Estonia. (Terveyden ja hyvinvoinnin laitos 2021)

2.5.3 Natural Reaction and Adverse Events

Currently, the BCG vaccine is injected intradermally to the left forearm. The vaccine commonly causes a localised *M. bovis* BCG skin infection that manifests as redness, a small lump or blister at the injection site. After few weeks, a small ulcer with discharge develops and typically heals within few weeks to months. A small flat scar might be visible at the injection site afterward. (Terveyden ja hyvinvoinnin laitos 2021)

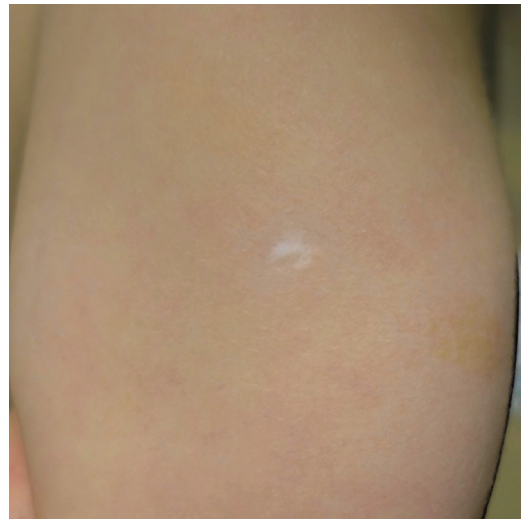


Figure 2 ***BCG vaccine scar in a three-year-old boy. (Photo by A. Kontturi)***

In some instances, an abscess of the injection site develops, or the healing of the ulcer is prolonged, and the discharge continues for several months. Lymphadenopathy or a lymph node abscess can also develop near the injection site. (Terveyden ja hyvinvoinnin laitos 2021) In rare cases, a more severe *M. bovis* BCG infection develops, such as osteitis or arthritis. In Finland, the incubation period for these remote infections has typically been over a year from the vaccination. (Kröger 1994, Korppi 2020) BCG can also cause a disseminated infection which typically indicates an underlying predisposing immunodeficiency.

2.5.4 Protective Efficacy

Cellular immunity is vital for the immune response against mycobacteria, and many antigens are similar between different Mycobacterium species. Therefore, the immune response achieved with the localized *M. bovis* BCG skin infection provides cross-mycobacterial effects against other mycobacterial infections. (Lopez-Varela 2015).

2.5.4.1 **Protective Efficacy Against Tuberculosis**

The protective effect of the BCG vaccine has been studied exhaustively with animal models, measuring the cellular immune response and in large trials. The efficacy of BCG vaccination against TB has varied substantially depending on the study design, BCG vaccine strain, age at vaccination, and climate zone. (Ritz 2008, Ritz 2012) In some settings, the protective efficacy of BCG against PTB has even been nonexistent. (Bull World Health Organ 1979, Rodrigues 1993, Aronson 2004) However, BCG has consistently been highly effective against severe childhood TB disease, and the protection against all TB forms has been weaker. (Rodrigues 1993, Abubakar 2013, Colditz 1995) It seems that the best results so far have been achieved with BCG vaccination at infancy in the north temperate zones such as Finland. Infant vaccinations likely result in better vaccine response because exposure to NTM before vaccination might decrease the cellular response achieved with the BCG vaccine. BCG Danish and Japan strains seem to be superior regarding the achieved cellular response compared to BCG Russia. (Ritz 2012)

The estimated duration of protection is 10–15 years, however, partial protection could persist as long as 50–60 years. (Aronson 2004, Moliva 2015) BCG vaccine has proven effective against drug-resistant TB strains, and some evidence suggests that BCG vaccine might also provide protection against LTBI. (Seaworth 2014, Soysal 2005, Roy 2014)

2.5.4.2 **Protective Efficacy Against Other Mycobacteria**

In animal models, the BCG vaccine has demonstrated a protective effect against NTM infections (Collins 1985, Engbaek 1966, Orme 1985). Furthermore, childhood NTM infections increased after the universal BCG vaccinations were discontinued in Sweden in the 70s, and a similar observation has been reported from the Czech Republic. (Romanus 1995, Trnka 1994) Compared to Sweden and the Czech Republic, universal BCG vaccinations continued much longer in Finland, during which childhood NTM infections were rare. (Katila 1987) Thus, it has been postulated that the BCG vaccine offers protection also against childhood NTM disease, but since recent publications on the issue are scarce, it has remained debated.

BCG vaccine has also been shown to protect from Buruli ulcers, a skin infection caused by *M. ulcerans,* and has demonstrated protective efficacy between 26–61% against leprosy caused by *M. leprae.* (Zimmermann 2018, Setia 2006, Merle 2010)

2.5.4.3 **Nonspecific Effects on the Immune System**

Shortly after the first BCG vaccinations, Calmette reported a reduction of overall mortality among the vaccinated children: the non-TB-related mortality within the first year after vaccination decreased from 16–26% to 4%. (Calmette 1931) Since then, multiple studies have reported a decrease in the overall childhood mortality, and the relative risk reduction caused by BCG is estimated to be 30%. (Benn 2013, Higgins 2016) Observational studies have also suggested that BCG vaccination reduces the number of childhood acute respiratory tract infections. (Hollm-Delgado 2014, de Castro 2015) It is believed that the BCG vaccine exhibits heterologous effects on the immune system independent

of the vaccine-specific immunity, such as activation of lymphocytes and enhanced cytokine production. (Flanagan 2013, Ritz 2013, Fritschi 2020, Zufferey 2013, Kleinnijenhuis 2014) Therefore, the BCG vaccine protects against other infectious pathogens such as other bacteria, viruses, and parasites. (Freyne 2015, Moorlag 2019, Messina 2019) BCG vaccine has also been shown to affect the antibody concentrations achieved with other vaccines. (Ritz 2013)

3 Aims of the Study

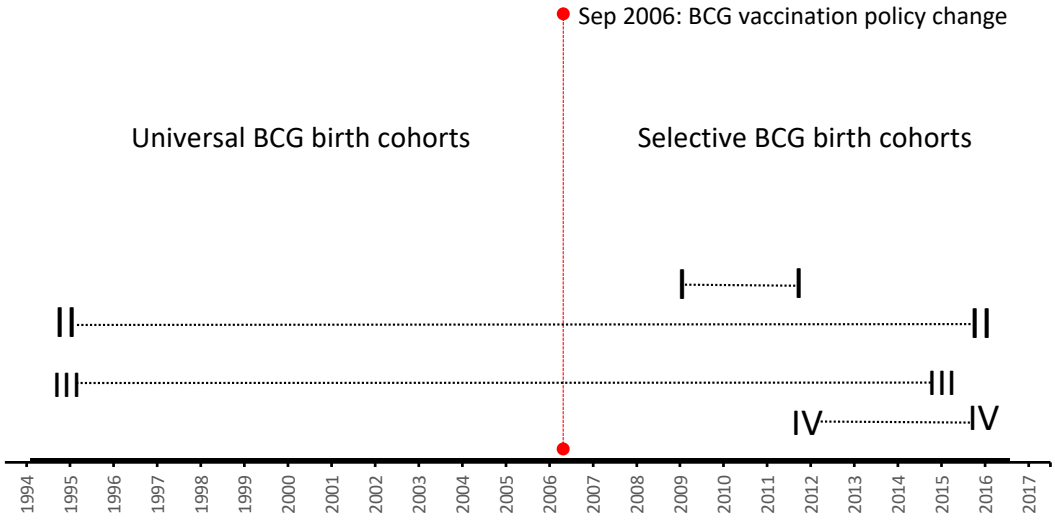
The primary aim of the thesis is to evaluate the disease burden of mycobacterial infections in the paediatric population in Finland and evaluate the effects of the BCG vaccination policy change. The specific aims of the studies were to:

I. Evaluate the potential of the HUSLAB modified T-SPOT.TB test for diagnosing cervical NTM lymphadenitis in children (I)

II. Evaluate the effect of the BCG vaccination policy change in Finland on the incidence of childhood NTM infections (II)

III. Evaluate the effect of the BCG vaccination policy change on the native under-five TB morbidity and describe the epidemiological trends of paediatric TB in Finland (III)

IV. Evaluate TB contact tracing among paediatric contacts exposed to TB in the HUS area (IV)



Graphic 4 *The retrospective timeline of the studies in reference to the BCG vaccination policy change in Finland.*

4 Materials and Methods

4.1 DATA SOURCES

4.1.1 Finnish National Infectious Diseases Register

The Finnish National Infectious Diseases Register (NIDR) collects nationwide data concerning infectious diseases as a part of national surveillance and control. The register has been maintained by the THL since 1995 and is based on the Communicable Diseases Act and Decree (the Finnish Communicable Diseases Act 1227/2016, 28 §). The data is collected through communicable disease notifications. Clinical microbiology laboratories are obligated to notify new TB or NTM isolates directly to the NIRD. Additionally, physicians are obligated to notify confirmed or clinically diagnosed TB cases directly to the NIDR. Previously the NIDR registered only bacteriologically or histologically confirmed TB cases. Clinically diagnosed TB cases have been registered since 2007 after the adoption of the standard European Union TB case definition. (Räisänen 2016) The public statistical database of the NIDR provides access to certain statistical data, and further registered data may be available with a research permit for research purposes.

4.1.2 Finnish Care Register for Health Care

The Finnish Care Register for Health Care (Hilmo), previously known as the Finnish Hospital Discharge Register, is a nationwide register of hospitalisations in Finland. (Sund 2014) The first data that has been collected to the register since 1956 is discharges from tuberculosis sanatoriums. (Sund 2012) The register has been broadened to include all nationwide inpatient hospital discharges since 1969, personal identification codes, and the International Classification of Diseases (ICD) diagnoses for each patient. (Sund 2012, Sund 2014) The ICD-9 coding has been used in 1987–1995 and ICD-10 since 1996. The coverage of the Hilmo is excellent, and virtually all inpatient visits from Finnish hospitals can be found from the register. (Sund 2012)

4.1.3 Population Data

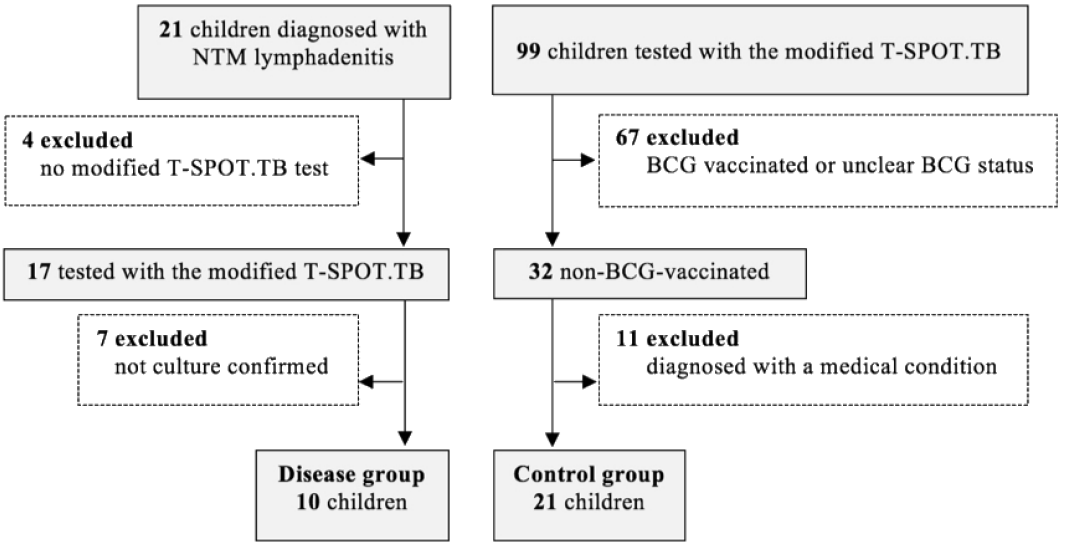
The Finnish Population Information System administrated by the Population Register Centre (VRK) contains the basic information of citizens residing in Finland. Personal identity codes allow the identification of each individual and linkage of data. The Statistics Finland public population database contains the official demographic statistics of Finland.

4.2 PATIENTS AND POPULATIONS

4.2.1 Study I

We retrieved a disease group of children tested with the HUSLAB modified T-SPOT.TB test and a culture-confirmed cervical NTM lymphadenitis diagnosed in the Helsinki University Central Hospital between March 2009 and January 2012. The data was obtained from the HUS medical records. A total of 21 children were identified. We excluded cases without modified T-SPOT.TB test or bacteriological isolation of NTM. A total of ten children were included in the data analysis.

We retrieved a control group of healthy non-BCG-vaccinated children under the age of five that had been tested with the modified T-SPOT.TB from the medical records. A total of 99 children had been tested with the modified T-SPOT.TB test in the Helsinki University Central Hospital between 2009 and 2010. We excluded 67 children who had been BCG-vaccinated or had an unclear BCG status. From the remaining 32 non-BCG–vaccinated children, we excluded 11 children diagnosed with a medical condition (e.g., rheumatoid arthritis, leukemia, mycobacterial or viral infection). The remaining total of 21 healthy non-BCG–vaccinated children was selected as the control group. Sixteen of the control group children had been tested with the modified T-SPOT.TB test because of TB contact tracing and five as a routine investigation performed before a BCG vaccination.



Graphic 5 *Flowchart of the disease and control groups in study I.*

4.2.2 Study II

We retrieved all NTM notifications of patients under five years of age between January 1995 and December 2016 from the NIDR. A total of 100 children were identified from the NIDR. We excluded three foreign-born children, and the remaining 97 Finnish-born children were included in the data analysis.

We retrieved the official birth rate of Finland in 1995–2016 from the Statistics Finland population database.

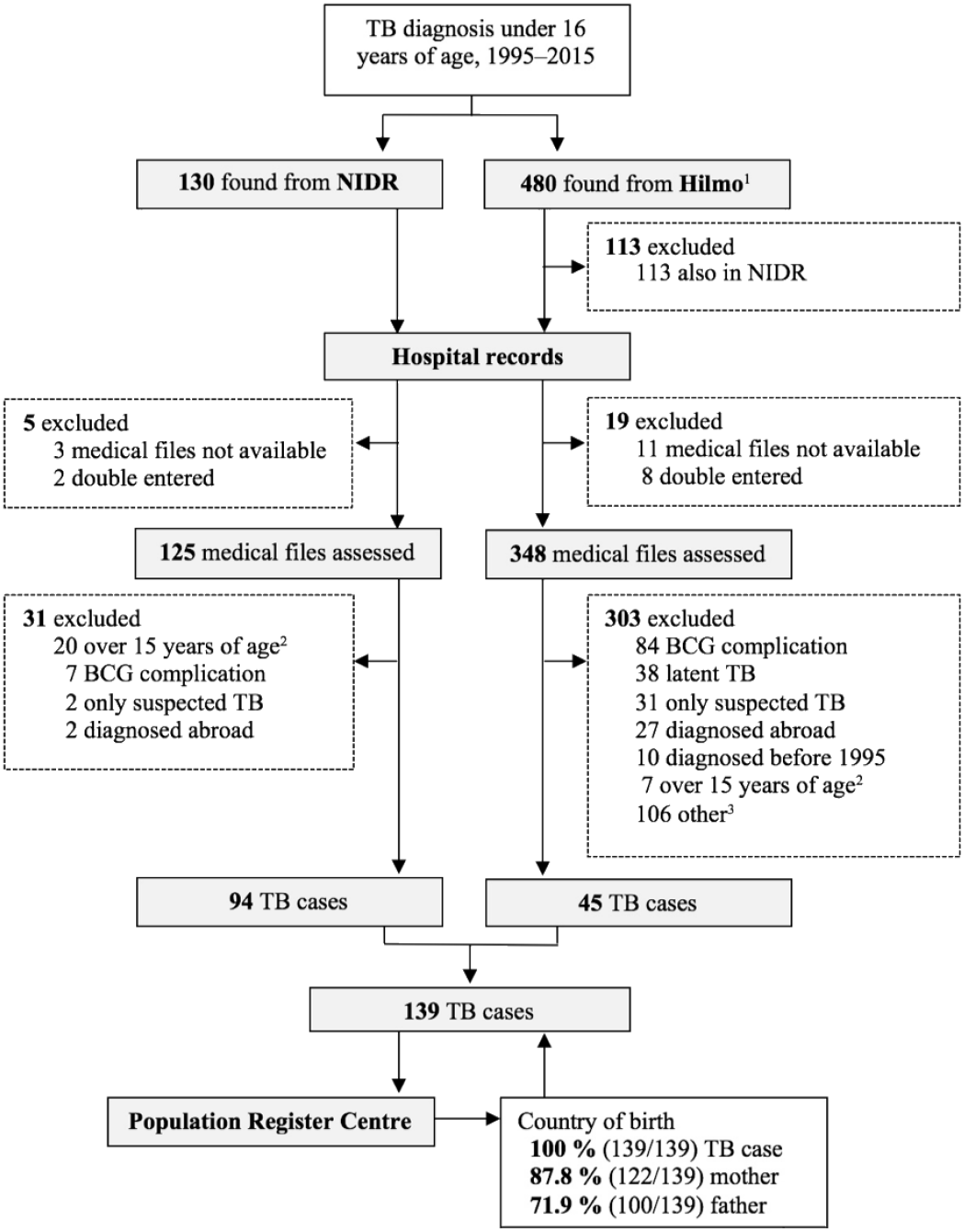
4.2.3 Study III

We retrieved all TB notifications of patients under 16 years of age between January 1995 and December 2015 from the NIDR. A total of 130 children were identified from the NIDR.

We retrieved all children under 16 years of age with any TB diagnostic code recorded to the Hilmo between January 1995 and December 2015. A total of 480 children were identified from the Hilmo.

The medical records of the identified cases were requested from the relevant hospitals. The medical records were evaluated to confirm the diagnosis and to collect relevant data for the study. We excluded cases whose medical records were not available for evaluation. We excluded cases deemed not to be active TB by the treating physician and TB cases that were diagnosed over the age of 15 or diagnosed initially abroad. A total of 139 children were included in the data analysis.

We retrieved the official country of birth of the children and their parents from VRK and the official birth rate and population data of Finland in 1995–2015 from the Statistics Finland population public database.



NOTE.

[1]Registered to the Hilmo with any diagnostic code for TB (ICD-9 010-018 or ICD-10 A15-19)

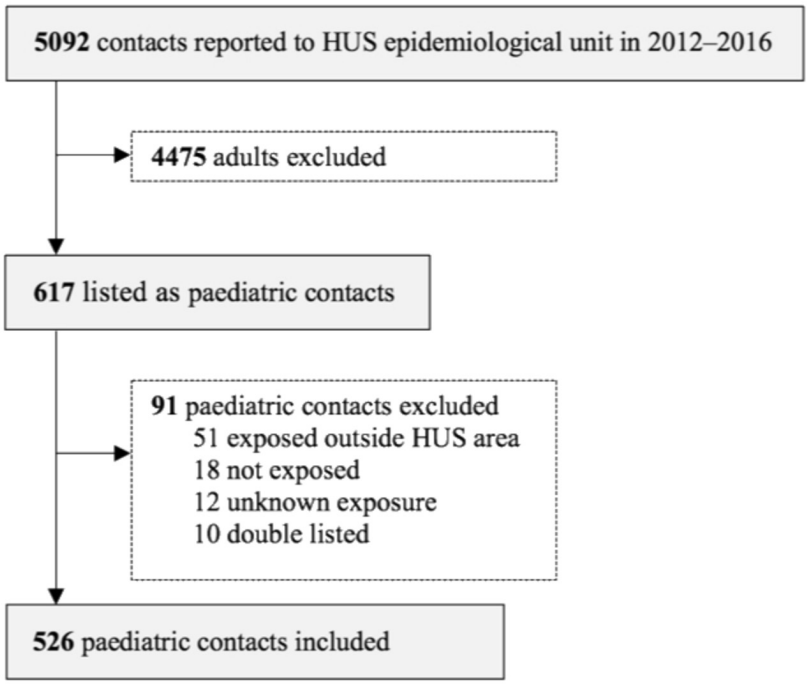
[2]Age at first contact with a public hospital or outpatient clinic

[3]Patient without any TB suspicion in medical records (i.e., TB diagnostic code used by mistake)

Graphic 6 *Flowchart of data collection in study III.*

4.2.4 Study IV

We retrieved all paediatric contacts exposed to TB under 16 years of age in the HUS area and their respective index cases between 2012 and 2016. The data was obtained from the epidemiological unit records. The medical records were evaluated to confirm eligibility and to collect relevant data for the study. We excluded contacts exposed outside the HUS area, deemed unexposed at the first contact investigation visit (i.e., no contact with the index case), or whose exposure was unknown. Contacts over 16 years of age at the time of exposure were excluded; however, older siblings aged 16–17 years were included if they underwent investigations together with their younger siblings in the paediatric clinic. A total of 5092 listed TB contacts were identified, of whom 526 were included together with their 121 index cases in the data analysis.



Graphic 7 *Flowchart of data collection in Study III.*

4.3 METHODS

4.3.1 Study Design and Setting

All studies were retrospective, and the patients or families were not contacted. Study I represent a small pilot study of an in-house diagnostic test used in the HUSLAB. Studies II and III represent population-based epidemiological studies in Finland. Study IV represents a cohort study evaluating TB contact tracing in the HUS area. The datasets were collected as available from the specified registries and medical patient records. The NIDR and Hilmo data searches (studies II–III) were conducted by THL, after which the datasets were accessible in electronic format. The HUS medical records (studies I and III–IV) were evaluated manually in electronic or paper format. In study III, additional medical records outside HUS were also reviewed. The medical records from the Turku University Hospital and Tampere University Hospital were evaluated manually in either electronic or paper format by visiting the respective hospital registries. Medical records from other hospitals in Finland were received by mail and evaluated manually in paper format. Datasets from different sources were combined through the personal identification number of each patient. The data was collected on a specified data collection Excel form and pseudonymised.

4.3.2 Outcomes and Classifications

All outcome data were collected retrospectively as available from the registry data and medical records.

4.3.2.1 **Study I**

The modified T-SPOT.TB test results were collected as *"spots per 10E6 lymphocytes",* as reported by HUSLAB. A test result of ≥25 spots per 10E6 lymphocytes was classified as *"positive"* and otherwise *"negative"*. The positive cut-off value was selected based on the manufacturers' recommendation for TB diagnostics.

4.3.2.2 **Study II**

All eligible NTM notifications were classified as *"a case of NTM disease"*. All birth cohorts born before September 2006 were classified as *"universal-BCG cohorts"* and those born thereafter as *"selective-BCG cohorts"*.

4.3.2.3 **Study III**

The outcome was classified according to the diagnosis at the last visit in the medical records. All patients diagnosed by the treating clinician and started on a full TB treatment regimen with or without bacteriological or histological confirmation were accepted as *"a case of active TB disease"*. Cases were defined as *"bacteriologically confirmed"* if *M. tuberculosis* had been isolated with culture or a NAAT and otherwise *"clinically diagnosed"*. Cases were also defined as *"PTB"* or *"extrapulmonary TB"*, and *"MDR-TB"* according to the World Health Organization guidelines. (World Health Organization 2019) We considered cases to be incident at first known contact due to symptoms or referral that led to the diagnosis of TB. The BCG status of the patients was classified from the medical records as *"BCG vaccinated", "non-BCG-vaccinated",* or *"unknown BCG status".* According to the medical records, the index case of the patients was classified as *"household contacts"* if the child had lived in the same household at any time during the transmission period. Patients were classified as *"native"* if they were born in Finland and otherwise *"immigrant"* based on data from VRK. Native cases were further classified as *"second-generation immigrants"* if at least one parent had been born abroad based on data from VRK. The patients were classified as *"BCG eligible"* if the child or at least one parent had been born in "*a TB endemic country"* that was classified as a country with a TB incidence ≥50/100,000 population. All birth cohorts born before September 2006 were classified as *"universal-BCG cohorts"* and those born thereafter as *"selective-BCG cohorts".*

4.3.2.4 **Study IV**

The outcome was classified according to the diagnosis by the treating physician at the last contact investigation visit as *"TB"* (i.e., infection with signs of disease),

"TBI" (i.e., infection without signs of disease), *"no infection", or "unknown"* (i.e., contact investigations not completed or data for outcome unavailable). The exposed children were classified retrospectively from the epidemiological unit records and medical records. The guidelines that dictated contacts included in the contact investigations during the whole retrospective study period were published in 2011 by the THL. The exposure of the children was classified based on the records as *"household"* (i.e., exposed in the same household*), "congregated"* (i.e., exposure in school or reception centre for asylum seekers), or *"other"* (i.e., all other contacts). We determined the delay in days from the initiation of index case TB treatment to the first contact investigation visit of the exposed children. The BCG status was classified from the medical records. Based on previous literature, we evaluated if the patient had any medical conditions considered to increased the risk for disease progression (e.g., HIV, malignancy, or immunosuppressive therapy). We determined the contact tracing delay as the interval in days from the diagnosis of the index case (i.e., the first date of TB treatment) to the date of the first TB contact investigation visit.

4.4 STATISTICS

The sensitivity and specificity (study I) were calculated with the following formula:

Sensitivity = true positive / (true positive + false negative)

Specificity = true negative / (true negative + false positive)

We determined range and interquartile range (IQR) for the continuous variables. For comparison of two groups (studies II and III–IV), we used a Mann-Whitney U or Kruskal-Wallis and two-tailed Fisher's exact or chi-square to analyse continuous and categorical data, respectively. A p-value < 0.05 was considered statistically significant. A logistic regression model and its predictive margins (study III) were used to calculate the adjusted proportions.

The annual incidence rates (studies II–III) were calculated by dividing the number of cases by the relevant population in Finland. The incidence trends (study III) were analysed with a negative binomial model.

The incidence rates per 100 000 person-years (studies II–III) were calculated by dividing the number of cases per cohort with the number of live-born children per cohort multiplied by the number of years of observation per cohort, then multiplying the whole number with 100,000. We assumed that the birth rate was equal year-round, so that, for example, all children born in 2015 and registered until December 31, 2016, were observed for 1.5 years. Birth-cohorts that turned five years during the selected retrospective period were observed for a maximum of five years.

We used a Poisson log-linear model (studies II–III) to calculate the incidence rate ratio (IRR) with confidence intervals (CI) between the universal and selective BCG birth cohorts. The goodness of fit criteria was examined so that the ratio of the deviance to the degree of freedom (value/df) was less than 1.50. To assess if there was overdispersion with respect to the Poisson model, we assessed if negative binomial regression was needed.

We used a binary logistic regression (study IV) to examine the potential risk factors for infection (odds ratio, OR). Factors for univariate analysis were

selected based on previous literature (Erkens 2010). Age, gender, and factors with significant association were selected for multivariable analysis (adjusted odds ratio, aOR). Because of the known association between cavitary disease and sputum smear positivity, these factors were combined so that a reference group without cavitation and smear-negative could be used in the multivariate model. Furthermore, because BCG vaccination is reflective of birth in a TB endemic country, it was excluded from the multivariable analysis.

Data were collected and analysed with Microsoft Excel (Version 15, Microsoft Corporation, Redmond, WA, US) and IBM SPSS Statistics (Version 24, IBM Corporation, Armonk, NY, US).

4.5 ETHICAL CONSIDERATIONS

The study protocols were approved by the Research Ethics Committee of the HUS (I, IV) or the Research Ethics Committee of the THL (III). Data in study II was analysed within the epidemiological research purposes authorized by the Finnish Communicable Diseases Act 1227/2016, 42 § and, therefore, ethics approval was deemed unnecessary for study II.

The procedures were carried out in accordance with the ethical standards described in the Helsinki Declaration revised in 2013. Access to identifiable information such as personal identity codes was limited, and the data was pseudonymised before statistical analysis.

5 Results

5.1 CHILDHOOD NTM LYMPHADENITIS IN FINLAND (I-II)

5.1.1 Patient Characteristics

In study I, five of the NTM lymphadenitis patients were female and five male, and the median age was 31 months (range 15 to 38 months). All of them were non-BCG-vaccinated, born in Finland to Finnish parents, and had exhibited a typical clinical presentation of NTM lymphadenitis: a unilateral nontender cervical lymphadenitis without general symptoms.

In study II, a total of 97 culture-confirmed NTM cases were identified from the NIDR among Finnish-born children under five years of age. The female-to-male ratio among the cases was 2:1. At the time of specimen collection, the median age was 27 months (range 4 to 59 months).

5.1.2 Microbiological Test Results

In study I, microbiological confirmation was achieved in 10/17 (58.8%) of the children diagnosed with NTM lymphadenitis. In study II, NTM species and source of the specimen of all the NTM isolates notified to NIDR among Finnish-born children are presented in Table 3.

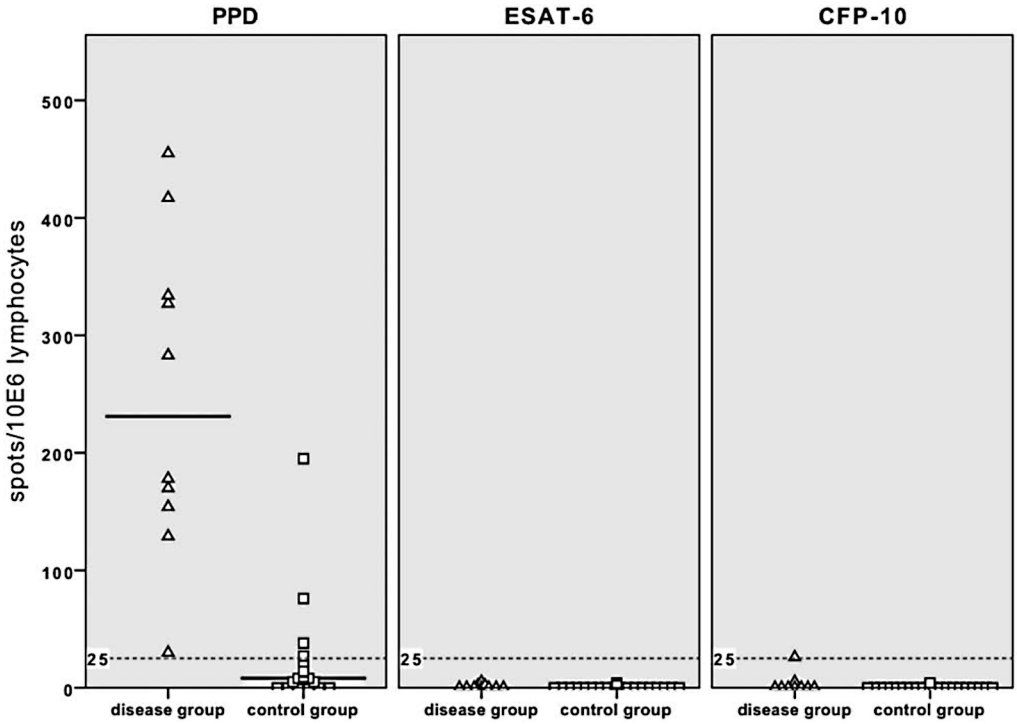
5.1.3 Modified T-SPOT.TB Test Results

In study I, the median result of PPD stimulation in the disease group was 231 spots/10E6 lymphocytes (range 30 to 455). All of the children in the disease group exhibited positive test results to the PPD stimulation (Graphic 8). The median par-

	Specimen					
Species	Needle etc.[1]	Other[2]	Skin	Naso-pharynx	Unsp.[3]	Total
M. avium	63	2	-	1	3	69 (71)
M. lentiflavum	6	-	-	-	1	7 (7)
M. malmoense	5	-	1	-	-	6 (6)
M. intracellulare	3	-	-	-	-	3 (3)
M. bohemicum	2	-	-	-	-	2 (2)
M. interjectum	1	-	1	-	-	2 (2)
M. fortuitum	-	1	-	-	-	1 (1)
M. abscessus	-	1	-	-	-	1 (1)
M. gordonae	-	-	-	-	1	1 (1)
M. scrofulaceum	1	-	-	-	-	1 (1)
M. simiae	1	-	-	-	-	1 (1)
M. kansasii	1	-	-	-	-	1 (1)
NTM, Unsp.[3]	1	1	-	-	-	2 (2)
Total	84 (87)	5 (5)	2 (2)	1 (1)	5 (5)	97 (100)

NOTE.
Data are N (%)
[1] Needle aspirate, tissue biopsy, or abscess material
[2] Other discharge material
[3] Unspecified

Table 3 *NTM species and source of the specimen of NTM isolate in 1995–2016.*



Graphic 8 *Modified T-SPOT.TB test results among children with culture-confirmed cervical NTM lymphadenitis (disease group) and healthy control group with a cut-off value of ≥25 spots/10E6 lymphocytes.*

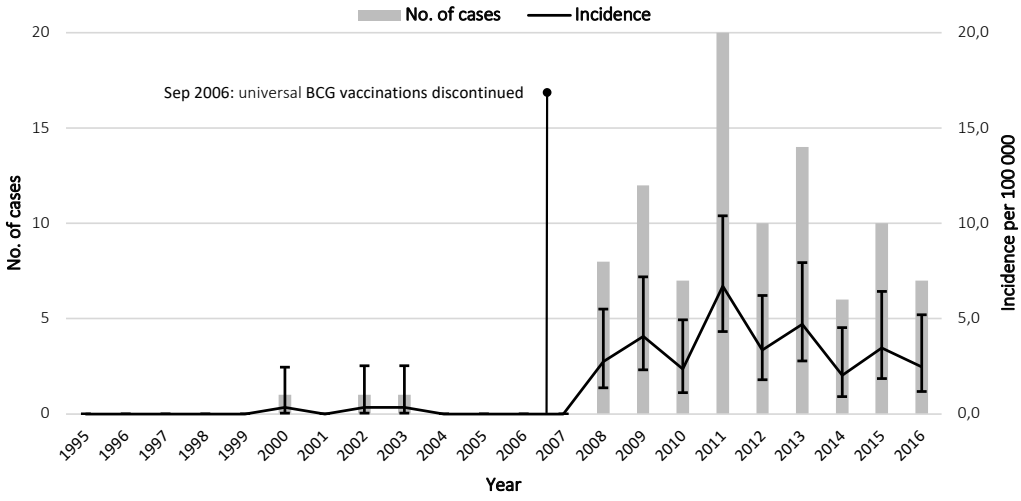
ent-reported duration of the lymphadenitis until the modified T-SPOT.TB test date was two months (range 1 to 8).

In the control group, the median result of PPD stimulation was eight spots/10E6 lymphocytes (range 0 to 195). In the control group, 4/21 (19.0%) exhibited a positive test result to the PPD stimulation. One child in the disease group exhibited borderline positive reactivity to CFP-10 antigen (26 spots/10E6 lymphocytes), no reactivity to ESAT-6 antigen, and high reactivity to PPD antigen stimulation (455 spots/10E6 lymphocytes).

Additionally, this child's lymph node specimen yielded *M. avium,* and the result of the Xpert MTB/RIF test was negative. The estimated sensitivity and specificity of the PPD stimulation test were 1.00 (95% CI, 0.72–1.00) and 0.81 (95% CI, 0.64–0.98), respectively.

5.1.4 Incidence

In 1995– 2016, the native-born under-five population ranged from 280,049 to 322,369. In study II, the number of NTM cases among native-born children under five years of age ranged from 0 to 20 per year, and the incidence of NTM infections among native-born children under five years of age ranged from 0 to 6.7 per 100,000 per year (Graphic 9).



Graphic 9 *The annual number of NTM infections and estimated incidence among native-born children under five years of age in Finland, 1995–2016.*

5.1.5 Estimated Effect of Bcg Vaccination Policy Change

In study II, the incidence rate of NTM infections among the birth cohorts ranged from 0 to 5.4 per 100,000 person-years (Table 4). The combined incidence rate of NTM infections under five years of age among children born during the universal BCG vaccination policy in 1995–2006 was 0.2/100,000 person-years (95% CI, 0.1–0.4/100,000 person-years) and among children born during the selective BCG vaccination policy in 2006–2015 was 3.9/100,000 person-years (95% CI, 3.2–4.8/100 000 person-years). The IRR of selective-BCG cohorts compared to universal-BCG cohorts was 19.03 (95% CI, 8.82–41.07).

5.2 CHILDHOOD TB IN FINLAND (III-IV)

5.2.1 Registry Data and Patient Characteristics

In study III, a total of 139 TB cases diagnosed in Finland in 1995–2015 under the age of 15 were found from the registers: 85 cases were found from both NIDR and Hilmo, nine from NIRD alone, and 45 from Hilmo alone. Of the paediatric TB cases diagnosed in 2007–2015, 11/67 (16.4%) were found from the Hilmo alone. Altogether, there were 50 native-born and 89 immigrant TB patients (Table 5).

BCG policy	Birth cohort	Cohort population	No. (%) with foreign-born mother	Total person-years[3]	No. of cases	Incidence rate[4]
universal	1995	63 067	1 881 (3.0)	315 335	0	0.0
	1996	60 723	1 989 (3.3)	303 615	0	0.0
	1997	59 329	2 133 (3.6)	296 645	0	0.0
	1998	57 108	2 267 (4.0)	285 540	2	0.7
	1999	57 574	2 382 (4.1)	287 870	0	0.0
	2000	56 742	2 381 (4.2)	283 710	0	0.0
	2001	56 189	2 633 (4.7)	280 945	0	0.0
	2002	55 555	2 696 (4.9)	277 775	1	0.4
	2003	56 630	2 825 (5.0)	283 150	1	0.4
	2004	57 758	2 959 (5.1)	288 790	0	0.0
	2005	57 745	3 220 (5.6)	288 725	0	0.0
	I/2006[1]	39 801	2 378 (6.0)	199 005	3	1.5
selective	II/2006[2]	19 039	1 138 (6.0)	95 195	5	5.3
	2007	58 729	3 690 (6.3)	293 645	16	5.4
	2008	59 530	3 923 (6.6)	297 650	14	4.7
	2009	60 430	4 290 (7.1)	302 150	11	3.6
	2010	60 980	4 760 (7.8)	304 900	16	5.2
	2011	59 961	4 969 (8.3)	299 805	9	3.0
	2012	59 493	5 415 (9.1)	267 719	7	2.6
	2013	58 134	5 625 (9.7)	203 469	8	3.9
	2014	57 232	6 219 (10.9)	143 080	3	2.1
	2015	55 472	6 363 (11.5)	83 208	1	1.2

NOTE.

[1]Born from January to August 2006.

[2]Born from September to December 2006.

[3]Total person-years in cohort observed up until December 31, 2016, or five years of age.

[4]Number of cases per 100,000 person-years.

Table 4 *Demographic of Finnish birth cohorts born from 1995 to 2015 and estimated incidence rates per 100,000 person-years of NTM infection in children under five years of age.*

	All	Native	Immigrant	P-value
Tuberculosis cases	139 (100)	50 (36.0)	89 (64.0)	N/A
Second-generation immigrant	24 (17.3)	24 (48.0)	–	N/A
Country of birth[1]				N/A
Somalia	59 (42.4)	7 (14.0)	52 (58.4)	
Ethiopia	10 (7.2)	4 (8.0)	6 (6.7)	
Thailand	5 (3.6)	3 (6.0)	2 (2.2)	
Afghanistan	6 (4.3)	1 (2.0)	5 (5.6)	
Other	33 (23.4)	9 (18.0)	24 (27.0)	
BCG eligible[2]	109 (78.4)	23 (46.0)	86 (96.6)	<0.0001
Gender, male	72 (51.8)	25 (50.0)	47 (52.8)	0.75
Age, years	9.3 (4.1–12.6)	3.7 (1.7–8.4)	11.4 (7.1–13.5)	<0.0001
Age group, years				<0.0001
<5	40 (28.8)	28 (56.0)	12 (13.5)	
5–14	99 (71.2)	22 (44.0)	77 (86.5)	
BCG vaccinated				0.08[3]
Yes	49 (35.3)	19 (38.0)	30 (33.7)	
No	27 (19.4)	5 (10.0)	22 (24.7)	
Unknown	63 (45.3)	26 (52.0)	37 (41.6)	0.24
Case finding				0.002[4]
Symptoms	67 (48.2)	21 (42.0)	46 (51.7)	
Contact tracing	48 (34.5)	29 (58.0)	19 (21.3)	
TB screening	24 (17.3)	–	24 (27.0)	
TB source				0.0005[3]
Household contact	45 (32.4)	19 (38.0)	26 (29.2)	
Other	19 (13.7)	17 (34.0)	2 (2.2)	
Unknown	75 (54.0)	14 (28.0)	61 (68.5)	<0.0001
Pulmonary TB	75 (54.0)	31 (62.0)	44 (49.4)	0.42[5]
Bacteriologically confirmed	63 (45.3)	16 (32.0)	47 (52.8)	0.15[6]
Smear-positive[7]	8 (8.1)	3 (8.3)	5 (7.9)	0.99
MDR-TB	1 (0.7)	0	1 (1.1)	0.99

NOTE. Data are N (%) or median (IQR).

[1]Birth country of the child if immigrant or parent(s) if second-generation immigrant.

[2]Patient or parent(s) born in a high TB incidence country (≥50/100 000 population)

[3]Unknown excluded from the analysis.

[4]TB screening excluded from the analysis.

[5]Adjusted for age.

[6]Adjusted for age and infection focus (pulmonary or extrapulmonary).

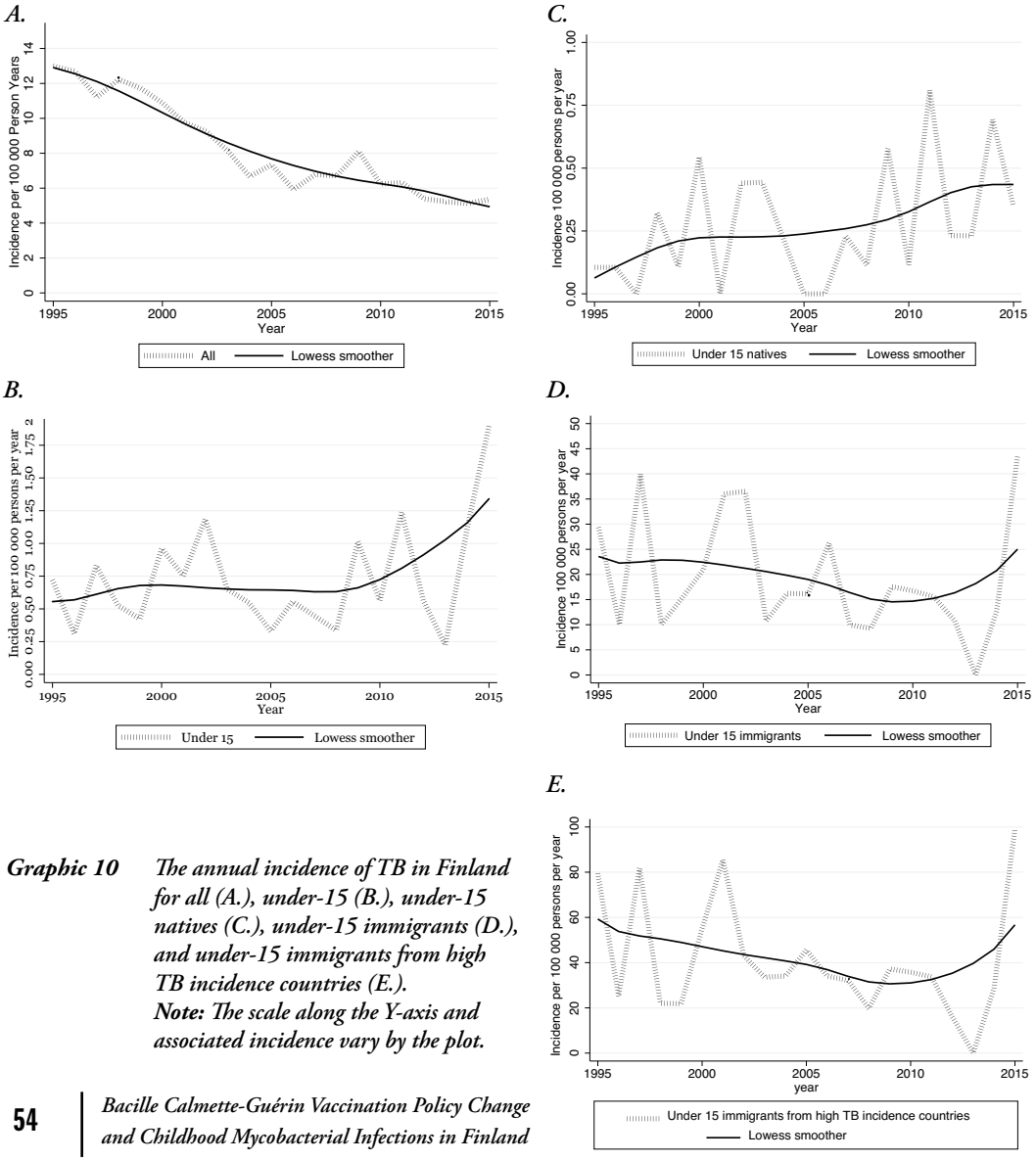
[7]Cases with a smear-positive respiratory sample out of patients with at least one respiratory smear sample.

Table 5 *Demographic and clinical characteristics of children under 15 with newly diagnosed TB in Finland, 1995–2015.*

5.2.2 Incidence

In 1995–2015, a total of 1,227,221 children were born in Finland: 678,221 children during the universal BCG vaccination policy and 549,000 children during the selective BCG vaccination policy. In study III, the total retrospective observation period for the birth cohorts was 5,681,926 person-years: 3,391,105 person-years for the birth cohorts born during the universal BCG vaccination policy and 2,290,821 person-years for the birth cohorts born during the selective BCG vaccination policy. The percentage of native-born children with foreign-born mothers in the annual birth-cohorts ranged from 3.0 to 11.5%, and the sex ratio (males/females) from 1.04 to 1.06.

In 1995–2015, the IRRs among the overall, under-15, under-15 immigrant, under-15 immigrants from high TB incidence countries, and native under-15 populations were 0.95 (95% CI, 0.95 – 0.96), 1.02 (0.99–1.06), 0.98 (0.94–1.02), 0.98 (0.94–1.02), and 1.06 (1.01–1.11), respectively. The annual incidence trends of TB among different groups in Finland are presented in Graphics 10.



Graphic 10 *The annual incidence of TB in Finland for all (A.), under-15 (B.), under-15 natives (C.), under-15 immigrants (D.), and under-15 immigrants from high TB incidence countries (E.).*
Note: *The scale along the Y-axis and associated incidence vary by the plot.*

5.2.3 Estimated Effect of Bcg Vaccination Policy Change

In study III, the IRR of TB under five years of age in the selective-BCG cohorts compared to universal-BCG cohorts was 1.3 (95% CI, 0.7–2.3). There were two cases of severe TB among the birth cohorts: one born in 2004 during the universal BCG vaccination policy and one born in 2009 during the selective BCG vaccination policy (Table 6). There was one death due to TB (1/139; 0.7%): an infant born in Finland during the selective BCG vaccination policy with native-born parents and not BCG vaccinated.

	All			Under 15		
Year	**Population** (% immigrants)	**TB cases**	**Incidence** (95% CI)[1]	**Population** (% of all)	**TB cases** (% of all)	**Incidence** (95% CI)[1]
1995	5010523 (2.1)	651	13.0 (12.0–14.0)	971770 (19.4)	7 (1.1)	0.7 (0.3–1.5)
1996	5021189 (2.2)	637	12.7 (11.7–13.7)	968567 (19.3)	3 (0.5)	0.3 (0.1–0.9)
1997	5029279 (2.3)	564	11.2 (10.3–12.2)	961350 (19.1)	8 (1.4)	0.8 (0.4–1.6)
1998	5034596 (2.5)	616	12.2 (11.3–13.2)	951145 (18.9)	5 (0.8)	0.5 (0.2–1.2)
1999	5040182 (2.6)	591	11.7 (10.8–12.7)	943001 (18.7)	4 (0.7)	0.4 (0.1–1.1)
2000	5044912 (2.7)	549	10.9 (10.0–11.8)	936333 (18.6)	9 (1.6)	1.0 (0.4–1.8)
2001	5049766 (2.9)	493	9.8 (8.9–10.7)	931587 (18.4)	7 (1.4)	0.8 (0.3–1.5)
2002	5054238 (3.0)	468	9.3 (8.4–10.1)	927009 (18.3)	11 (2.4)	1.2 (0.6–2.1)
2003	5060865 (3.1)	411	8.1 (7.4–8.9)	920097 (18.2)	6 (1.5)	0.7 (0.2–1.4)
2004	5070250 (3.3)	338	6.7 (6.0–7.4)	914560 (18.0)	5 (1.5)	0.5 (0.2–1.3)
2005	5078968 (3.5)	373	7.3 (6.6–8.1)	906904 (17.9)	3 (0.8)	0.3 (0.1–1.0)
2006	5089045 (3.7)	301	5.9 (5.3–6.6)	901181 (17.7)	5 (1.7)	0.6 (0.2–1.3)
2007	5097956 (4.0)	346	6.8 (6.1–7.5)	894590 (17.5)	4 (1.2)	0.4 (0.1–1.1)
2008	5107688 (4.3)	343	6.7 (6.0–7.5)	891162 (17.4)	3 (0.9)	0.3 (0.1–1.0)
2009	5118244 (4.6)	415	8.1 (7.3–8.9)	888323 (17.4)	9 (2.2)	1.0 (0.5–1.9)
2010	5127141 (4.8)	320	6.2 (5.6–7.0)	887677 (17.3)	5 (1.6)	0.6 (0.2–1.3)
2011	5135119 (5.2)	324	6.3 (5.6–7.0)	888982 (17.3)	11 (3.4)	1.2 (0.6–2.2)
2012	5141203 (5.6)	277	5.4 (4.8–6.1)	891392 (17.3)	5 (1.8)	0.6 (0.2–1.3)
2013	5146991 (5.9)	269	5.2 (4.6–5.9)	895021 (17.4)	2 (0.7)	0.2 (0.03–0.8)
2014	5149776 (6.3)	264	5.1 (4.5–5.8)	896608 (17.4)	10 (3.8)	1.1 (0.5–2.1)
2015	5150146 (6.5)	276	5.4 (4.7–6.0)	896023 (17.4)	17 (6.2)	1.9 (1.1–3.0)
Total		8826			139 (1.6)	

Note.
[1] Cases per 100,000 person years.
[2] TB incidence ≥50/100,000 population

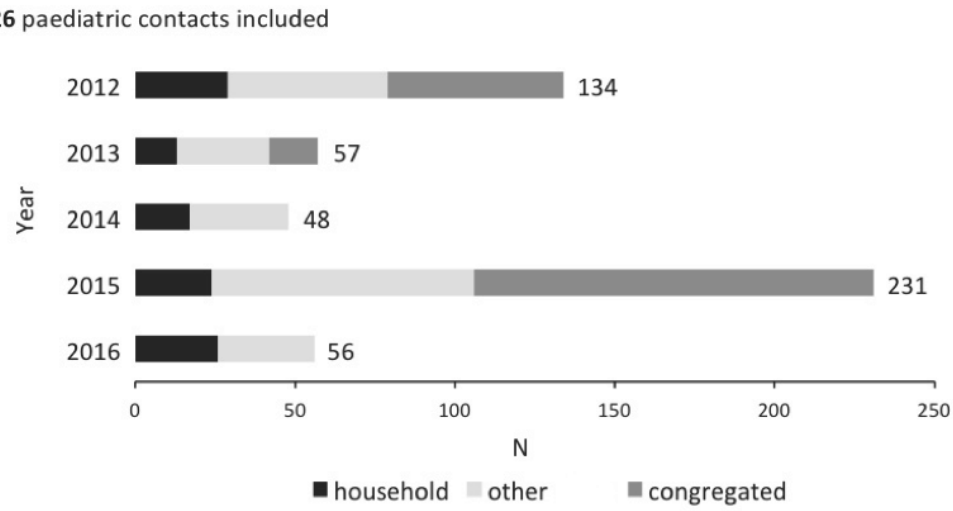
Table 6 *Demographic and annual TB incidence per 100,000 in Finland, 1995–2015.*
Table continues on the next page.

Year	Under 15 natives			Under 15 immigrants			Under 15 immigrants from high TB incidence country[2]		
	Population (% of <15)	TB cases (% of <15)	Incidence (95% CI)[1]	Population (% of <15)	TB cases (% of <15)	Incidence (95% CI)[1]	Population (% of <15)	TB cases (% of <15)	Incidence (95% CI)[1]
1995	951329 (97.9)	1 (14.3)	0.1 (0.003–0.6)	20441 (2.1)	6 (85.7)	29.4 (10.8–63.9)	7553 (0.8)	6 (85.7)	79.4 (29.2–172.9)
1996	948549 (97.9)	1 (33.3)	0.1 (0.003–0.6)	20018 (2.1)	2 (66.7)	10.0 (1.2–36.1)	7946 (0.8)	2 (66.7)	25.2 (3.0–90.9)
1997	941353 (97.9)	0 (0.0)	0.0 (0.0–0.4)	19997 (2.1)	8 (100.0)	40.0 (17.3–78.8)	8556 (0.9)	7 (87.5)	81.8 (32.9–168.6)
1998	931238 (97.9)	3 (60.0)	0.3 (0.1–0.9)	19907 (2.1)	2 (40.0)	10.0 (1.2–36.3)	9107 (1.0)	2 (40.0)	22.0 (2.7–79.3)
1999	923477 (97.9)	1 (25.0)	0.1 (0.003–0.6)	19524 (2.1)	3 (75.0)	15.4 (3.2–44.9)	9136 (1.0)	2 (50.0)	21.9 (2.7–79.1)
2000	917219 (98.0)	5 (55.6)	0.5 (0.2–1.3)	19114 (2.0)	4 (44.4)	20.9 (5.7–53.6)	9088 (1.0)	4 (44.4)	55.0 (17.9–128.4)
2001	912151 (97.9)	0 (0.0)	0.0 (0.0–0.4)	19436 (2.1)	7 (100.0)	36.0 (14.5–74.2)	9352 (1.0)	7 (100.0)	85.5 (36.9–168.6)
2002	907850 (97.9)	4 (36.4)	0.4 (0.1–1.1)	19159 (2.1)	7 (63.6)	36.5 (14.7–75.3)	9284 (1.0)	7 (63.6)	43.1 (11.7–110.3)
2003	901492 (98.0)	4 (66.7)	0.4 (0.1–1.1)	18605 (2.0)	2 (33.3)	10.7 (1.3–38.8)	8939 (1.0)	2 (33.3)	33.6 (6.9–98.1)
2004	896075 (98.0)	2 (40.0)	0.2 (0.03–0.8)	18485 (2.0)	3 (60.0)	16.2 (3.3–47.4)	8805 (1.0)	3 (60.0)	34.1 (7.0–99.6)
2005	888302 (97.9)	0 (0.0)	0.0 (0.0–0.4)	18602 (2.1)	3 (100.0)	16.1 (3.3–47.1)	8777 (1.0)	3 (100.0)	45.6 (12.4–116.7)
2006	882116 (97.9)	0 (0.0)	0.0 (0.0–0.4)	19065 (2.1)	5 (100.0)	26.2 (8.5–61.2)	8805 (1.0)	5 (100.0)	34.1 (7.0–99.6)
2007	874507 (97.8)	2 (50.0)	0.2 (0.03–0.8)	20083 (2.2)	2 (50.0)	10.0 (1.2–36.0)	9332 (1.0)	2 (50.0)	32.2 (6.6–93.9)
2008	869589 (97.6)	1 (33.3)	0.1 (0.003–0.6)	21573 (2.4)	2 (66.7)	9.3 (1.1–33.5)	10104 (1.1)	2 (66.7)	19.8 (2.4–71.5)
2009	865555 (97.4)	5 (55.6)	0.6 (0.2–1.3)	22768 (2.6)	4 (44.4)	17.6 (4.8–45.0)	10755 (1.2)	4 (44.4)	37.2 (10.1–95.2)
2010	863798 (97.3)	1 (20.0)	0.1 (0.003–0.6)	23879 (2.7)	4 (80.0)	16.8 (4.6–42.9)	11182 (1.3)	4 (80.0)	35.8 (9.7–91.6)
2011	863308 (97.1)	7 (63.6)	0.8 (0.3–1.7)	25674 (2.9)	4 (36.4)	15.6 (4.2–39.9)	11851 (1.3)	4 (36.4)	33.8 (9.2–86.4)
2012	863544 (96.9)	2 (40.0)	0.2 (0.03–0.8)	27848 (3.1)	3 (60.0)	10.8 (2.2–31.5)	12540 (1.4)	2 (40.0)	16.0 (1.9–57.6)
2013	865126 (96.7)	2 (100.0)	0.2 (0.03–0.8)	29895 (3.3)	0 (0.0)	0.0 (0.0–12.3)	13244 (1.5)	0 (0.0)	0.0 (0.0–27.9)
2014	864993 (96.5)	6 (60.0)	0.7 (0.3–1.5)	31615 (3.5)	4 (40.0)	12.7 (3.4–32.4)	13944 (1.6)	4 (40.0)	28.7 (7.8–73.4)
2015	863838 (96.4)	3 (17.6)	0.3 (0.1–1.0)	32185 (3.6)	14 (82.4)	43.5 (23.8–73.0)	14132 (1.6)	14 (82.4)	99.1 (54.2–166.2)
Total		50 (36.0)			89 (64.0)			86 (61.9)	

5.2.4 Paediatric Contact Tracing in the HUS Area

5.2.4.1 Characteristics of Contacts and Index Cases

Altogether, there were 5092 listed TB contacts in 2012–2016, of whom 526 (10.3%) were included in the data analysis together with their 121 index cases in study IV. The annual number of paediatric contacts ranged from 48 to 231 (Graphics 11).



Graphic 11 *The annual number of contacts by exposure.*

The characteristics, contact tracing delays, and outcome of the exposed children in Table 7 and the characteristics of the index cases are presented in detail in Table 8.

		Total	Exposure group			Difference between groups (p)	
			Household (1)	Other (2)	Congregate (3)	1 vs. 2	1 vs. 2 vs. 3
Contact	**N**	526	109 (20.3)	221 (41.1)	196 (36.4)	N/A	N/A
	Age, years					0.32	<0.0001
	median	6.57	5.05	4.03	12.02		
	IQR	2.5–13.5	2.2–10.8	1.7–10.0	4.0–14.3		
	Age group, years					0.52	<0.0001
	<5	224 (42.6)	51 (46.8)	118 (53.4)	55 (28.1)		
	5–9	105 (20.0)	28 (25.7)	48 (21.7)	29 (14.8)		
	≥10	197 (37.5)	30 (27.5)	55 (24.9)	112 (57.1)		
	Gender					0.77	0.03
	male	273 (51.9)	63 (57.8)	124 (56.1)	86 (43.9)		
	female	249 (47.3)	46 (42.2)	98 (44.1)	105 (53.8)		
	unknown	4 (0.8)	0	0	4 (2.1)		
	Birth					0.001	<0.0001
	native	411 (78.1)	85 (78.0)	198 (89.6)	128 (65.3)		
	immigrant	106 (20.2)	24 (22.0)	20 (9.0)	62 (31.6)		
	TB endemic country[1]	96 (18.3)	19 (17.4)	15 (6.8)	62 (31.6)	0.003	<0.0001
	unknown	9 (1.7)	0	3 (1.4)	6 (3.1)		
	BCG					<0.0001	<0.0001
	yes	234 (43.5)	87 (79.8)	120 (54.3)	27 (13.8)		
	among <5 age group[2]	92 (48.7)	41 (80.4)	43 (40.2)	8 (25.8)		
	no	121 (23.0)	16 (14.7)	77 (34.8)	28 (14.3)		
	unknown	171 (32.5)	6 (5.5)	24 (10.9)	141 (71.9)		
	Delay, days						
	median	18	10	14	30	0.02	<0.0001
	IQR	10–35	4–19	8–25	22–42		
	<7 days[2]	92 (19.0)	44 (40.4)	44 (21.6)	4 (2.3)	0.04	<0.0001
	among <5 age group[2]	35 (15.3)	27 (52.9)	26 (23.9)	2 (4.1)		
	unknown	41 (7.8)	0	17 (7.7)	24 (12.2)		

Table 7 *The characteristics, contact tracing delays, and outcome of the paediatric TB contacts exposed to TB in the HUS area 2012–2016.*

		Total	Exposure group			Difference between groups (p)	
Outcome	**Infection**	34 (6.5)	19 (17.4)	14 (6.3)	1 (0.5)	0.003	<0.0001
	TB	9 (1.7)	5 (4.6)	3 (1.4)	1 (0.5)	0.13	0.07
	<5 years	3 (1.3)	2 (3.9)	1 (0.8)	0	0.22	0.19
	immigrant	5 (4.7)	4 (16.7)	0	1 (1.6)	0.11	0.02
	TBI	25 (4.8)	14 (12.8)	11 (5.0)	0	0.02	<0.0001
	<5 years	7 (3.1)	1 (2.0)	6 (5.1)	0	0.68	0.20
	immigrant	6 (5.7)	5 (20.8)	1 (5.0)	0	0.20	<0.0001
	No infection	431 (81.9)	87 (79.8)	189 (85.5)	155 (79.5)		
	received prophylaxis	41 (7.8)	14 (12.8)	26 (11.8)	1 (0.5)	N/A	N/A
	Unknown	61 (11.6)	3 (2.8)	18 (8.1)	40 (20.4)		
	outside HUS[3]	23 (4.4)	0	13 (5.9)	10 (5.1)		
	lost to follow-up	36 (6.8)	3 (2.8)	5 (2.3)	28 (14.4)	0.99	<0.0001
	no data	2 (0.4)	0	0	2 (1.0)		
Index case	**N**	121	53	80	11	N/A	N/A
	Contacts/Index ratio						
	mean	4.03	2.01	2.08	17.08	N/A	N/A
	range	1–94	1–5	1–14	1–89		
	Relation to contact					N/A	N/A
	parent	61 (11.6)	56 (51.4)	5 (2.3)	0		
	sibling	16 (3.0)	16 (14.7)	0	0		
	grandparent	82 (15.6)	18 (16.5)	64 (29.0)	0		
	aunt /uncle	35 (6.7)	7 (6.4)	28 (12.7)	0		
	other	332 (63.1)	12 (11.0)	124 (56.1)	196 (100.0)		

NOTE.
Categorical data are N (%).
N/A, not applicable
IQR, interquartile range
[1] Born in a high TB incidence (≥50/100,000) country.
[2] Percentage out of those with BCG or delay data available.
[3] Contact investigations outside the Hospital District of Helsinki and Uusimaa.

Characteristic	Total	Contacts with TB infection		Difference (p)
		≥1	None	
N	114	21 (17.4)	93 (76.9)	N/A
Age, years				0.21
median	39.8	38.5	40.7	
IQR	27.8–65.3	25.7–44.9	28.8–66.4	
Gender				0.99
male	63 (55.3)	12 (57.1)	51 (54.8)	
female	49 (43.0)	9 (42.9)	40 (43.0)	
unknown	2 (1.7)	0	2 (2.2)	
Birth				0.16
native	60 (52.6)	8 (38.1)	52 (55.9)	
immigrant	54 (47.4)	13 (61.9)	41 (44.1)	
TB focus				0.46
pulmonary	110 (96.5)	20 (95.2)	90 (96.8)	
extrapulmonary	3 (2.6)	1 (4.8)	2 (2.2)	
unknown	1 (0.9)	0	1 (1.1)	
Smear				0.01
positive	(59.6)	17 (81.0)	51 (54.8)	
negative	45 (39.5)	3 (14.3)	42 (45.2)	
unknown	1 (0.9)	1 (4.8)	0	
Cavitation[1]				<0.0001
yes	29 (25.4)	14 (66.7)	15 (16.1)	
no	82 (71.9)	7 (33.3)	75 (80.6)	
unknown	3 (2.6)	0	3 (3.2)	
MTB drug susceptibility				0.25[2]
sensitive	94 (82.5)	17 (81.0)	77 (82.8)	
resistant	12 (10.5)	4 (19.0)	8 (8.6)	
mono resistant	7 (6.1)	2 (9.5)	5 (5.4)	
MDR	4 (3.5)	2 (9.5)	2 (2.2)	
XDR	1 (0.9)	0	1 (1.1)	
unknown	8 (7.0)	0	8 (8.6)	

NOTE.
Data on seven index cases with all contact outcomes unknown not presented.
Categorical data are N (%).
N/A, not applicable
IQR, interquartile range
[1] Cavitation on chest radiograph
[2] sensitive vs. resistant

Table 8 *The Characteristics of the Index Case and Relation to the Outcome of the Contacts.*

5.2.4.2 **Characteristics of TB and TBI cases**

In study IV, nine cases of TB and 25 cases of TBI were identified among the exposed children. None of the exposed children were diagnosed with TB in the HUS area within two years after the exposure outside the contact investigations. The maximum contact tracing delay for the children under five years of age with either TB or TBI was seven days among the household exposure group and 12 days among the other exposure group.

The median age of the exposed children with TB and TBI was 10.9 years (IQR, 4.3–13.7 years) and 8.9 years (IQR, 3.9–12.9 years), respectively. None of them had medical conditions considered to increase the risk for disease progression. Among the cases, 5/9 with TB and 6/25 with TBI were immigrants born in a high TB incidence country.

At the time of diagnosis, the median TST result of the TB and TBI cases were 18 mm (IQR, 8–19 mm) and 17 mm (IQR, 11–20 mm), respectively. Eight of the cases with TB were diagnosed at the first visit and one during follow-up after IGRA conversion and development of prolonged cough. Among the TBI cases, 19/25 were diagnosed at the first visit and the rest during follow-up visits due to conversion of TST (3/25), IGRA (1/25), or both (2/25). All of them were diagnosed within four months of the first contact investigation visit, and none receive preventive treatment before the diagnosis. Three of the cases diagnosed with TBI during follow-up were under the age of five.

Eight of the cases with TB were diagnosed with PTB, of which four had an abnormal chest x-ray and the rest had prolonged cough with or without fever. None of the PTB cases were microbiologically confirmed. One contact in the congregated exposure group had culture-confirmed abdominal TB. None of the children had severe disease forms such as meningeal or miliary disease.

All of the children with TB made a full recovery: 8/9 completed full TB treatment, and in one case, the treatment was discontinued slightly before full completion due to elevated liver enzymes. Among the children with TBI, 22/25 completed full preventive treatment, one moved abroad during the treatment, and two with an MDR-TB index case were followed up for over two years without preventive treatment or progression to disease.

5.2.4.3 **TB and TBI yields**

In study IV, the overall contact investigation yield for TB or TBI was 6.5% (34/526). The overall TB and TBI yields were 1.3% and 3.1% among children under five years of age, respectively.

The TB and TBI yields were 4.6% and 12.8% among the household contact group, respectively. Among the household contact group children under five years of age, there were two cases with TB (one BCG-vaccinated and one non-BCG-vaccinated) and one case with TBI (non-BCG-vaccinated) resulting in a TB and TBI yields of 3.9% and 2.0%, respectively.

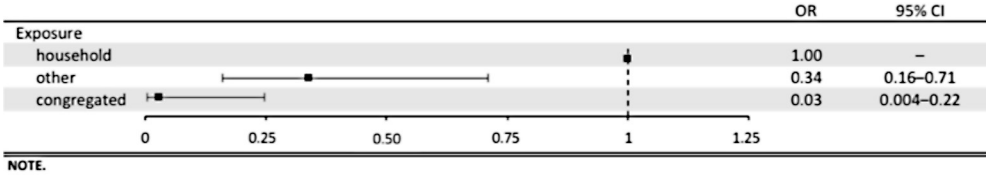
Among the other contact group, the TB and TBI yields were 1.4% and 5.0%, respectively. Among the other contact group children under five years, there was one case with TB (BCG-vaccinated) and six cases with TBI (five BCG-vaccinated and one non-BCG-vaccinated), resulting in TB and TBI yields of 0.8% and 5.1%, respectively.

5.2.4.4 **Factors associated with TB or TBI**

In study IV, the OR for either TB or TBI per exposure group is presented in Graphic 12. Due to low TB and TBI yield in the congregate exposure group, this group was excluded from further analysis.

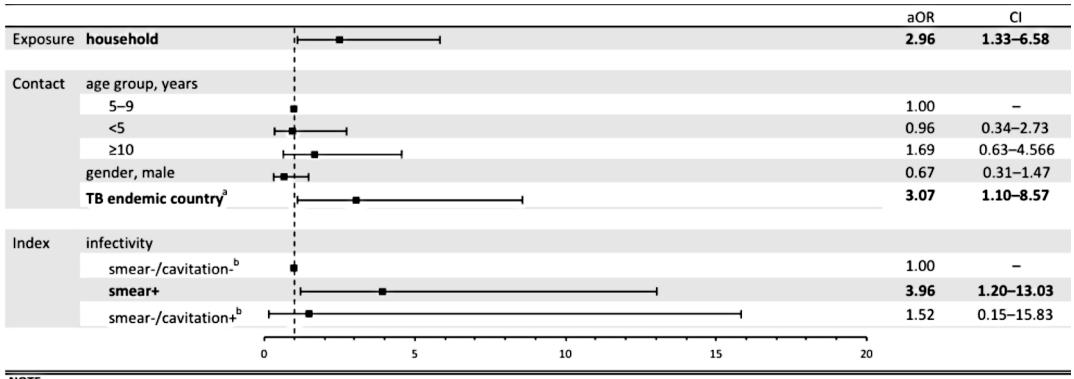
The ORs per different factors among the household and other contact group is presented in Graphic 13. Household exposure, BCG vaccination and birth in a TB endemic country of the contact, and smear positivity and pulmonary cavitation on chest x-ray of the index case were associated with TB or TBI of the exposed children.

Because BCG vaccination reflects birth in a TB endemic country, it was excluded from the multivariable analysis. In the multivariable analysis, household exposure and birth in a TB endemic country of the contact and smear positivity of the index case were associated with TB or TBI of the exposed children (Graphic 14).



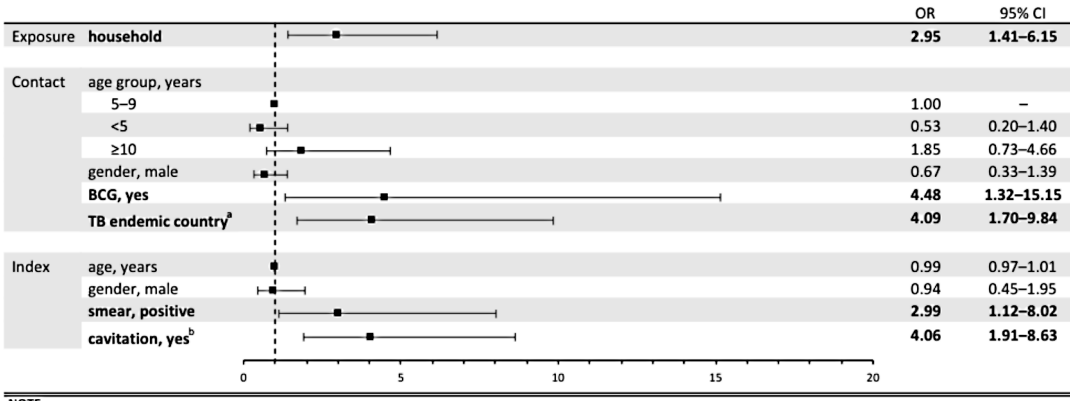
NOTE.
OR, odds ratio
CI, confidence interval

Graphic 12 *Unadjusted odds ratios for tuberculosis disease or infection among the paediatric contacts per exposure group.*



NOTE.
Bold indicates statistically significant factor in multiple logistic regression.
aOR, adjusted odds ratio
CI, confidence interval
[a]Immigrant born in a high TB incidence country (≥50/100,000).
[b]Cavitation on chest radiography

Graphic 13 *Unadjusted odds ratios for tuberculosis disease or infection among the household and the other contact group per different factors.*



NOTE.
Bold indicates statistically significant factor in logistic regression.
OR, odds ratio
CI, confidence interval
[a]Immigrant born in a high TB incidence country (≥50/100,000).
[b]Cavitation on chest radiography

Graphic 14 *Adjusted odds ratios for tuberculosis disease or infection among the household and the other contact group per different factors.*

6 Discussion

6.1 CHILDHOOD NTM LYMPHADENITIS IN FINLAND

6.1.1 Epidemiology

The estimated annual incidence of NTM infections among native-born children under five, most of which manifest as lymphadenitis, was based on registered cases with a positive NTM culture. It should be noted that approximately only half of the NTM lymphadenitis cases are generally culture-confirmed. Therefore, if clinically diagnosed cases were captured, the true incidence of childhood NTM lymphadenitis in Finland might be at least double. The estimated incidence rate of culture-confirmed childhood NTM infections presented in our study compares well with other published incidence rates when BCG vaccination policy is taken into consideration (Table 9).

Study	Country	Study period	BCG policy	Age group (years)	Frequency/100,000
our study	Finland	1995–2016[1]	universal	0–4	0.2[3]
Katila 1987	Finland	1977–86	universal	1–4	0.6
Romanus 1995	Sweden	1969–74	universal	0–4	0.06
our study	Finland	2007–2016[2]	selective	0–4	3.9[3]
Romanus 1995	Sweden	1975–80	selective	0–4	2.5
Romanus 1995	Sweden	1981–85	selective	0–4	5.7
Romanus 1995	Sweden	1986–90	selective	0–4	4.5
Hermansen 2017	Denmark	1991–2015	selective	0–4	5.4
Haverkamp 2004	The Netherlands	2001–03	selective	0–4	2.3

NOTE.
[1]Birth cohort from January 1995 to August 2006.
[2]Birth cohort from September 2007 to December 2015.
[3]The annual incidence rate presented is for native-born children.

Table 9 *Published incidence rates of childhood NTM disease and BCG vaccination policies during the study periods.*

There was a clear female predominance among the patients in our data and the previous study from Finland (female-to-male ratio of 2:1 and 3:1, respectively). (Katila 1987) The clear female predominance in Finland seems to have remained relatively the same after the BCG vaccination policy changed. Female predominance among adults with pulmonary NTM disease and children with NTM disease has also been observed elsewhere but to a much lesser extent. (Romanus 1990, Grange 1995, Trnka 1994, Pham-Huy 2010, Reuss 2009, Wolinsky 1995, Cassidy 2009) It remains unclear why females are more affected and why the female predominance in Finland is more distinct.

The NTM infections among native-born children under five years of age increased drastically after the BCG vaccination policy change in Finland. Because the BCG vaccination policy change resulted in a sharp decrease in infant BCG coverage in Finland, our observation supports the hypothesis that BCG offers protection against childhood NTM lymphadenitis. The IRR between selective-BCG and univer-

sal-BCG birth cohorts suggests that non-BCG-vaccinated children have a 19-fold higher risk for NTM lymphadenitis than BCG-vaccinated children.

The estimated effect of BCG compares well with other published studies. In Sweden, the ratio between non-BCG-vaccinated and BCG-vaccinated birth cohorts was 5.9 (95% CI, 1.6–48.5). (Romanus 1995) Furthermore, a systematic review and meta-analysis published the same year with our study, which did not include our results, estimated that BCG vaccinations greatly reduce childhood NTM lymphadenitis and that the estimated risk ratio of BCG-vaccinated compared to that of non-BCG-vaccinated was 0.04 (95% CI 0.01–0.21). (Zimmermann 2018)

6.1.2 NTM Culture Results

The culture-confirmed proportion in study I was approximately 60% and compares well with the expected sensitivity of cultures in the literature. (Zimmermann 2017, Willemse 2018) Similarly, the most common pathogen in studies I and II was *M. avium* which seems to be the most prevalent pathogen in most published studies, although some geographical variation exists. (Lopez-Varela 2015, Zimmermann 2015)

Interestingly, *M. lentiflavum* was the second most common species in our data accounting for 7% of the isolates. In sharp contrast, a review including 1274 culture-confirmed paediatric cases with NTM lymphadenitis found only one case caused by *M. lentiflavum,* and the pathogen seemed to be very rare. (Zimmermann 2015) The review, however, included studies since the 1960s, and *M. lentiflavum* was not recognized as a new species until 1996. (Springer 1996) At least one paediatric case of lymphadenitis caused by an unknown NTM species has been reported before *M. lentiflavum* was recognised as a species, and since its recognition, several cases of human *M. lentiflavum* infections, some of which were co-infections with a MAC species, have been reported in both adults and children. (Haas 1993, Tortoli 2002, Haase 1997, Cabria 2002, Suffys 2006, Jiménez-Montero 2014) Thus, *M. lentiflavum* might not be as rare a pathogen as previously suggested. Furthermore, a study from Spain noted that *M. lentiflavum* isolates accounted for most NTM lymphadenitis cases in the Madrid region. (Jiménez-Montero 2014) Swimming pools and potable water sources in certain regions have been proposed as a source for *M. lentiflavum* exposure and could explain the regional difference of NTM isolates in clinical samples. (Marshall 2011, Jiménez-Montero 2014) Although the exact places of transmission of the cases in our study are unknown, the *M. lentiflavum* isolates came from five different hospital districts without any specific region standing out. Thus, a regional difference of *M. lentiflavum* in Finland was not evident.

6.1.3 The Modified T.SPOT.TB Test

In our pilot study evaluating the modified T-SPOT.TB test, all children with culture-confirmed NTM lymphadenitis demonstrated a positive reaction to PPD stimulation yielding a high estimated test sensitivity. However, four patients in the healthy control group demonstrated a positive PPD reaction, i.e., a false-positive test result for NTM lymphadenitis. TST studies with PPD have shown that some children exhibit positive reactions even without mycobacterial disease or BCG vaccination. (Lind 1991) Because NTM are ubiquitous in the environment, virtually all children are exposed to NTM at some point in their life, but the majority of them do not develop clinical disease. NTM exposure is, however, believed to affect the cellular responses to BCG vaccination. (de Lisle 2005, Demangel 2005, Young 2007, Mendoza-Coronel 2011) This suggests that asymptomatic NTM exposure leads to some cellular memory affecting reactions to mycobacterial proteins. Thus, it is likely that the false-positive reactions were a result of an asymptomatic NTM exposure. Compared to TST, the response to PPD stimulation in the modified T-SPOT.TB test is performed *ex-vivo* and measured more accurately at a cellular level. Therefore, the false-positive rate of the modified T-SPOT.TB test is likely less compared to TST.

Notably, one child with *M. avium* lymphadenitis also exhibited a positive reaction in the CFP-

10 antigen stimulation. Some NTM species express the RD-1 region that encodes ESAT-6 and CFP-10, but *M. avium* is not one of them. (Arend 2005) A review of culture-confirmed NTM cases and their QFT-GIT results in Denmark found that 4% of patients with an infection caused by an NTM species exhibited positive QFT-GIT reactions. (Hermansen 2014) However, none of the children with NTM lymphadenitis caused by a species lacking the RD-1 region exhibited a positive QFT-GIT result. (Hermansen 2014) Some NTM species, including *M. avium*, might express genes encoded in genomic regions other than RD-1 for homologues of ESAT-6 that might cause positive reactions to ESAT-6 stimulation. (Hur 2014) Nevertheless, it is puzzling why the patient in our study exhibited positive reactivity to CFP-10 alone.

The parent-reported duration of lymphadenitis ranged from one to eight months at the time of testing. It is unclear how early during NTM lymphadenitis the PPD stimulation in the modified T-SPOT. TB test converts to positive. In TB, it can take several months for T-SPOT.TB to convert to positive. (Starke 2016) As NTM lymphadenitis progresses slowly, the initial exposure and infection are likely to occur well before the clinical disease. Thus, it is likely that the immunological memory enabling a positive PPD reaction would be present at the time of the testing of a child with clinical disease.

Altogether, the modified T-SPOT.TB test results in our pilot study were promising. The test's high sensitivity suggests a good negative predictive value for NTM infection among children with prolonged lymphadenitis. In such an event, further investigations for different aetiological reasons are warranted. Given the false positive rate in the control group and the unspecificity of PPD, a positive result should be viewed cautiously in the clinical context.

6.2 CHILDHOOD TB IN FINLAND

6.2.1 Epidemiology

The TB incidence or severe TB cases among native-born children under five did not increase after the BCG vaccination policy change in Finland. In contrast, a transient increase of TB and severe TB disease among children under five years of age has been previously reported from Sweden and the Czech Republic after changing to a targeted BCG vaccination policy. (Romanus 1992, Trnka 1993) A more recent observation from France, where universal BCG vaccinations were discontinued in 2007, noted minimal impact on the incidence of paediatric TB or TB meningitis. (Van Bui 2015, Guthmann 2011) There are factors that might explain why the TB morbidity of children under five years of age did not increase in Finland compared to Sweden: the overall lower TB incidence in the general population at the time of the BCG vaccination policy change and higher concentration of TB morbidity to the indigenous older population. (Salo 2006) Capturing the target group for selective BCG vaccinations is also decisive but can be unpredictable. (Faust 2019, Erkens 2014) In Finland, the implementation of selective vaccinations was planned meticulously, and BCG eligibility is determined in advance at public maternity clinics with very high attendance. (Salo 2006) Thus, the target population's immediate coverage was likely higher compared to Sweden, where it was reported to be nonoptimal immediately after the policy revision. (Romanus 1992)

The overall TB rate in Finland decreased in 1995–2015, but the negative binomial model did not show a decreasing trend in the under-15 population. The trend among the native children under 15 years of age was, in fact, slightly increasing with a relative annual increase of 6%. At the same time, the demographics in Finland have changed drastically. Thus, the observed trends likely reflect an increasing proportion of immigrants under 15 years of age and second-generation immigrants with a higher risk for TB. The TB incidence among children under 15 years of

age was expected to increase slightly in Finland after the BCG vaccination policy change. (Hersh 2003, Brantsaeter 2009) The under-15 population in our study included children born both during universal and selective BCG vaccination policy. Thus, further evaluation of the impact of the BCG vaccination policy change among children under 15 years of age is possible when the selective BCG cohorts grow older.

Altogether, over 80% of the paediatric TB patients were immigrants or second-generation immigrants. Thus, similar to other countries in Western Europe, childhood TB morbidity in Finland is highly concentrated to the immigrant and second-generation immigrant population. (Romanus 1992, Mohiyuddin 2019, Marx 2015, Erkens 2014) Native-born TB cases were more frequently found through TB contact tracing compared to immigrant TB cases. Most immigrant children were diagnosed because of symptoms and less than a third through immigrant TB screening. The index case of an immigrant child was also more frequently unidentified. The median age of the immigrant TB cases was significantly older than the native-born TB cases. The usual clinical presentation was PTB. Extrapulmonary disease was more common among immigrant children, but the difference was not significant after adjusting for age. A minority of the paediatric PTB cases were smear-positive, suggesting a low transmission risk for paediatric TB. Clinical diagnoses were common, and less than half were bacteriologically confirmed, underscoring the shortcomings of the current diagnostic techniques for paediatric TB. Bacteriologically confirmed proportion was higher among immigrants, but the difference was not significant after adjusting for age and infection focus. The smear-positive rate of paediatric TB in Finland compares well with studies from other countries. (Kunkel 2016) Furthermore, the PTB and bacteriologically confirmed proportions show similar tendencies with a recent UK study: young children are more likely to have PTB and bacteriological confirmation is less likely among young, native TB cases. (Mohiyuddin 2019)

6.2.2 TB Registry Data

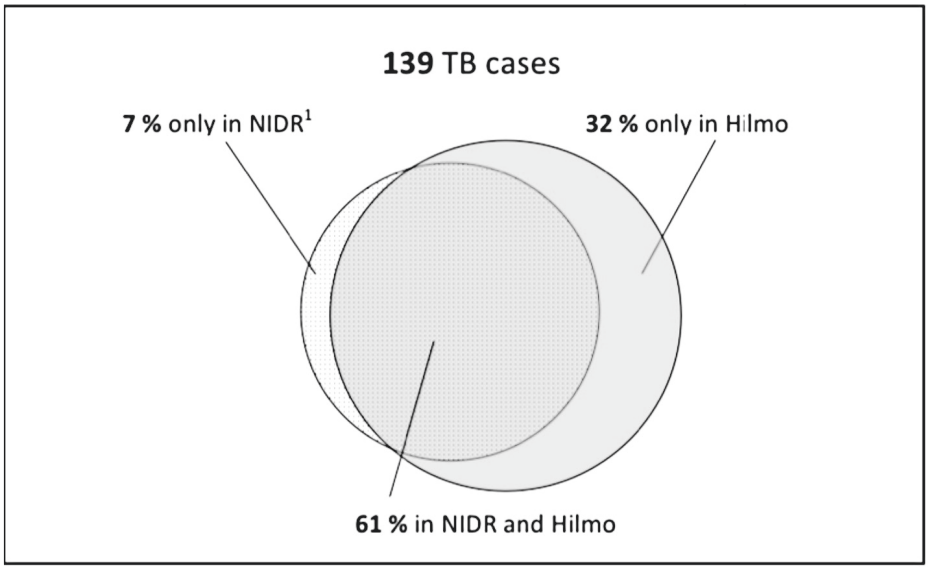
Notifications to the NIDR are the only source of national TB data reported from Finland. Almost a third of the actual paediatric TB cases were missing from the NIDR. Thus, a significant case detection gap is evident in the previously reported paediatric TB data from Finland. Most of the missing cases were clinically diagnosed before 2007 and likely missing due to the former strict register criteria. However, over 16% of the cases diagnosed in 2007–2015 were also missing from the NIDR. Because cases diagnosed clinically during that period were also accepted to the NIDR, the missing cases were apparently not notified at all. These missing cases had been diagnosed in seven different hospital districts suggesting human error rather than an institutional one. Approximately half of the patients registered to the Hilmo with a diagnostic code for TB disease were not actual TB cases. This indicates a high error rate in the Hilmo data regarding TB and that inclusion of TB data from Hilmo without verification is unreliable. The proportion of the cases and their respective registry is presented in Graphic 15.

6.2.3 TB Contact Tracing In HUS Area

Compared to previously published figures, the TBI yield in our study seems lower among the young children (Table 10).

Although an extensive systematic review of contract tracing among young children in high-income countries found a much higher prevalence of TB and LTBI, the review also noted substantial heterogeneity in the published data. (Fox 2013) The heterogeneity suggests that the combined estimate might not be comparable to a single country. BCG vaccinations might explain some of the variances in the published studies: in our study, most of the under-five household contacts were BCG-vaccinated, whereas in the United States the BCG vaccine is not commonly used.

Epidemiological studies suggest that eventually, 30–80% of young children with household exposure are infected. (Grzybowski 1975) Thus, our study's relatively low yield among the young household con-



NOTE.
[1]Registered to the Hilmo with other than diagnostic code for TB (ICD-9 010-018 or ICD-10 A15-19).

Graphic 15 *Venn diagram of the TB cases found in NIDR or Hilmo registry.*

Study	**Country**	**Exposure**	**Age group** (years)	**TB yield** (%)	**TBI or LTBI yield** (%)
our study	Finland	household	0–4	3.9	2.0
Fox 2013	review[1]	not reported	0–4	4.7	16.3
Pavic 2011	Croatia	close contact	0–4	not reported	26.4
Marks 2000	USA	close contact	0–4	5.0	not reported
Marks 2000	USA	close contact	0–5	not reported	21.0[2]
our study	Finland	household	5–15	5.2	22.4
Fox 2013	review[1]	not reported	5–14	2.9	18.4
Bennet 2020	Sweden	household	0–17	3.5	17.1
Aissa 2008	France	closed room[3]	0–14	1.4	13.1
Cavany 2017	UK	close contact	0–14	2.6	10.0
Diel 2008	Germany	household	0–15	not reported	4.5–9.0[4]

NOTE.
[1]data from high-income countries
[2]TST positive
[3]exposure time over 40 hours
[4]depending on the selected TST cut-off value

Table 10 *Published TB contact tracing yields among children exposed to TB.*

tacts suggests that index cases in the HUS area are promptly diagnosed and isolated before transmission mostly occurs. Young children are a high priority in contact tracing: the European consensus is that TB contacts should be informed within a week after identifying the index case. (Erkens 2010) Contact tracing in the HUS area identified exposed young children quickly: most children under five years of age in the household exposure group had first contact investigation visit within a week, and the maximum delay for those with either TB disease or infection was seven days. Additionally, most of the TB infections among the children under five years of age were found before progression to disease, and none had severe disease forms. In the HUS area, contact tracing is performed immediately after a diagnosis of a new TB case, and exposed young children are notified directly to the paediatric clinic. The HUS model shows that prompt investigations and early diagnosis of those infected can be achieved with well organised contact tracing structure.

In agreement with previous studies, household contacts, contacts with a sputum smear-positive index case and those born in a TB endemic country were more likely to be infected. (Aissa 2008, Bennet 2020, Marks 2000) In the univariate analysis, cavitation on the chest radiograph of the index case was a risk factor for TB or TBI of the exposed children. Cavitary PTB disease increases the bacterial load of sputum and the likelihood for positive sputum smear. (Palaci 2007) Therefore, we combined these factors for the multivariable analysis, and the association of cavitation without positive sputum smear was not significant. However, our results should be viewed cautiously as it is likely that our retrospective study was underpowered to achieve significant association for a cavitary disease alone. BCG vaccination was, paradoxically, associated with a higher risk among the contacts. Since the BCG vaccination policy in Finland is currently selective, the association of BCG likely reflects higher TB exposure risk and the likelihood of household exposure.

6.3 PUBLIC HEALTH SIGNIFICANCE

6.3.1 Childhood TB Prevention

6.3.1.1 **Surveillance Data**

Accurate national TB data is crucial for effective TB surveillance and prevention. Awareness of the global paediatric TB under-reporting has increased, however national TB surveillance in Europe is considered high performing and statutory notifications a good proxy indicator of the true incidence. (World Health Organization 2020) For example, in 2007, the European Centre for Disease Prevention estimated that TB under-reporting from Finland was at 5%. (European Centre for Disease Prevention 2012)

Our study represents the first extensive validation of the paediatric TB registry data in Finland, and much higher under-reporting was evident. Our findings compare well with the UK, where an estimated 20% of paediatric cases were missing from the national surveillance system. (Teo 2009) Childhood TB cases are also more likely culture- or smear-negative, and as such, more likely to remain unnotified. (Morales-Garcia 2015, van Hest 2007, Perez-Velez 2012) Therefore, despite broadly adopted obligatory reporting legislation, the European TB surveillance data is likely missing clinically diagnosed paediatric TB cases. The adjustment factor used by the World Health Organization that accounts for under-reporting in most of Europe is generally standard for all age groups. (World Health Organization 2020) Thus, it fails to account for the shortcomings of paediatric data separately. A separate adjustment factor would likely increase the reliability of the estimates, and further inventory studies focusing on paediatric TB

notification data within Europe should be encouraged.

6.3.1.2 **Immigrant Families**

Screening high-risk immigrants from TB endemic countries might capture TBI and TB disease at an early stage. However, the utility of LTBI screening of all immigrant children remains debated. (Rendon 2018) After individual risk assessment, asylum seekers and refugees in Finland are offered a selective TB screening with a chest x-ray. However, LTBI screening is only performed as a pre-BCG-vaccination investigation to a few. In our data, most of the immigrant children with TB were found through symptoms. It is unclear how many of them were infected abroad or attended entry TB screening. Recent studies from Finland indicate that 67% of asylum seekers under 18 are screened with chest x-ray, yet only 20% of all immigrant TB cases had undergone TB screening. (Räisänen 2018, Tiittala 2018) Whether TB or TBI among high-risk immigrant children could be captured earlier with a more active case detection and meticulous TB screening with immunological tests warrants further investigations. The high proportion of second-generation immigrants among the native-born TB cases suggests that higher TB exposure risk remains in the immigrant community and passes to the children born in Finland.

Close-contact investigations among the immigrant population are cost-effective, but at the same time, foreign-born contacts are more likely to remain unidentified. (Borraccino 2014, Dasgupta 2000) It is likely that investigations among the immigrant community also identify old infections resulting from TB exposure abroad. However, in our data majority of the immigrant TBI cases had an active disease or exhibited TST or IGRA conversion suggesting recent infection. A minority of the immigrant TB cases were found through contact investigations, and the index case for them remained largely unidentified. This can indicate that immigrant children infected with TB abroad are not captured in entry TB screenings, their index cases in Finland are not diagnosed early, or immigrant children exposed to TB are not identified during contact tracing.

6.3.1.3 **BCG Vaccinations**

The first years of life are the most critical for children at TB risk: young children are vulnerable to severe TB and benefit from BCG vaccinations the most. (Perez-Velez 2012, Sloot 2014) After the BCG vaccination policy change, we did not observe an increase in TB incidence or severe TB cases among native children under five years of age. This suggests that returning to universal BCG vaccinations is not warranted in Finland in the current epidemiological landscape.

Nevertheless, as adult TB remains uneradicated, TB exposures and infections among young children are inevitable and regular evaluation of the BCG vaccination policy remains essential. Selective BCG vaccinations depend decidedly on the identification and capture of the at-risk infant population. Previously it was estimated that the TB reduction with BCG vaccinations of risk groups with an annual TB incidence of approximately 24 per 100,000 is close to that achieved with universal BCG. (Hersh 2003) In our data, the TB incidence among the under-15 immigrant population from high TB incidence countries was well above this threshold. The majority of the TB cases in our data were eligible for BCG under the current target groups for selective vaccination. However, BCG coverage was relatively low among the young children undergoing contact investigations in the HUS area, considering they have evidently been at risk for TB exposure. In Finland, the older indigenous population remains a major reservoir for TB reactivation as they contracted TB infections when TB was endemic in their youth and still account for the majority of all TB cases. (Räisänen 2016, Smit 2014) This likely causes transmission risk directly, or indirectly through the parents, to the grandchildren. Indeed, TB exposure from grandparents was substantial and most of the children under five years of age with such exposure were non-BCG-vaccinated. This indicates that this risk group of grandchildren is not

comprehensively captured under the current selective BCG vaccination policy. In the future, the national vaccination registry can further clarify the capture of our current selective vaccinations.

6.3.1.4 **Contact Tracing**

Approximately 10% of all listed contacts were paediatric, and 4% were under-five years, indicating that the exposure of this vulnerable age group in the HUS area is ongoing. With the majority of the infant population non-BCG-vaccinated, identifying young children exposed to TB quickly is imperative.

The annual number of listed contacts varied extensively, and contact tracing efforts were remarkably higher in 2012 and 2015 due to few large-scale congregated exposures. The yields in the congregate exposure group were meager, indicating that the large-scale investigations after these exposures were inefficient. Nevertheless, TB transmission can occur through social interaction, and substantial TB outbreaks in schools have been reported. (Fox 2013, Hoskyns 2003, Smit 2015) In the national guidelines, the exposure criteria for TB investigations are essentially the same for all out-of-household contacts. Thus, it seems that the exposure risk in congregate settings was difficult to quantify and may be overestimated compared to the other non-household contacts. Because fruitless large-scale investigations deplete valuable contact tracing resources, investigations after an extensive congregate exposure should be more cautiously targeted.

6.3.2 Childhood NTM Lymphadenitis Prevention

6.3.2.1 **Surveillance and Diagnostics**

The global burden of childhood NTM lymphadenitis and other NTM infections, especially in high-TB-incidence countries where environmental exposure to NTM, BCG coverage, and HIV prevalence are likely higher, remains largely unknown. (Lopez-Varela 2015) Understanding NTM epidemiology and the interactions between NTM and the immune system will advance the understanding of other mycobacterial infections and prevention also.

The optimal treatment scheme for NTM lymphadenitis remains unsettled as most observational studies include an invasive biopsy to achieve the diagnosis. (Zimmermann 2015) Epidemiological and prospective treatment studies are largely limited by the lack of an alternative, better diagnostic test to NTM cultures. The invasive diagnostic methods also increase the burden on the patient and families. Thus, noninvasive methods are urgently needed, and the modified T.SPOT.TB with commonly available PPD antigen is a promising diagnostic test for childhood NTM lymphadenitis.

6.3.2.2 **BCG Vaccinations**

BCG vaccinations prevent childhood NTM lymphadenitis. However, vaccinations with Statens Serum Institut BCG vaccine during Finland's universal BCG vaccination policy resulted in lymph node abscesses caused by the BCG vaccine in 150 per 100,000 vaccinees. (Kilpi 2006) Thus, the incidence of BCG lymphadenitis was far higher than the estimated incidence of childhood NTM lymphadenitis after the BCG vaccination policy change. Furthermore, during the previous universal BCG vaccination policy, osteitis, arthritis, and generalized BCG infections were observed in 14 per 100,000 vaccinated. (Kilpi 2006) Though other BCG vaccine strains may be less reactogenic, BCG remains a live vaccine that may cause serious adverse effects. In contrast, the nature of NTM lymphadenitis is generally self-limiting. Therefore, utilizing BCG vaccinations for NTM lymphadenitis prevention should not be recommended.

6.4 ETHICAL CONSIDERATIONS

A selective BCG vaccination policy has ethical considerations, especially as the BCG vaccine is not commercially available in Finland, and the only source for vaccination is the public health sector. Childhood TB can be a debilitating and lethal disease, and the BCG vaccine effectively prevents severe TB disease in children. Although TB in Finland is rare, it is far from eliminated. Determining the exact risk for TB exposure is also challenging as TB can be transmitted even in a brief and random contact. Therefore, it is likely that some non-BCG-vaccinated children are exposed to TB and ultimately might develop serious TB disease. Furthermore, childhood NTM lymphadenitis cases would likely be avoided with a universal BCG vaccination policy, and, although childhood NTM lymphadenitis is usually self-limiting, the full resolution of the disease can take months.

In contrast, as a live attenuated vaccine, BCG itself can cause complications, and only a part of the population is subjected to the potential harms of the vaccine. The risks of BCG vaccination should be regularly weighed against the benefits. Furthermore, all decisions regarding childhood vaccinations are decided in an agreement with the parents, and preserving the confidence of the parents in the national vaccination program is essential. Thus, the natural BCG vaccination reaction and potential adverse events should be carefully discussed with the family as soon as the need for BCG vaccination is recognised.

TB can also stigmatize and cause public fear through a perceived risk of transmission from an infected individual; media outlets commonly report cases of TB in schools or other institutions. Thus, it is vital that TB epidemiology and risk group-based approaches avoid labeling nomenclature. Regarding childhood TB, it is essential to highlight that immigrant children themselves are, in fact, at-risk and not a risk.

6.5 STRENGTHS AND LIMITATIONS

The major strength of our studies is the nationwide capture of childhood NTM and TB cases. National NTM registries are exceptionally scarce, and our study provides valuable insight into childhood NTM epidemiology. Because TB and NTM isolates are reported directly from the laboratories, the NIDR data is very robust regarding culture-confirmed cases. Because the BCG vaccination policy change resulted in a rapid decrease of BCG coverage among cohorts living in an otherwise similar environment, we provide a rare window into NTM epidemiology and add to the growing evidence that BCG provides protection against childhood NTM lymphadenitis. Due to the capture of childhood TB cases from two separate nationwide registers, in theory, we captured all diagnosed childhood TB cases in Finland: our study provides convincing data regarding the true incidence of childhood TB in Finland.

The number of TB and NTM cases was relatively small. Although we did not observe an increasing trend for native under-five TB rate, minor changes might be concealed under the overall decreasing TB trend in Finland or not achieve statistical significance. However, an observable decrease from the previous very low rate would essentially mean close to zero TB cases. As TB in Finland remains uneradicated, some TB cases among the native under-five population are inevitable, and, most importantly, severe TB cases among native children under five years of age have remained very rare.

Although other NTM infections than lymphadenitis are very rare among young children, some isolates might no represent a case of NTM lymphadenitis. We did not verify the NTM cases from the medical records, and some of the NTM isolates might also reflect colonization or contamination. Nevertheless, a Danish study determined that 95%

of NTM isolates in children under five years of age represent definite NTM disease. (Hermansen 2017) There is no reason to assume otherwise regarding NTM isolates in this age group in Finland.

Because all of the studies were retrospective, the available data was limited. In our study of the modified T.SPOT.TB test the control group was also retrospectively collected and, therefore, not actively matched to the disease group. The BCG vaccination status of the patients and comprehensive BCG coverage of the birth cohorts were not available from the national vaccination registry. The infectiousness of the index case and level of exposure was based on available retrospective data. Index cases were also based on the epidemiological link and not molecular epidemiology.

6.6 FUTURE CONSIDERATIONS

Our pilot study regarding the utility of the modified T.SPOT.TB test for diagnosing childhood cervical NTM lymphadenitis showed promise. However, further validation of the test among a larger cohort of children with different aetiology for cervical lymphadenitis and in different epidemiological settings should be conducted. Different positive cut-off values should also be evaluated to attain the best sensitivity and specificity for the test. Since childhood NTM lymphadenitis cases are rare, this would likely require multinational collaboration so that a large prospective cohort would be feasible. Given the estimate that only approximately half of the actual NTM lymphadenitis cases are culture-confirmed, an additional diagnostic reference method should be considered to avoid the confounding effect of the culture false-negative cases.

It will be possible to carry out a follow-up study on the paediatric NTM incidence in Finland in the future. This would add valuable data concerning whether the incidence stabilizes. Furthermore, the protective effect of the BCG vaccine against adult NTM infections remains unknown. As the non-BCG-vaccinated cohorts grow older, it will be possible to evaluate whether pulmonary NTM infections among the adult population will increase due to the decreasing BCG vaccination coverage.

Monitoring of childhood TB cases should be constant. The selective BCG vaccination policy should be evaluated regularly as the risk groups might change over time. The capture of the risk groups is of the utmost importance. The BCG vaccination coverage of the different populations should be closely monitored: the national vaccination registry will add valuable data regarding this issue in the future. At this point, the effects of the BCG vaccination policy change on under-15 TB morbidity cannot be evaluated. This will be possible in the future when the selective BCG vaccination policy cohorts grow older.

Rapid TB contact tracing is even more crucial since the BCG vaccination coverage of the population decreases. Further studies evaluating why most childhood immigrant TBI cases are not captured until the infection progresses to disease should be conducted.

7 Conclusions

In summary, the epidemiological landscape of childhood mycobacterial infections in Finland has changed. The BCG vaccination policy change in 2006 resulted in an increase in childhood NTM infections, but childhood TB infections did not increase, and restarting universal BCG vaccinations seems unwarranted. Childhood TB, however, remains an essential public health issue, and future surveillance is essential. The focus of childhood TB prevention in Finland should be further targeted to those with an immigrant background.

The main findings of the thesis are as follows:

1. The modified T.SPOT.TB test with additional PPD stimulation is a promising non-invasive diagnostic test for childhood NTM lymphadenitis.

2. NTM infections among native-born children under five years of age have increased after universal BCG vaccinations were discontinued, suggesting a protective effect of the BCG vaccine against childhood NTM lymphadenitis.

3. Childhood TB morbidity or severe TB cases have not increased after the universal BCG vaccination policy changed to a risk group-based approach.

4. Childhood TB cases in Finland are highly concentrated in the immigrant and second-generation immigrant population.

5. The NIDR is missing childhood TB cases indicating a case detection gap in the national TB data.

6. Prompt investigations for paediatric TB contacts and early diagnosis of infected children can be achieved with a well-organised contact tracing structure.

7. Household contact, index case infectivity, and birth in a TB endemic country increase the risk of TB or TBI among paediatric TB contacts.

8. Paediatric TB and TBI yields of large-scale contact investigations after TB exposure in congregated settings are low, and investigations after such exposure should be targeted cautiously.

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Antti Kontturi
Helsinki, August 2021

Alvar Kontturi: ***Dad lying on the bed and writing on*** *his laptop* (2021)

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