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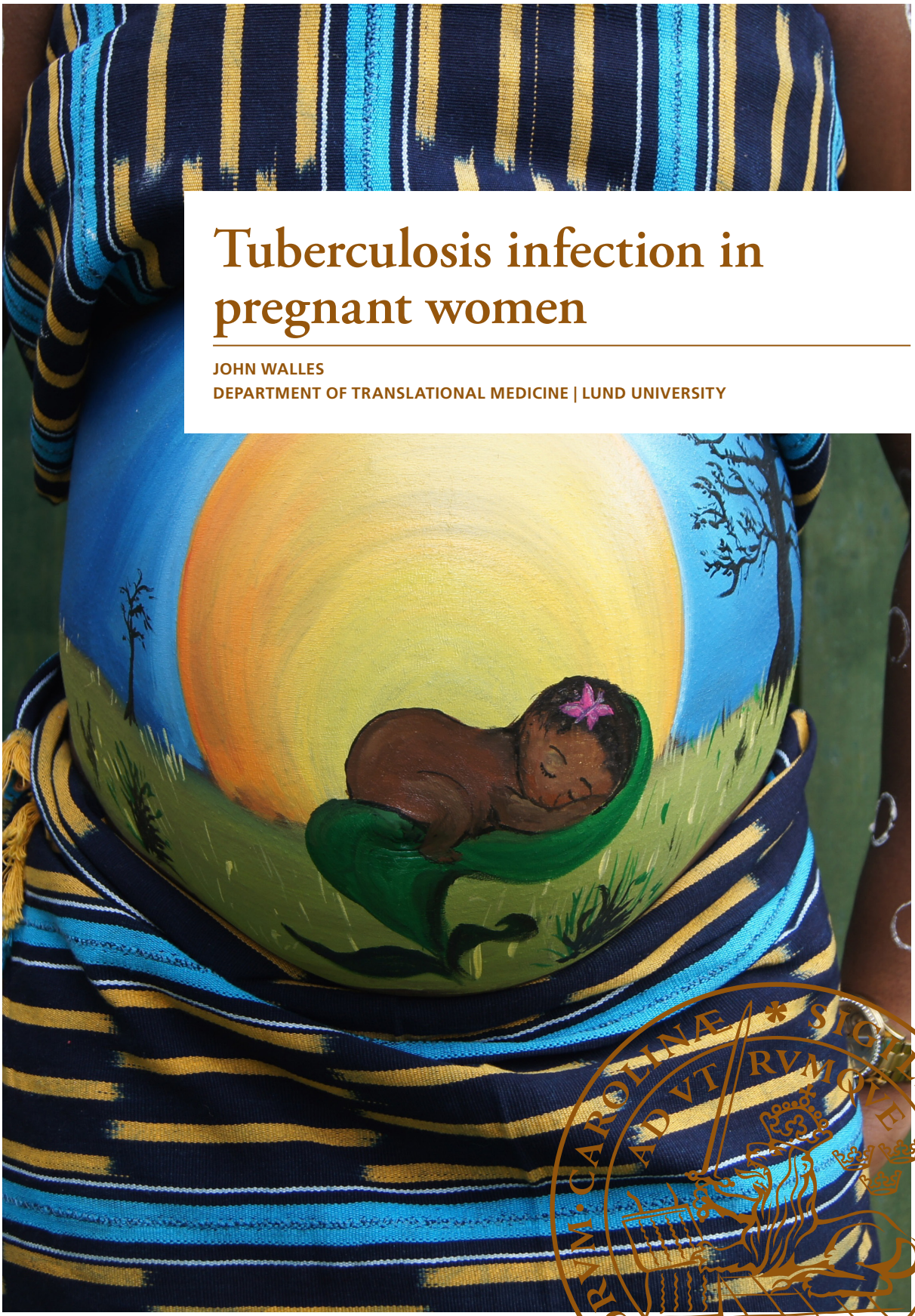

Tuberculosis infection in pregnant women

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LUND
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Abstract

Tuberculosis (TB) is among the top killers of infectious diseases. It causes disease in around 10 million people annually, with a strong predilection for settings and populations burderned by poverty, malnutrition and immunosuppressive comorbid conditions, and with poor access to adequate care, and caused more than 1.4 million deaths in 2018, and 1.7 billion people are estimated to have an asymptomatic carriage of tuberculosis known as TB infection (previously latent TB). This work revolves around the interaction between the physiological immunomodulations occurring during pregnancy, and TB infection. Previous studies have indicated that TB disease is more frequently diagnosed after delivery compared to periods unaffected by pregnancy, and that TB disease is associated with adverse pregnancy outcomes, maternal and neonatal mortality, especially in the context of HIV coinfection. Here, we have investigated the following aspects of TB infection in association to pregnancy:

1. the effect of pregnancy on the performance of immune based tests to detect TB infection, specifically the excess sensitivity of the novel TB2 antigen formulation in the QuantiFERON TB GOLD Plus (QFT) assay
2. the occurrence and associated characteristics of TB infection in pregnant women in a high-burden setting, and
3. the consequences of maternal TB infection on pregnancy complications

Aims 1-3 have been investigated in a prospective cohort of women recruited from antenatal care in Adama, Ethiopia, during 2015-2018. Study participants were subjected to extensive interview, physical examination, HIV rapid test, QFT as well as investigation for TB disease (using PCR and culture) for women with symptoms suggestive of TB disease at any time during the study. Women and their subsequent offspring were followed until at least 4 years after delivery. Aim 3 was also investigated in a Swedish retrospective material of immigrant women screened for TB infection during routine antenatal care 2014-2018. QFT results were linked to the Swedish Pregnant and Pregnancy Registers to define pregnayc outcomes. We made the following observations:

1. The TB1 and TB2 antigen formulations elicited similar levels of interferon-γ in pregnant women, discordance was rare and the additional sensitivity from the addition of the TB2 antigen formulation was limited in this context. HIV positive women had significantly lower TB-antigen stimulated levels of interferon-γ.
2. More than one-third of the pregnant women in the study had acquired TB infection (TB infection [32.4%] or past [4.4%] or present [0.3%] TB disease). TB disease was more common in women with HIV coinfection, and the risk of TB infection was strongly associated with increasing maternal age.
3. Stillbirth, severe preeclampsia, emergency caesarean section and low birthweight were independently associated with TB infection in the large Swedish register-based study (n=12 443), while no significant differences in pregnancy outcome were observed in the smaller Ethiopian cohort (n=1456).

We conclude that women of fertile age in and Ethiopian city are continuously exposed to contagious TB. Increased efforts are required to investigate the source of transmission and to develop effective strategies to identify and treat individuals with contagious TB in the community in high-buden settings.

Furthermore, we have observed an excess risk of pregnancy complications in women with TB infection. Due to the observational nature of the studies, it is impossible to ascertain causality. Considering that TB infection has been estimated to occur in 1.7 billion people, these findings motivate further studies to verify our findings, clarify pathogenetic mechanisms and explore if any excess risk can be modulated. It also supports the WHO ambitions of a rapid reduction of global TB incidence, but novel strategies and tools may be required to achieve this.

Key words Tuberculosis, infection, LTBI, pregnancy, stillbirth, preeclampsia, Africa

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To Maria and Erik, with love and gratitude

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II. John Walles, Fregenet Tesfaye, Marianne Jansson, Taye Tolera Balcha, Erik Sturegård, Mestawet Kefeni, Gadissa Merga, Stefan R. Hansson, Niclas Winqvist, and Per Björkman. Tuberculosis infection in women of reproductive age - a cross-sectional study at antenatal care clinics in an Ethiopian city. Clin Infect Dis. 2021;73(2):203–10.

III. John Walles, Laura García Otero, Fregenet Tesfaye, Asmamaw Abera, Marianne Jansson, Taye Tolera Balcha, Erik Sturegård, Niclas Winqvist, Stefan R. Hansson and Per Björkman. Tuberculosis infection and stillbirth in Ethiopia-A prospective cohort study. PLoS One. 2022;17(4):e0261972.

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Papers not included in thesis

I. König Walles J, Balcha TT, Winqvist N, Björkman P. Growth pattern in Ethiopian infants – the impact of exposure to maternal HIV infection in relation to socio-economic factors. Glob Health Action [Internet]. 2017;10(1):1296726.

II. Tesfaye F, Sturegård E, Walles J, Winqvist N, Balcha TT, Karlson S, et al. Alternative biomarkers for classification of latent tuberculosis infection status in pregnant women with borderline Quantiferon plus results. Tuberculosis. 2020;124(April).

III. Flanagan E, Mattisson K, Walles J, Abera A, Eriksson A, Balidemaj F, et al. Air Pollution and Urban Green Space: Evidence of Environmental Injustice in Adama, Ethiopia. Front Sustain Cities. 2021;3(September).

IV. Tesfaye F, Walles J, Winqvist N, Balcha TT, Kefeni M, Jansson M, et al. Longitudinal Mycobacterium tuberculosis-Specific Interferon Gamma Responses in Ethiopian HIV-Negative Women during Pregnancy and Postpartum. J Clin Microbiol. 2021;59(10):1–10.

V. Flanagan E, Oudin A, Walles J, Abera A, Mattisson K, Isaxon C, et al. Ambient and indoor air pollution exposure and adverse birth outcomes in Adama, Ethiopia. Environ Int [Internet]. 2022;164(February):107251.

VI. Tesfaye F, Sturegård E, Walles J, Bekele B, Bobosha K, Björkman P. Dynamics of Mycobacterium tuberculosis -Specific and Nonspecific Immune Responses in Women with. Microbiol Spectr. 2022;epub ahead of print.

VII. Szanyi J, Walles JK, Tesfaye F, Gudeta AN, Björkman P. Intrauterine HIV exposure is associated with linear growth restriction among Ethiopian children in the first 18 months of life. Trop Med Int Heal. 2022;6–8.

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IX. Giuseppe Zenatti, Mario Raviglione, Fregenet Tesfaye, Kidist Bobosha, Per Björkman, and John Walles. High variability in tuberculosis treatment outcomes across 15 health facilities in a semi-urban area in central Ethiopia. Manuscript, submitted to Journal of Clinical Tuberculosis and Other Mycobacterial Diseases.

Populärvetenskaplig sammanfattning

Tuberkulos är en sjukdom som i mångt och mycket glömts bort i Sverige. För 150 år sedan var situationen en annan, tuberkulos skördade en betydande del av de totala dödsfallen bland personer i arbetsför ålder. I Sverige förbättrades levnadsvillkoren betydligt efter förra sekelskiftet; frånvaro av krig, mer hygieniska levnadsförhållanden, tillgång till näringsrik mat samt tillgång till vaccin och isoleringsvård ledde till en kraftig nedgång, innan de första läkemedlen blev tillgängliga under mitten av 1900-talet. Andra delar av världen har inte haft samma gynnsamma utveckling, och globalt sett har tuberkulos varit den infektionssjukdom som orsakat flest dödsfall årligen, 1.4 miljoner år 2019. År 2022 är situationen förändrad av Covid-19-pandemin som nu dominerar den globala dödligheten i infektioner.

Tuberkulos orsakas av mykobakterier (*Mycobacterium tuberculosis* och närbesläktade arter), som främst orsakar lungsjukdom och där luftburen smitta är den helt dominerande vägen. Tuberkulos kan dock även sprida sig till kroppens alla organ och vävnader om immunförsvaret är försvagat. Effektiv behandling finns tillgänglig i nästan alla världens hörn, förutsatt att bakterien inte förvärvat några resistensmekanismer för att skydda sig mot antibiotika. Dock uppskattas att runt en tredjedel av tuberkulosfallen inte upptäcks och därmed inte heller kan erbjudas behandling. Att hitta och behandla sådana personer är av stor vikt för dem men även för deras medmänniskor som riskerar att smittas. Runt en fjärdedel av jordens befolkning tros vara infekterad av tuberkulosbakterier, som kan ligga inkapslade av immunförsvaret i ett slags dvala i flera decennier och utan att orsaka sjukdomssymptom, ett tillstånd som ofta kallas ”vilande” eller ”latent” tuberkulosinfektion.

Vid graviditet är fostret och moderkakans vävnader inte immunologiskt helt lika moderns övriga vävnader, fostrets genetiska uppsättning kommer ju i lika delar från båda föräldrarna. Man kan likna fostret och moderkakan vid en transplanterad njure, och följaktligen behöver immunförsvaret regleras ner under kontrollerade former för att undvika ”bortstötningsreaktioner” eller i detta fall graviditetskomplikationer som havandeskapsförgiftning. En gravid kvinna kan därför försvara sig sämre mot infektionssjukdomar, och som flera andra infektionssjukdomar förefaller tuberkulos vara något vanligare i anslutning till graviditet och dessutom vara förknippat med ett mer allvarligt förlopp, och även en rad graviditetskomplikationer.

Huruvida vilande tuberkulosinfektion kan orsaka graviditetskomplikationer är inte känt, men är värt att undersöka med tanke på hur vanligt tillståndet är och inte minst att den moderna litteraturen betraktar vilande tuberkulosinfektion som ett spektrum som i ena änden gränsar till tuberkulossjukdom.

I denna avhandling har vi bland annat studerat

1. förekomsten av tuberkulos bland gravida kvinnor i Etiopien, och
2. huruvida vilande tuberkulosinfektion ökar risken för graviditetskomplikationer.

Bland 1834 gravida kvinnor rekryterade under 2015-2018 från tre mödrahälsovårdskliniker i Adama, en stad i centrala Etiopien, hade 37% tecken på att ha blivit infekterade med tuberkulosbakterier. De flesta (32.4%) hade vilande tuberkulos, 4.4% rapporterade att de tidigare behandlats för tuberkulossjukdom, och fem (0.3%) diagnosticerades med tuberkulossjukdom i anslutning till graviditeten. Förekomst av tuberkulosinfektion ökade med ålder (2.1% infekterade per levnadsår), och var även högre hos kvinnor med hiv-infektion, men var inte tydligt kopplad till socioekonomiska eller demografiska karakteristika. Detta tyder på förekomst av utbredd samhällsspridning av tuberkulos.

Sammanlagt 7 408 kvinnor som invandrat till Sverige från länder med hög förekomst av tuberkulos, och som provtagits för tuberkulosinfektion i samband med graviditet under 2014-2018 undersöktes avseende tuberkulosinfektionens inverkan på graviditetsutfall. Av dessa kvinnor kunde 12 443 graviditeter analyseras, varav 2536 var hos kvinnor med tuberkulosinfektion. Förekomsten av såväl dödfödsel som svår havandeskapsförgiftning, akut kejsarsnitt och låg födselvikt var högre bland kvinnor med tuberkulosinfektion, även med justering för att kvinnor med tuberkulosinfektion hade en annan fördelning av ålder och geografiskt ursprung än kvinnor utan tuberkulosinfektion.

Vidare studier krävs för att verifiera detta fynd och att utreda möjliga mekanismer, samt i förlängningen om en riskökning går att påverka med exempelvis förebyggande behandling mot vilande tuberkulos.

Studierna understryker vikten av att minska tuberkulosbördan i världen. Ytterligare studier är motiverade för att utreda hur smittsamma individer som ej självmant tar initiativ till sin tuberkulosutredning bäst kan identifieras och erbjudas effektiv behandling.

Introduction

Mycobacterium tuberculosis and tuberculosis infection

Characteristics of mycobacteria and Mtb

Members of the genus *Mycobacterium* include some of the most important human pathogens, and is constituted of the *Mycobacterium tuberculosis* complex (MTBC), the causative agent of tuberculosis (TB) [1], *Mycobacterium leprae,* the causative agent of leprosy [2,3], and the nontuberculous mycobacteria (NTM), a heterogenous group of bacteria which may colonize or cause a range of diseases, most notably in immunosuppressed individuals [4,5]. Mycobacteria are aerobic non-sporulating and non-motile rods, but differ from other bacteria in several important regards. Perhaps the most distinguishing feature is the composition of the cell wall. It's high content of mycolic acids, free lipids, and waxes, provides a thick and hydrophobic protection from harsh environments including drought and chemical agents, and bacilli may remain viable and infectious in the environment for extended periods of time (Figure 1). These properties also make them difficult to stain with the traditional Gram method, and after staining with carbolfuchsin (used for Ziehl-Neelsen staining) or auramine (for fluorescence microscopy), they are not easily decolorized by acid or alcohol treatment [6]. The term "acid-fast bacilli" is therefore almost synonymous with mycobacteria. Owing to the complex cell wall, their growth is considerably slower than that of most other bacteria, often requiring 20 hours per generation under optimal conditions [7].

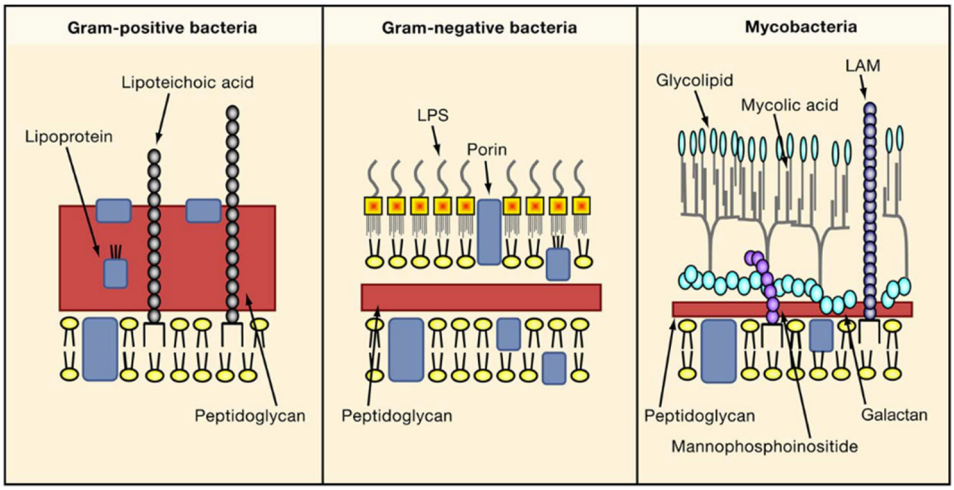

Figure 1. Schematic overview of the mycobacterial cell wall compared to typical Gram-positive and Gram-negative bacteria. Reprinted with permission. Copyright 2006 Elsevier. [8]

The major mycobacterial human pathogens – members of the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae* - are obligate pathogens and require humans or animals for their replication. In contrast, non-tuberculous mycobacteria (NTM), of which around 200 species have been described, are living freely in their environmental niches, especially in temperate regions, and may invade and inhabitate humans with no or variable capacity and predilection for causing disease. MTBC is a group of 12 closely related species of mycobacteria capable of causing tuberculosis disease, most notably *M. tuberculosis sensu stricto* which is in absolute dominance, *M. africanum* (causing a significant proportion of cases in Western Africa [9]), *M. bovis* (known for causing zoonotic tuberculosis through consumption on un-pasteurized dairy products [10]) and its artificially attenuated form, *M. bovis bacille Calmette-Guerin* (BCG), which can cause iatrogenic disease through vaccination or intravecisal instillation for treatment of uroepithelial cancer [11–13]. *Mycobacterium canetti* is a rare East-African species which has enjoyed particular attention as being considered to be close to the common ancestor of the MTBC [14–16].

Early host-pathogen interaction

Infection with *Mycobacterium tuberculosis* typically starts with the inhalation of infectious aerosol ranging 1-5 µm (expectorated or generated through speaking, singing or sneezing) from an individual with pulmonary tuberculosis [17,18]. After inhalation, bacilli reach the alveolar space where their first interaction with players of the innate immune system occurs. This involves recognition by cell surface

receptors, leading to initiation of diverse proinflammatory signalling cascades as well as ingestion of bacilli by antigen presenting cells, most importantly alveolar macrophages [19].

At this stage, perhaps the most important virulence factor of Mtb, the ESAT-6 secretory system-1 (ESX-1), comes into play. With rare exceptions (including *M. marinum*), the ESX-1 secretion system is specific to MTBC among mycobacteria, but has been removed from the *M. bovis BCG* strains through countless generations of *in vitro* cultivation, substantially limiting its pathogenetic potential [20]. Of note, the ESX-1 system is a complex structure comprised of several proteins, including Culture Filtrate Protein 10 (CFP-10) and Early Secreted Antigenic Target pf 6 kDa (ESAT-6) [21]. Apart from preventing intracellular killing of bacilli through hindering the fusion of phagosomes with lysosomes, this also triggers recruitment of a range of immune cells and possibly also triggers migration of Mtb-infected alveolar macrophages away from the primary alveolus across the respiratory epithelium to the pulmonary interstitium [10,21,22]. Bacilli may also translocate to the interstitium through infection of the epithelial cells [1,23]. The transepithelial migration also allows the spread of bacilli from tissue resident macrophages to migratory macrophages and neutrophils, which may be less resistant to intracellular killing and permit intracellular replication [19,24].

Infected dendritic cells and monocytes migrate to draining lymph nodes to present Mtb antigens to CD4+ T lymphocytes, leading to their activation and proliferation as well as contribution to a Th1-response [1]. This mechanism triggers an adaptive immune response aiming at clearing the infection, but may also provide a route for bacilli to escape the site of inoculation [19]. Priming of T cells lead to the recruitment of T and B cells to the site of infection, which may lead to the sterilization of the infection or, the encapsulation of infected cells in a granuloma. The granuloma is the pathologic hallmark of mycobacterial disease and possesses the dual functionality of imprisoning the pathogen in a mechanical and immunological cage while still providing a protected environment in which bacilli may hibernate and remain viable for decades, as an infectious reservoir for the individual as well as the community [1,25–28].

Spectrum of tuberculosis infection

Following exposure infectious Mtb bacilli, containment of viable bacilli in a granuloma is one among several potential courses. In most instances, bacilli are killed at an early stage by the innate immune system with or without involvement of players of the adaptive immune system. If the adaptive immune response is involved, CD4+ T cells are primed to recognize Mtb antigens and form an immunological memory which can be assessed at a later stage using immunological tests such as the tuberculin skin test (TST) or interferon-γ release assays (IGRAs) [28].

Surviving bacilli may be contained in granulomas, in physical as well as immunological imprisonment. This may occur at the primary site of infection, but in parallel, infected macrophages and other antigen presenting cells may translocate lymphatically or haematogenously (especially in immunosuppressed individuals) to regional lymph nodes or distant tissues, which may give rise to persistent controlled infection either intracellularly or within granulomas [28]. Recruitment of primed CD4+ and CD8+ T cells leads to localized inflammatory responses at these sites, striving to achieve immune control and sterilization at the primary site as well as any metastasized foci [7]. Depending on the efficacy, the infection may either be sterilized, progress to clinically overt disease, or be controlled in an asymptomatic condition where bacilli with zero or limited reproductive activity may hibernate. The latter state has until recently been known as latent TB infection (LTBI), but owing to the variability in bacillary burden and activity within this entity as elaborated below, is currently labelled simply TB infection. Failing to achieve sterilization and immunological control of the infection leads to active TB, or with current terminology, TB disease.

Increasing bacillary burden triggers increasing inflammatory responses leading to signs and symptoms from the affected tissues as well as systemic symptoms, as determined by the interaction between the host immune system and the invading pathogen. Consequently, clinical manifestations may be highly heterogenous.

Historically, LTBI and active TB were considered to be two distinct entities, and as discussed below, more delicate subclassification may still be challenging in clinical practice. However, studies from the past decades have indicated that the dichotomous view was indeed an oversimplification. Instead, tuberculosis may be better represented by a continuous spectre ranging from a completely sterilized infection through a true latent state, to varying degrees of active bacillary replication or metabolism, and with interaction with the host immune system causing clinical signs and symptoms ranging from none to fulminant disease (Figure 2) [29].

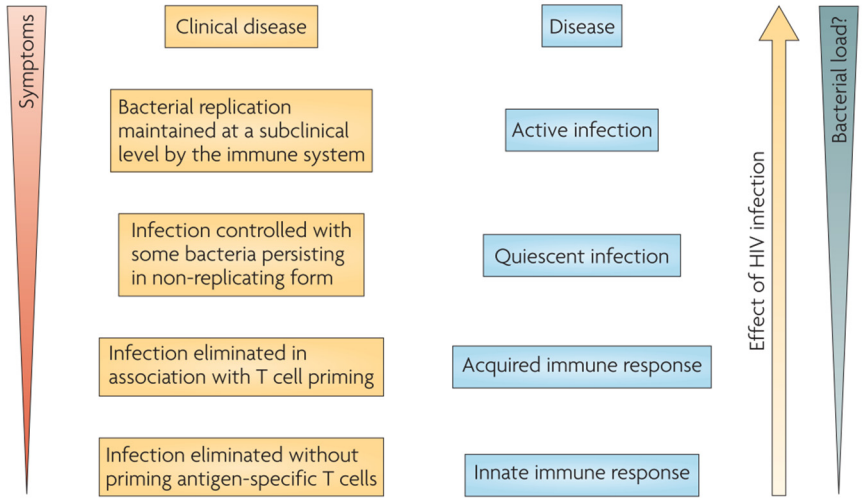

Figure 2. The spectrum of TB infection with respect to immune competence and symptom intensity. Reproduced with permission. Copyright 2009 Springer Nature. [29]

Importantly, the distinction between TB infection and TB disease may not always be sharp, and individuals considered to have TB infection may have features similar to that seen in individuals with TB disease. For example, imaging studies using 18-fluorodeoxyglucose positron emission tomography (PET-CT) have indicated that individuals with TB infection may have visible laesions with similar characteristics and metabolic profile as can be seen in individuals with active TB disease [30]. Similarly, in a subset of TB contacts without radiological, microbiological or clinical evidence of TB disease, metabolic uptake may be detected in minimal lung infiltrates as well as draining regional lymph nodes [31]. Furthermore, non-human primate models of LTBI have revealed a range of granuloma phenotypes including the caseating granuloma to non-necrotic or fibrotic granulomas with or without mineralization [29], highlighting that the border between latent and active TB may not be sharp and obvious.

Modern terminology of tuberculosis infection

For clinical purposes, immunological evidence of TB infection in absence of signs of disease has been referred to as LTBI, a term originally proposed in 1909 [32]. Acknowledging that this is indeed an oversimplification, WHO has adopted a modern terminology to acknowledge infection with *Mycobacterium tuberculosis* as a continuous spectre rather than a binary distribution of latent and active TB, a revised terminology has been adopted; “TB infection” to mean the concept of immunological evidence of infection in absence of clinical, radiological or microbiological signs of disease, as opposed to the term “TB disease” [18,33]. A

further subclassification of the spectre of tuberculosis has been proposed to include "incipient TB" to mean infection with viable bacilli with a high likelihood of progression to disease in the absence of prophylactic therapy but which has not yet incited development of any signs of disease, and "subclinical TB" to mean infection causing microbiological or radiological evidence of disease but not disease symptoms (Figure 3) [34].

Apart from revised terminology, the recognition of tuberculosis as a continuous spectre also puts emphasis on the progression or regression along the axis of bacillary burden and activity and local and systemic inflammatory response. This also may complicate the clinical decision-making around asymptomatic cases with a positive IGRA test, especially pertaining to contagiousness and whether single drug prophylaxis aimed at TB infection rather is sufficient to prevent disease [34].

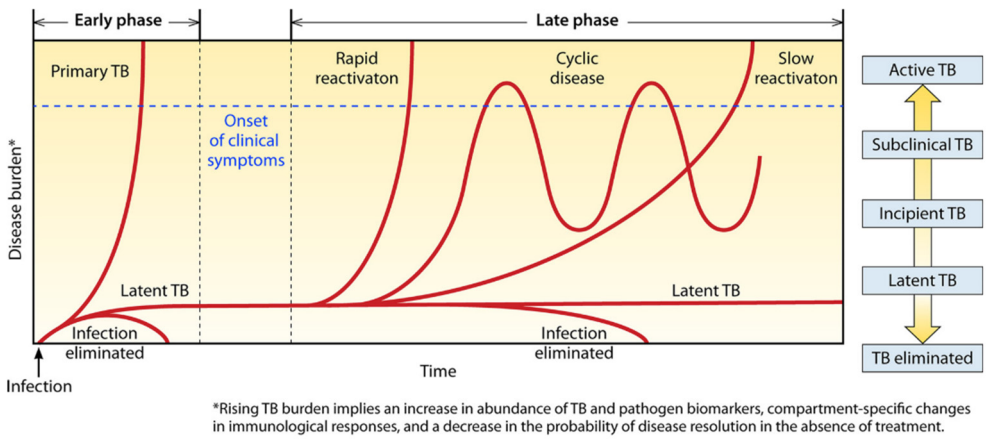

Figure 3. Spectre and dynamics of TB infection and disease. Reproduced with permission. Copyright 2018 American Society for Microbiology. [34]

Clinical forms of TB disease

Tuberculosis, being transmitted through the respiratory route, most frequently affects the lungs, but differences in factors such as occurrence and distribution of metastatic spread, host immune competence and other comorbid conditions, creates a myriad of possible presentations [7]. Tuberculosis can affect virtually any organ and tissue of the body, with host immune competence perhaps the most significant determinant of pulmonary containment, but the symptoms are also largely determined by the host capacity to mount an inflammatory response in the affected tissues. Consequently, the clinical picture is often ambiguous, especially with extrapulmonary TB or TB in immunosuppressed individuals.

Even pulmonary disease comes in a range of clinical forms. Primary pulmonary TB, which is also commonly known as childhood TB, is considered to be a direct continuation of the primary infection into active disease without any substantial delay. Due to the anatomical circumstances, inhaled contagious aerosol is prone to deposit contagious particles in the midlung zones, and this is also where the infection usually starts. It is usually associated with hilar lymphadenopathy but rarely mineralization of the granulomas. The immature host immune system typically does not instigate adult type encapsulation with necrosis and liquefaction, and there is rarely any breakthrough of contagious material to the bronchi. Tissue destruction may therefore be more limited, and studies from the pre-chemotherapy era revealed that this disease manifestation often is self-limiting. Due to the functionality of the immune system, concomitant haematogenous and lymphatic spread is common and may lead to extrapulmonary disease, most notably miliary or meningeal TB [7,35,36].

The most common type of pulmonary tuberculosis is the adult or post-primary type, which most often starts with a local inflammation in the apical upper lobes. This is typically not the site of the primary infection, but a secondary focus reached through haematogenous spread in temporary connection to the primary infection. The predilection for this specific location has been debated, proposed mechanisms include that (1) the abundance of oxygen in this part of the lung to favour the aerobic mycobacteria, and (2) that the minimal respiratory motion obstructs the lymphatic flow, therefore also the antigen presentation and the local hypersensitivity reaction [7]. Increasing bacillary burden triggers the recruitment of immune cells which apart from assisting intracellular killing also attempts to encapsulate the infection. With sufficient size, this leads to central hypoxia in the infectious hearth, necrosis, caseation and liquefaction of cells, exudate and bacilli. With break-through to the bronchial tree, leaving a radiologically characteristic cavity, the highly contagious sputum and aerosol may transmit the infection both to other parts of the lungs and respiratory tract, and to the surrounding air. [7,25,27,37]

Early stages of pulmonary tuberculosis may be asymptomatic, but with increasing disease burden and inflammatory response, classic systemic symptoms including low grade fever, weight loss, fatigue and nocturnal sweating ensue, as well as a cough [1,7]. With progressive disease, haemoptysis may ensue as a consequence of bronchial inflammation or bronchocavitary communication. Chest pain may also arise from subpleural infection with secondary inflammation of the parietal pleura, but pleural infection with effusion may also be the primary manifestation if a subpleural hearth breaks through to the pleural cavity, with or without simultaneous pulmonary involvement.

Any organ may be affected by TB, but although lympho-haematogenous spread would be expected to expose bodily tissues to reasonably similar degrees, certain organs and tissues are considerably more prone to disease development, especially vertebrae and long bone epiphyses, kidneys, lymph nodes and meninges (as well as

dorsoapical lung) [7]. Important clinical manifestations are pleural and lymph node TB, which are relatively common, as well as pericardial TB, TB of the central nervous system as miliary TB. Miliary TB is a severe form of disseminated TB, characterized by a dense seeding of <2 mm intraparenchymal lesions detected in radiological or histopathological investigations, and is the result of lympho-haematogenous dissemination from a pulmonary or extrapulmonary focus. It typically involves several organs, and is most often found in liver, spleen and lungs. Extrapulmonary TB and especially disseminated and miliary TB are closely associated with immunosuppression, including a relative dominance of Tregs over CD4+ and CD8+ effector T cells, and is associated mostly with HIV/AIDS but also malnutrition, congenital and neonatal acquisition of TB, as well as iatrogenic immunosuppression [38,39]. Whether pregnancy, which is also associated with immunomodulation including a relative abundance of Tregs, also increases the risk of extrapulmonary disease is has been subject to controversy [38,40–42].

Diagnostic tools

Diagnosing TB disease

Timely diagnosis of TB disease allows for prompt initiation of treatment, which reduces mortality and morbidity as well as transmission. Under optimal conditions, individuals with signs or symptoms suggestive of active TB, such as low grade fever, cough, weight loss and nocturnal sweating, should be undergo a careful investigation including a thorough physical examination as well as radiological and microbiological investigations [43,44].

A positive mycobacterial culture for Mtb is considered as reliable proof of TB disease and can be performed on any bodily fluid- or tissue specimens; it also allows for phenotypic resistance testing to antimycobacterial agents, which is increasingly important with the global rise of multidrug resistant TB. However, TB culture comes with a number of limitations, which are of special concern in high-burden resource constrained settings [45]. Mtb is classified as a biosafety level 3 (BSL3) agent and culture must be performed in an appropriate BSL3 laboratory with negative air pressure and other rigorous safety precautions to protect personnel from contagion [46]. It is also technically challenging and labour intense, and requires skilled staff as well as expensive equipment. Mycobacterial culture is considerably slower than that of most other types of bacteria, and requires several weeks without growth to be considered negative [47,48].

Rapid molecular tests such as the Xpert® MTB/RIF (Cepheid) require in comparison limited laboratory resources and experience, and provide rapid (within a few hours) results as to whether Mtb DNA is present in the sample. The sensitivity

is satisfactory, around 80-(90)% of that of culture (depending on which generation and which clinical setting), and the assay may also detect mutations conferring resistance to rifampicin [43,49]. It has been increasingly used in high-burden settings including sub-Saharan Africa during the past decade [43]. It cannot, however, distinguish DNA from living and dead Mtb bacilli, limiting its use as marker for treatment efficacy.

Microscopy on respiratory specimens is technically simple to perform in terms of laboratory equipment and still widely used, but its use for making the diagnosis has largely been overtaken by rapid molecular tests and culture. The sensitivity is indeed limited for traditional Ziehl-Neelsen as well as (to a lesser extent) for fluorescence microscopy [44,50], but the microscopical detection of acid-fast bacilli in a respiratory sample indicates that the patient is highly contagious ("smear-positive TB"), which remains of clinical value.

All microbiological tests rely on the production of adequate samples from the site of infection. Spontaneously expectorated sputum is the most commonly used material, but frequently patients may not be able to produce representative sputum samples, especially children. There are a number of innovative approaches to retrieve representative respiratory samples in such occasions, such as sputum induction through inhalation of nebulized saline, gastric lavage, "string test" – swallowing one end of a string which can then be retracted, and stool (DNA of acid-resistant mycobacteria in swallowed sputum may retrieved in faecal matter) [51–56]. In extrapulmonary disease, invasive methods are frequently required to obtain a sample of the affected tissue, which may be associated with discomfort, a risk of complications and additional costs, and the diagnostic yield is often lower. Furthermore, clinical diagnosis may be especially challenging in extrapulmonary disease in the context of immunosuppressive comorbid conditions, and there may be a disproportionate diagnostic failure of extrapulmonary TB in low-resource settings where culture usually is not available.

Diagnosing TB infection

In animal models, autopsy studies and other exceptional circumstances, microbial isolation as proof of TB infection may be possible. All currently available methods to diagnose TB infection in a clinical context rely on measuring the host immunological memory; measurement of the dermal reaction to Koch's tuberculin was proposed by von Pirquet more than a century ago [32], the first QFT version was approved by the US Food and Drug Agency in 2001 [57,58]. In modern QFT assays, the myriad of mycobacterial antigens in the tuberculin mixture have been replaced with a limited number of antigens specific for the *Mycobacterium tuberculosis* complex except for *Mycobacterium bovis BCG*, reducing false-positive reactions due to infection with NTM or previous BCG vaccination.

Lacking a method of microbial or other direct proof of TB infection, no “gold standard” exists for diagnosis of TB infection to which such assays can be compared. Furthermore, neither IGRAs nor TST can discriminate presence of viable bacilli from immunological memory of a sterilized infection, or to discriminate between TB disease and TB infection. The sensitivity is also limited by conditions affecting the CD4 lymphocyte count or function, most notably poorly controlled HIV infection [59–61]. Another important pitfall of IGRAs is the limited sensitivity in TB disease; Mtb-primed CD4+ and CD8+ T cells may be exhausted during TB disease with reduced capacity for responsive interferon-γ secretion in the assay [62–65].

The practical purpose if immune based tests are the prediction or development of TB disease. Among all people infected with Mtb, only a small minority will develop TB disease, although this differs considerably depending on the clinical context (Figure 4.) [66]. It has been described that systemic inflammatory response and transcriptomic profile may be different between individuals with TB infection, some of which resemble patterns that are seen in TB disease and which also forego progression to TB disease [67,68]. Attempts are ongoing to develop tests with capacity to better predict incident TB based on blood transcriptomic signatures [68–72].

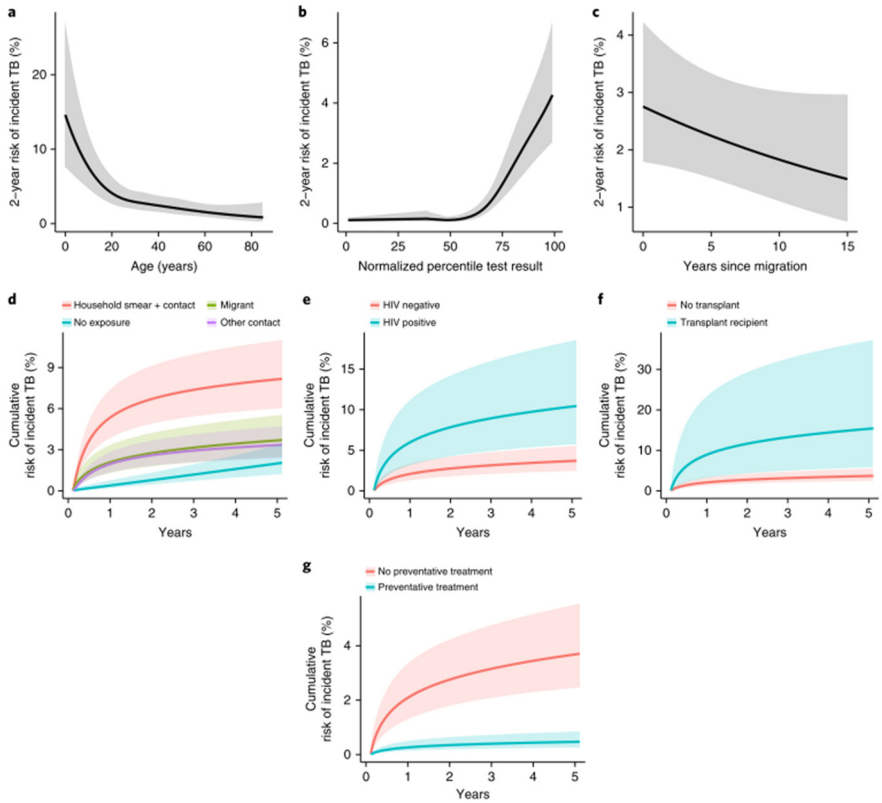

Figure 4. Prediction of incident TB. Reproduced with permission. Copyright 2020, The Authors, under exclusive licence to Springer Nature America, Inc. [66]

QFT-Plus is an elaboration of the earlier QuantiFERON-TB Gold In Tube (QFT-GIT), designed for improved sensitivity in immunosuppressed individuals particularly with low numbers or function of CD4+ T cells, as well as individuals with recent infection or TB disease [73]. Compared to earlier versions, it is comprised of two antigen formulations; TB1, containing whole CFP-10 and ESAT-6 proteins, and TB2 containing whole proteins as well as peptide derivatives of CFP-10 and ESAT-6. The shorter peptides in the TB2 formulation were designed to stimulate sensitized CD8+ T cells to contribute to the interferon-γ release by CD4+ T cells [73,74].

Considering the physiological immune modulations occurring during pregnancy, there is also a concern that pregnancy may influence the sensitivity of immune based tests for TB infection [75–82].

Current global epidemiology

In the year 2019, around 10 million new cases of TB disease occur worldwide, causing 1.4 million deaths, making TB the top infectious killer globally until recently [83]. Furthermore, 1.7 billion are estimated to have acquired the infection, acting as a reservoir for disease [28]. History has seen top incidence rates in connection to extreme poverty, overcrowding, famine, and war, and while living standards improved in Europe at the end of the 19th century, cases dropped gradually long before effective treatment became available. In modern days, TB is still a disease of poverty, and the highest incidence rates occur in low- and middle-income countries, and especially vulnerable populations within these countries [1,84]. Since the 90's, the WHO has considered TB as a global priority, leading to a series of ambitious strategies to improve care and disease burden [85].

In 2013, the End TB Strategy was launched by the WHO, and included aiming for a 50% decline in TB incidence and a 75% reduction in TB deaths by 2025 compared to 2015, through an ambitious package of improved coverage of microbiological testing and systematic screening of contacts and other high risk groups; universal access to treatment, integration of TB care with that of HIV and comorbidities, and prophylactic measures as such as provision of vaccination and preventive treatment [86]. Until 2019, the incidence had fallen 9% in total since 2015, far less than targeted by the End TB Strategy [83].

This sinister title of "leading infectious killer" has since been overtaken by the present Covid-19 pandemic, which to date (October 2022) has caused more than 6.5 million deaths since the first reports in 2019 [87]. At the same time, TB case notifications have dropped by 18 percent in the past year [88]. Although restrictions and hygiene recommendations may have had some effect on the transmission of TB, another plausible explanation may lie in a diagnostic deficit owing to overburdened medical facilities as well as that patients and caregivers may have been biased to misinterpret respiratory symptoms as Covid-19 [89]. The Covid-19 pandemic has also led to a surge in the use of systemic corticosteroids, particularly in the elderly and comorbid individuals [90,91]. The extent to which this practice may have influenced the risk of development of TB disease is not yet fully understood on a population level, but examples of disseminated TB have been reported in connection to Covid-19 and anti-inflammatory therapy [89,91].

Sex differences in TB

TB notification rates are consistently higher in men compared to women [83]. The reason for this difference remains debated; biological differences as well as patterns of predisposing conditions, socio-cultural factors affecting exposure and care

seeking behaviour may be considered. For example, male mice have been shown to be more susceptible to infection with Mtb and other mycobacteria, and the influence of sex hormones has been demonstrated experimentally through castration and cis- or trans- sex hormone replacement [92–95]. In another mouse model of pulmonary TB model, male mice exhibited lower pulmonary and systemic inflammatory responses and despite higher bacillary burden, but this could be reversed with orchidectomy [96]. A number of mechanisms have been proposed, but the extent to which these and other similar observations are relevant to human disease is has yet to be established [97,98].

Horton *et al.* demonstrated in a systematic review that on average, men are disproportionately burdened by TB disease, and that despite this, are often disadvantaged in accessing TB care as indicated by prevalence to notification ratio 2.6 *vs.* 1.6 for men and women, respectively [99]. However, both prevalence ratios and sex disaggregated prevalence to notification ratios differ substantially between different settings (Figure 5.) [100].

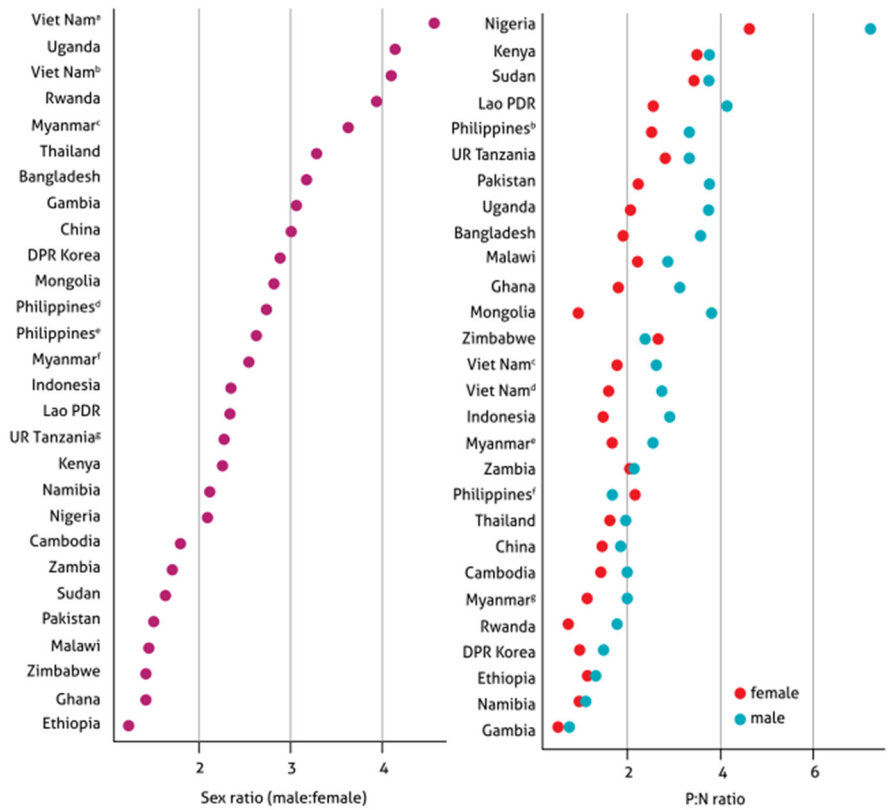

Figure 5. Sex differences in prevalence (left) and prevalence to notification ratios (right) of bacteriologically confirmed TB in selected countries 2007-2018. Adapted from the WHO Global Tuberculosis Report 2019 [100].

The prevalence ratio also differs by age, with male predominance increasing with age [99,101,102]. Compared to other regions, sub-Saharan Africa has exhibited higher numbers of TB in young women, sometimes referred to as the feminization of the TB epidemic [102]. The HIV epidemic of Africa has disproportionately affected women, and a female predominance of TB has been observed in settings with high HIV prevalence (mainly before the availability of effective ART) [99,102]. Women of sub-Saharan Africa have a high fertility rate compared to other parts of the world and spend significant parts of their lives as pregnant or postpartum, periods which have recently been associated with increased TB incidence [103,104] (Figure 6, based on data from the World Bank [105]).

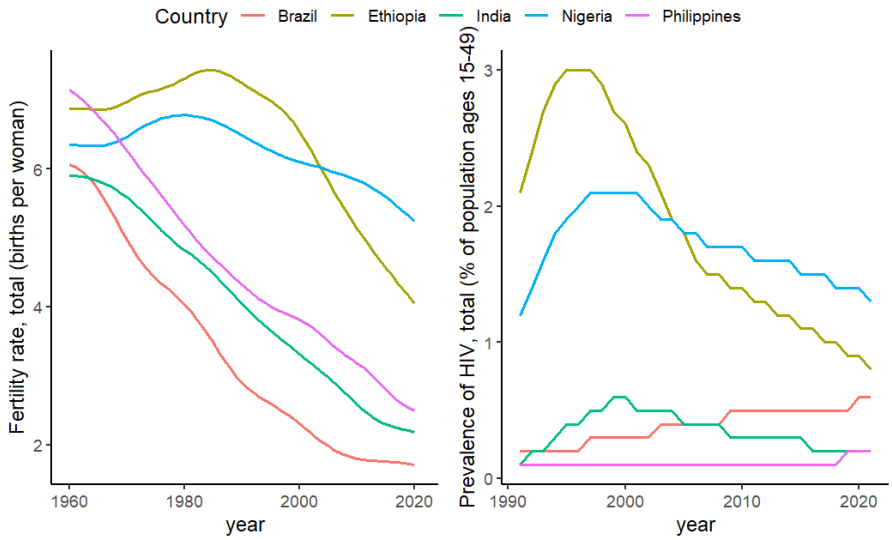

Figure 6. Fertility rates and adult HIV prevalence in selected TB endemic countries. The figure was based on data extracted from the World Bank [105].

Tuberculosis infection and pregnancy

A brief history of an ancient controversy

For as long as medicine existed, several questions pertaining to tuberculosis in the context of pregnancy have been subject to controversy and debate. W. Brooks [106] and D. Snider [107] have summarized how Hippocrates and Galen observed symptom improvement of tuberculosis during pregnancy. This led to them advocating pregnancy as a treatment for tuberculosis, which was generally accepted until the mid-nineteenth century. Later physicians speculated that the uterine

expansion could lead to compression and collapse of pulmonary cavities, similarly to what was pursued through artificial pneumothorax and thoracoplasty.

The beneficial effect on tuberculosis was however challenged by case series in the mid- and late nineteenth century showing deleterious effects of pregnancy on the course of tuberculosis, particularly aggravation or reactivation following delivery. Consequently, it was recommended that pregnancies in women suffering from tuberculosis should be terminated, which was frequently practiced in Europe. With modern perspective this might seem excessive, but some contemporary case series reported maternal or neonatal mortality up to 30-40%. In fact, tuberculosis was a common medical indication for abortion in Sweden in the early 1900's. In 1953, E. Hedvall reviewed available evidence from the pre-antibiotic era and concluded that pregnancy did not exert positive or negative effects on tuberculosis and that the offspring usually had a successful outcome [108]. However, Hedvall and others noted frequent reactivation of "inactive" tuberculosis during the postpartum period [107]. As increasingly effective chemotherapeutic treatment regimens revolutionized the prospect of a successful treatment outcome, therapeutic abortion was abandoned, and pregnant women with tuberculosis were managed largely similar to other adults.

The immune system in pregnancy

Pregnancy is indeed a vulnerable occasion in the human physiology. Of special importance to this work is the required immune modulations and their consequences in terms of susceptibility to infectious agents. Tissues of the developing placenta and foetus exhibit antigens that may be recognized as foreign to the maternal immune system, the metaphor "natural allograft" has been used to highlight the risk for T cell-mediated rejection [109]. Profound but transient immune modulations are carefully orchestrated throughout pregnancy to promote tolerance to these tissues, occurring systemically and at the maternofoetal interface. Importantly, T regulatory cells (Tregs) increase from early pregnancy, and promote immune tolerance through suppression of circulating CD4+- and CD8+ T lymphocytes and NK cells (Figure 7) [110–113]. The Tregs also control the immune balance through stimulating production of anti-inflammatory cytokines, such as IL-10 and TGF-β. These and other mechanisms reduce the uterine inflammatory activity [114,115] to protect the delicate implantation process [116,117].

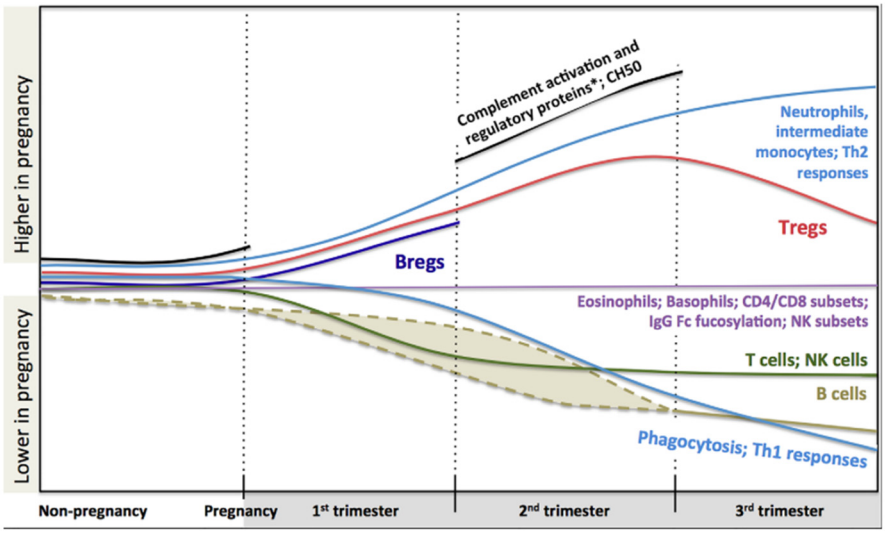

Figure 7. Physiologic dynamics of components of the immune system during a normal pregnancy. Copyright (CC BY), 2020 the authors. [112]

An unwanted consequence is the increased susceptibility to infectious diseases, including a number of viral, bacterial and protozoal diseases [112,118]. Principally, infectious agents may cause pregnancy complications through direct invasion of foetus (such as the TORCH [toxoplasma, rubella, cytomegalovirus and herpesvirus] group) or placenta (i.e. *Plasmodium falciparum)*, or through indirect mechanisms, including interference with the immune balance [119–122].

The vascularization of the developing placenta is a delicate process, throughout the course of pregnancy. The systemic inflammatory milieu is an important determinant for the balance of pro- and antiangiogenic signalling which is required for a successful placental development. Inadequate placental development may lead to foetal haemodynamic changes, foetal oxidative stress and complications such as preeclampsia, stillbirth and growth restriction [122,123]. Both malaria and HIV infection have been associated with altered angiogenic balance and placental morphologic changes [121,124–126], and are known to contribute to pregnancy complications [125,127,128]. It has been proposed that similar mechanisms may be involved in adverse pregnancy outcomes in women with active TB [129].

Epidemiology and clinical manifestations of tuberculosis in connection to pregnancy

Based on distributions of sex, age groups, fertility rates and TB incidence in individual countries, tuberculosis was estimated to coincide with pregnancy in

around 200'000 women annually around the globe and in 3.6 cases per 1000 pregnancies in the African region in a mathematical modelling study [130]. This estimation did not take into account any influence of pregnancy on the incidence of active tuberculosis, or the disproportionate burden of HIV infection among reproductive women in sub-Saharan Africa. Considering the immune modulations of pregnancy, it would be reasonable to hypothesize that the immune control of TB infection would be suppressed during pregnancy. Numerous studies in the era of effective chemotherapy with different study designs and -populations have yielded somewhat heterogenous results [131–136]. There has been some concern about residual bias [137,138]. In the past decade, however, two population-based retrospective cohorts in UK [104] Sweden [103] have provided support for this hypothesis, reporting moderately increased incidence rate ratios 1.6 and 1.9 during the postpartum period, less so during the pregnancy itself (IRR 1.03 and 1.4), respectively. The predilection for TB disease presentation in the postpartum rather than gestational period has been proposed to stem from postpartum normalization of the immune modulations, i.e. an unmasking phenomenon mechanistically resembling the immune reconstitution syndrome occurring after ART initiation in HIV positive individuals with unrecognized TB disease [139,140].

Whether the excess rate of diagnosis in these women represent reactivation or *de novo*-infection during pregnancy is not known but considering that most have migrated from TB-endemic countries to Europe before pregnancy, it seems likely that the bulk of TB-exposure in the study populations occurred before pregnancy.

Characteristics of maternal TB disease

Tuberculosis is among the leading causes of death among women of fertile age and non-obstetric maternal deaths [141–144]. Several studies from high-burden settings report that TB in connection to pregnancy frequently is associated with delayed maternal diagnosis of TB [132,144,145]. Diagnostic delay of TB in pregnancy can result from symptoms being masked by or confused with physiological changes during pregnancy. Furthermore, symptoms may be altered or attenuated due to pregnancy-related immune modulations, leading to mild or atypical presentations, especially in HIV-coinfected women [132,146–148]. It has been subject to debate whether pregnancy may predispose to extrapulmonary TB, similar to other states of immune modulation. Some studies have found similar distributions, while a retrospective nation-wide Danish study found increased risk of extrapulmonary disease [42]. A literature review of cases with early (within one month) postpartum unmasking revealed high proportions of extrapulmonary TB including TB meningitis and miliary TB [149]. In HIV-positive women, postpartum TB disease has frequently been reported [150,151], but large systematic studies are lacking from high-burden settings.

Consequences of TB in pregnant women

The effect of TB disease on pregnancy is modulated by a range of circumstances and co-prevalent conditions, such as clinical manifestation, disease severity and gestational age at diagnosis, as well as HIV coinfection (importantly, access to effective antiretroviral therapy) and other comorbidities. Tuberculosis in pregnancy is a rare phenomenon in the industrialized world, and predominantly affecting vulnerable populations, most importantly recent migrants. Consequently, most studies are retrospective or register-based, and require consideration of confounding and other potential sources of bias. Studies from high-burden settings, on the other hand, often include subjects with late diagnosis and advanced disease, and (often recently diagnosed) HIV-co-infection.

Tuberculosis and maternal outcomes

Most women diagnosed with TB disease in connection to pregnancy have a successful vital and pregnancy outcome with access to effective therapy [148]. However, maternal mortality and other unfavourable outcomes have been reported frequently in HIV positive women [136,151,152] and in the context of drug-resistant TB [153,154]. There is also some evidence to suggest that the risk of drug-induced hepatotoxicity, sometimes fatal, may be higher in pregnant women [155]. In autopsy-series from endemic countries, TB has often been causing or contributing to maternal mortality, of which the vast majority has been recorded in HIV-coinfected women with low ART uptake (some from the pre-ART era) and low CD4-count [141,152,156,157]. Significant maternal mortality has also been reported in case-series from endemic countries, especially with advanced disease at diagnosis or with HIV-coinfection [145,158–160]. Retrospective cohort or case-control studies however have been scarce, small and heterogenous. A 2016 systematic review could only include three studies with 374 subjects and four maternal deaths, all in women with TB, not reaching statistical significance [161]. The burden of maternal morbidity, as reflected by antenatal hospital admission, anaemia and a range of other conditions, was however frequently reported to be increased in pregnancies affected by TB [161]. Two subsequent large register-based American studies later found six- and 37-fold increased odds of maternal mortality in women with active TB [142,143].

Vertical transmission of tuberculosis

Congenital tuberculosis infection is a rare but severe complication of maternal tuberculosis. The foetus can be infected either through haematogenous spread through the umbilical vein, typically causing hepatic primary infection, or through ingestion or inhalation of infected amniotic fluid with gastrointestinal or pulmonary infection [162,163].

In a series of 170 cases with congenital TB in the antibiotic era, more than half of the mothers had miliary TB and 45% had proof of genital or placental involvement

[164]. Still, many had been asymptomatic throughout pregnancy and >70% were diagnosed after delivery, and in many cases the child was the first to receive diagnosis. Children were often born prematurely (41%) and usually had atypical presentations and were often initially diagnosed with bacterial pneumonia, sepsis or meningitis. Almost ¼ died before diagnosis and initiating TB treatment, while almost 80% of those initiating treatment survived.

It is generally considered to be rare, as only a few hundred cases have been reported throughout the literature. However, apart from the challenges of reaching a bacteriological diagnosis in a neonate, criteria to ascertain *in utero* transmission have been difficult to fulfil in clinical routine – e.g. Beitzki's criteria from 1935 (presentation in the first few days of life, hepatic primary complex, or separation from the mother immediately after birth) [163]. Cantwell's revised criteria from 1994 also accepted maternal genital or placental involvement as proof of *in utero* infection, attempting at increasing the sensitivity [165]. Urogenital involvement is not uncommon in extrapulmonary TB or even concomitant with pulmonary TB and is often subclinical [166,167]. It is also found as a cause of infertility [168,169]. Furthermore, most perinatal deaths in high-burden settings are rarely subjected to autopsy [170], let alone mycobacterial investigation. It seems possible that congenital TB may be more common that suggested by the scarce reports in the literature.

Even in the absence of hematogenous or amniotic transmission of *Mtb*, women with pulmonary TB are at considerable risk of transmitting the infection to their neonates after delivery, which is in similarity to congenitally acquired infection, associated with nonspecific presentation and significant diagnostic delay [35,41,171].

Pregnancy complications in women with tuberculosis

Even in the absence of direct mycobacterial invasion of foetal or placental tissue, maternal tuberculosis has been associated with a range of pregnancy complications, where the causal mechanisms are less obvious. The most consistent findings across studies include preterm delivery and low birthweight [159,160,172–179]. Miscarriage, stillbirth, neonatal deaths, preeclampsia and placental abruption have often been reported in connection to maternal TB, mostly in complicating circumstances such as HIV-coinfection, drug-resistant or extrapulmonary TB [143,145,153,158,174,177,178,180–187]. The pathophysiological mechanisms (especially the relative importance of invasion of uterine, placental or foetal tissues as compared to systemic inflammatory activity or other indirect effects) underlying these complications in the context of TB has never been systematically investigated.

TB infection and pregnancy outcome – glimpses into a hitherto unexplored chapter

Studies from Sweden and other low-burden settings have indicated a disproportionate burden of stillbirth and other severe pregnancy complications in immigrants [188,189]. Immigrants may be at increased risk of adverse pregnancy outcome through a range of possible mechanisms. Socioeconomic conditions have been suggested [188,190], but also infectious conditions may be worthwhile consideration [191,192]. TB deserves special attention in this regard, as it is one of the leading infectious causes of death globally [88], is known to cause a range of pregnancy complications, and increased TB incidence in the postpartum period indicate that immune control over a TB infection may be negatively influenced by the immune modulations of pregnancy [103,104]. Furthermore, immigrants account for 83% of Swedish TB notifications [193]. TB infection is currently considered to be a dynamic condition represented by a spectre of bacillary burden and host-pathogen interaction, part of which is on the border of creating the signs and symptoms that are required for the definition of TB disease [18,29,34]. The immune modulations of a normal pregnancy are carefully orchestrated and may be disturbed by infectious and inflammatory condition, leading to pregnancy complications [122]. It has been described that TB infection may be associated with inflammatory markers [194–199]. Still, whether TB infection exerts any effect on the risk of pregnancy complications is not known.

In this thesis work, we have explored the possible consequences of TB infection in pregnancy, both in a prospective cohort in a high-endemic setting and in a large retrospective material of immigrant women screened for TB infection during routine antenatal care in Sweden.

Aims

1. To investigate the diagnostic performance of QuantiFERON TB GOLD PLUS in pregnant women
2. To investigate the prevalence and associated characteristics of TB infection if women of fertile age in Ethiopia
3. To investigate the effects of TB infection on pregnancy outcome

Methods

The Adama TB-pregnancy cohort

Papers (I-III) are based on the Adama TB-pregnancy cohort, which was launched in 2015 from the Adama-Lund University Research station, founded in 2010 by Professor Per Björkman. Since the start, the research station has continuously hosted prospective observational studies focusing on HIV and tuberculosis from epidemiological and translational perspectives, as well as several smaller cross-sectional or register based studies. These studies have been run in close collaboration with Armauer Hansen Research Institute, Oromia Regional Health Bureau, the Adama Regional Laboratory, as well as local public hospitals and health centres.

Adama is a city of more than 300 000 inhabitants, the capital of the Oromia region and situated in central Ethiopia, a country with a rapidly growing population exceeding 100 000 000. Ethiopia is heavily burdened by TB, but has successfully been able to bring down the incidence to 132 per 100 000 person-years. During the past decades, poverty has fallen steadily, as well as fertility rates, and access to antenatal care and obstetric services as well as maternal and neonatal survival are improving.

The present cohort was started with the objective to study the interaction of TB infection and pregnancy from a series of perspectives including prevalence and characteristics of TB infection in women of reproductive age, the effect of pregnancy on the immune control over TB infection, and effects of TB infection on pregnancy outcome and longitudinal child health.

Women were enrolled prospectively at three public ANC clinics during November 2015 to February 2018, by nurses employed at the ANC clinics after specific training in the study protocol. At these sites, which also provide HIV and TB care, around 8000 women register for antenatal care annually. For enrolment, women were required to provide written informed consent for all study procedures, including tracing by telephone and follow-up of the offspring resulting from the current pregnancy. At inclusion, study participants were subjected to a detailed structured interview covering demographic, socioeconomic, medical and obstetric history, and symptoms suggestive of TB disease, as well as a structured physical examination with focus on obstetric health and signs of TB disease. They were also

subject to laboratory investigations; including HIV testing using rapid tests (with CD4+ T cell count and viral load for HIV-positive individuals) and venous blood collection for QuantiFERON TB GOLD PLUS (QFT). Participants with signs or symptoms suggestive of active TB as well as HIV-positive participants irrespective of symptoms, were asked to submit two consecutive morning sputum samples, for microbial analysis using GeneXpert MTB/RIF and mycobacterial liquid culture. Diagnosis of TB disease could also be accepted on clinical grounds if made by an experienced clinican. All participants were informed about TB and offered to be investigated as part of the study, without any cost, should such symptoms occur during the duration of the study.

Up to three subsequent study visits were performed as coinciding with routine ANC visits. At the first postnatal visit, coinciding with the routine 6-week child immunisation, data on pregnancy outcome as well as offspring health and growth parameters were collected. Study participants (mothers and offspring) are still under continuous follow-up, with specific emphasis on signs suggestive of development of active TB, but also on growth and other health parameters.

QuantiFERON TB GOLD PLUS assay

For immune based diagnosis of TB infection, QFT-Plus was utilized in accordance with the manufacturers recommendations [73]. Venous blood was collected from study participants into a lithium-heparin tube, transported to the Adama Regional Laboratory and transferred to the four respective tubes of the assay (nil, TB1, TB2 and mitogen). Following approximately 16 hours of incubation, samples were centrifuges and supernatant plasma was stored in – 20 degrees for interferon-γ ELISA which was performed in batches and interpreted using software provided by Qiagen.

Ethical considerations

Ethical permission was granted from the Ethical Review Committee, Lund Sweden, as well as the national ethical review board of the Ministry of Science and Technology, Addis Ababa, Ethiopia. Individual consent was sought from the pregnant women on behalf of themselves and their offspring. Latent TB infection or QFT were not recognized by the Ethiopian TB guidelines, and neither results of the QFT assay nor preventive therapy could therefore be provided to study participants. Still, participation in the study provided participants with opportunities for screening for TB disease at study visits and on demand, as well as detailed information about signs suggestive of TB disease irrespectively of QFT result.

The Swedish register material

Since 2014, it is recommended that pregnant women originating in TB-endemic regions are screened for TB-infection using QFT upon ANC registration in Sweden. In Paper (IV), we exploited this to investigate the effect of TB infection on pregnancy outcome. Data on QFT-results obtained through ANC TB-screening during 2014-2018 were extracted from five clinical microbiology laboratories, with a total uptake area of 4.8 million. Due to the low transmission of TB in Sweden, it was assumed that women who were QFT-reactive had been infected prior to immigration to Sweden, and that women who were QFT-negative would not become TB-infected during the study period.

Using unique Swedish Personal Identification Numbers, these results were subsequently linked to data from national registers, the Patient Register, Pregnancy Register, and the Population Register, as well as TB notification data from the Swedish Public Health Agency. This combined data was used to define pregnancies and link them to a series of pregnancy outcomes. Each participant could have one or more pregnancies during the study period, all classified regarding maternal TB-infection irrespective of during which pregnancy the QFT testing was performed. Pregnancies occurring in participants with record of active TB before the pregnancy were excluded, and pregnancies temporally connected to active TB were managed as a separate group. Pregnancies occurring more than 10 years after immigration to Sweden were also excluded. Participants with HIV infection were excluded.

Study definitions

TB infection was defined as QFT reactivity ≥0.35 IU/mL and was (for the purpose of this study) considered to remain unchanged during all pregnancies since immigration to Sweden.

Pregnancy outcomes as well as model covariates were defined based on records of the Pregnancy Register, the Patient Register, or both.

Statistical analyses

The main analyses were logistic regression for binary outcomes, and linear regression for continuous outcomes. These models included adjustment for maternal age and origin as these were *à priori* judged to be causally linked to both the risk of TB infection and pregnancy outcome, but sensitivity analyses using an expanded set of variables (mainly risk factors for pregnancy complications with no apparent causal association with TB infection) for which data was less complete were used. Models included random effects for maternal identity to adjust for the possible inflation of apparent statistical power when analysing repeated pregnancy outcomes

in the same women. Finally, quantile regression was utilized to investigate effect of TB infection on the lower quantiles of gestational age, birth weight and deviation from expected birth weight (i.e. a more pronounced effect in a subset of women with TB infection rather than a general effect). Statistical analyses were performed in R, and the lme4 and quantreg packages were used for mixed effect regression modelling and quantile regression, respectively [200–202].

Results

Paper I

The first paper included data from the first 829 pregnant women included in the cohort. Among these women, 637 (76.8%) were aged 20-29 years old, 467 (58%) had been pregnant at least once before, 547 (67%) were at the second trimester of gestation at QFT-Plus sampling. Forty-nine (5.9%) were HIV-positive, of which 46 (94%) were on ART and only 2 (6%) had CD4 count <200. One (0.1%) woman was clinically diagnosed with pleural TB in connection to the study visit.

In all, 277 (33%) were classified as QFT-Plus positive using the manufacturers recommendation (TB1- or TB2 Mtb antigen stimulated IFN-γ ≥0.35 IU/ml after nil-subtraction). Both the correlation between the IFN-γ responses elicited by the two Mtb antigen formulations (Spearman's Rho 0.89; $p<0.0001$, Figure 8), and the agreement using the standard cut-off 0.35 IU/mL ($\kappa=0.92$, $p<0.0001$), were strong.

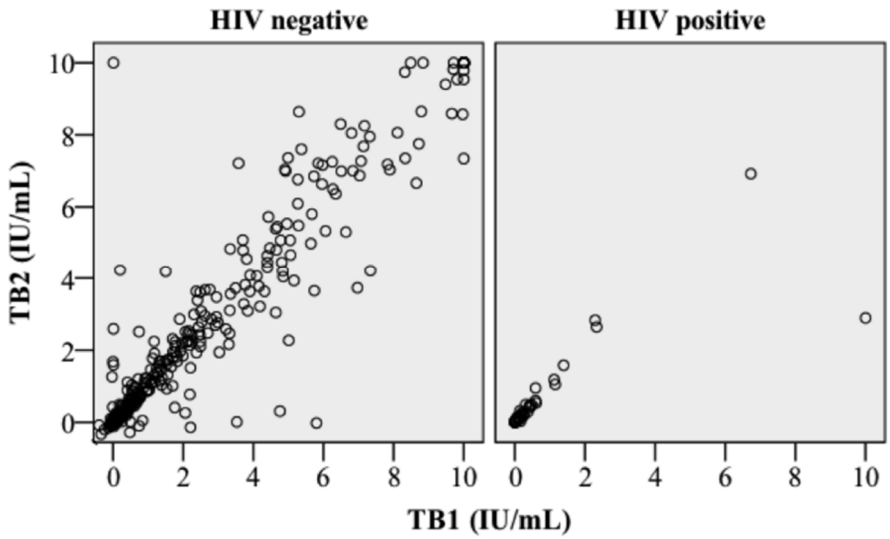

Figure 8. Correlation between interferon-γ levels elicited by TB1 and TB2 antigen formulations respectively, with stratification by HIV serostatus.

Discordant values were rare (27, 3.4%) and often associated with values around the cut-off in both antigen formulations, and with similar distribution of TB1-/TB2+ and TB1+/TB2- (12, 1.5% *vs.* 15, 1.9%, respectively). Similarly, a Bland-Altman plot could not identify any systematic differences in the distribution of interferon-γ levels elicited by the two antigen formulations in this population (Figure 9).

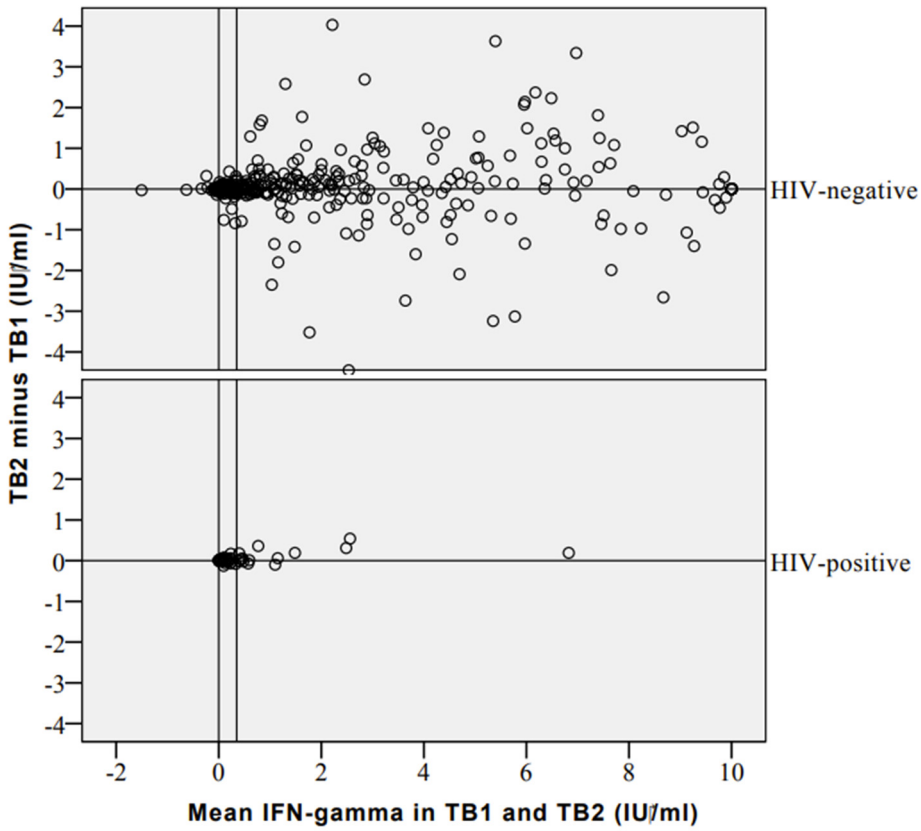

Figure 9. Bland-Altman plot divided by HIV serostatus. TB2 minus TB1 plotted against the mean of TB1 and TB2.

The distribution of values with respect to the borderline zone (0.20-0.70 IU/mL) was also investigated. In all, 90 (10.9%) has interferon-γ levels within the borderline range elicited by at least one of the antigen formulations; this proportion was significantly higher for HIV positive participants (15/49, 30.6%, *vs.* 75/780, 9.6%, respectively, $p<0.001$).

Paper II

The second study described 1834 participants of the cohort with regard to TB infection status and associated characteristics. In all, 679 (37.0%) fulfilled criteria for having acquired TB infection (TB+), of which five (0.3%) were diagnosed with active TB in connection to the current pregnancy, 80 (4.4%) reported previous treatment for active TB, and the remaining 596 (32.4%) had positive QFT-Plus result in absence of past or present active TB and were considered to have LTBI (Table 1). In univariable analysis, TB+ was associated with increasing maternal age, as well as number of previous pregnancies, level of education, number of rooms in the home and HIV infection (Table 1). In multivariable analysis, however, built in a forward selection strategy, only maternal age and HIV infection remained associated with TB+ (Table 2). However, further exploration of these associations revealed that the association between age and TB+ was confined to the HIV negative group, the proportion of TB+ was similar across age categories in the HIV positive group (Table 2, Figure 10). In HIV negative women, the proportion of TB+ increased from 19.7% for women aged <20 years to 45.6% in those aged >26, and an annual rate of infection of 2.1% was derived from the regression models.

Disaggregation of the TB+ category revealed that the excess proportion of TB+ in the HIV positive groups was explained by higher burden of active TB; (previous active TB: 33/170 [19.4%] *vs.* 47/1664 [2.8%]; current active TB: 3 [1.8%] *vs.* 2 [0.1%]).

Table 1. Characteristics of pregnant women stratified by TB infection.

	Total		TB-		TB+		p
	N	%	N	%	N	%	
Total	1834		1155		679		
Previous active TB	80	4.4	-	-	80	11.8	
Current active TB	5	0.27	-	-	5	0.7	
Age (years)							<0.001
≤ 20	350	19.1	255	22.1	95	14	
21-25	725	39.5	490	42.4	235	34.7	
26-30	610	33.3	334	28.9	276	40.7	
31-35	114	6.2	57	4.9	57	8.4	
>35	34	1.9	19	1.6	15	2.2	
NA	1	0.1					
Haemoglobin (g/DL, [Mean and SD])	12.37	1.2	12.39	1.22	12.35	1.17	0.61
Marital status							0.16[a]
Married	1757	95.8	1102	95.6	655	96.6	
Single	56	3.1	35	3	21	3.1	
Divorced	15	0.8	13	1.1	2	0.3	
Widowed	3	0.2	3	0.3	0	0	
NA	3	0.2					
Education							0.017
Higher education	201	11	117	10.1	84	12.4	
6-12 grades	1038	56.6	669	57.9	369	54.4	
<6 grades	360	19.6	239	20.7	121	17.8	

	Total		TB-		TB+		p
Illiterate	234	12.8	130	11.3	104	15.3	
NA	1	0.1					
Family size							
<4	1348	73.5	883	77.1	465	68.7	<0.001
4-6	401	21.9	221	19.3	180	26.6	
>6	74	4	42	3.7	32	4.7	
NA	11	0.6					
One room	995	54.3	658	57.2	337	49.9	0.003
NA	8	0.4					
No solid fuel combustion for cooking	441	24	264	23	177	26.3	0.12
NA	12	0.7					
No electricity	76	4.1	47	4.1	29	4.3	0.90
NA	13	0.7					
Occupation							0.044
Housewife	1165	63.5	747	64.8	418	61.8	
Employed	234	12.8	141	12.2	93	13.8	
Self-employed	148	8.1	97	8.4	51	7.5	
Daily labourer	219	11.9	122	10.6	97	14.3	
Student	39	2.1	31	2.7	8	1.2	
Unemployed	23	1.3	14	1.2	9	1.3	
NA	6	0.3					
Previous pregnancies							<0.001
0	671	36.6	465	41.6	206	31	
1	641	35	400	35.8	241	36.3	
2	293	16	155	13.9	138	20.8	
>2	176	9.6	97	8.7	79	11.9	
NA	53	2.9					
Gestational age (weeks)							0.36
<14	301	16.4	178	19.6	123	22.7	
14-27	1070	58.3	681	74.8	389	71.6	
>27	82	4.5	51	5.6	31	5.7	
NA	381	20.8					
MUAC <23 cm	398	21.7	259	22.8	139	20.9	0.38
NA	30	1.6					
HIV-positive	170	9.3	86	7.4	84	12.4	0.001

Abbreviations: QuantiFERON-TB Gold-Plus (QFT), Middle Upper Arm Circumference (MUAC), Standard Deviation (SD), Not Available (NA).a Fisher´s exact test was used.

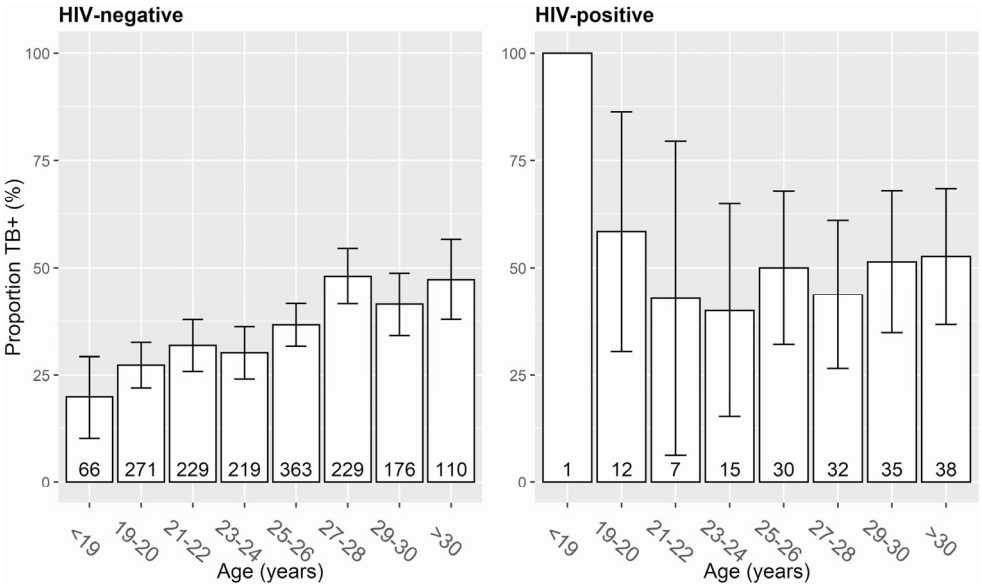

Figure 10. Proportion of tuberculosis (TB) infection stratified by age and human immunodeficiency virus (HIV) serostatus. Bar chart depicting the distribution of TB+ across age categories in HIV-negative and HIV-positive study participants. TB+ was defined as past or present active TB and/or positive QuantiFERON-TB Gold-Plus using the recommended cutoff of 0.35 IU/mL. Whiskers represent 95% confidence intervals for the proportion; the group size is denoted at the bottom of each bar.

Table 2. Multivariable logistic regression models for tuberculosis (TB) infection (TB+), defined as either latent TB infection, previous, or current active TB infection.

MODELS	AOR	95% CI		p
Model A				
HIV-status				
HIV-negative	Ref	Ref	Ref	
HIV-positive	1.43	1.03	1.99	0.031
Age, (years)	1.069	1.045	1.093	<0.0001
Model B				
HIV status, at age 25 years				
HIV-negative	Ref	Ref	Ref	
HIV-positive	1.76	1.21	2.54	0.0029
Age in HIV-negative (years)	1.079	1.053	1.105	<0.0001
Age in HIV-positive (years)	0.998	0.931	1.069	0.028[a]

Model A was constructed in a stepwise forward selection method, with variables with univariate P < .10 eligible for inclusion and likelihood ratio test used to determine the contributed model fitness. Model B was an extension of Model A exploring the interaction between age and HIV status on TB infection. Bonferroni- adjusted level of significance: 0.0038.

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; HIV, human immunodeficiency virus.

[a]p values indicate the level of evidence that the age association was different in HIV- positive and HIV-negative individuals (*i.e.*, the interaction).

Paper III

The third paper investigated the effect of latent TB infection on pregnancy outcome, specifically stillbirth and neonatal death. Out of 1857 women with available QFT-Plus results, these outcomes could be defined for 1456 women with record of pregnancy outcome from a physical study visit after delivery, as well as 30 women who reported foetal or neonatal death at tracing by telephone and therefore did not wish to participate further in the study. Out of these, 21 twin pregnancies as well as two induced abortions were excluded from the analysis, leaving 1463 (78.8%) included for analysis. Of these, 470 (32.1%) were categorized as having LTBI, 68 (4.6%) reported previous active TB and four (0.3%) were diagnosed with active TB in connection to the pregnancy.

Stillbirth (defined as occurring after 20 weeks of gestation to and including the day of delivery) occurred in 47 (3.2%) pregnancies, and neonatal death (defined as occurring from the second to the 28th day of life) occurred in 15 (1.0%) (Table 3). Proportions of stillbirth (19 [4.0%] *vs.* 25 [2.7%]) were higher in women with LTBI compared to TB uninfected women, although not reaching statistical significance in univariable logistic regression (OR 1.51, p = 0.18) or multivariable logistic regression with adjustment for HIV status, age and gravidity (AOR 1.38, 95% CI 0.73–2.57, p = 0.30).

Table 3. Pregnancy outcomes with regard to TB infection category.

Characteristics	TB-uninfected[1] N=921		LTBI[2] N=470		Previous active TB[3] N=68		Active TB diagnosed during pregnancy[4] (n=4)	
Offspring vital outcome	N	%	N	%	N	%		
Spontaneous abortion[5]	5	0.5	5	1.1	1	1.5	0	0
Stillbirth[6]	25	2.7	19	4.0	3	4.4	0	0
Foetal demise, unknown gestational age	4	0.4	2	0.4	0	0	0	0
Neonatal death ≤28 days [7]	10	1.1	5	1.1	0	0	0	0
Neonatal death ≤7 days	7	0.7	0	0	0	0	0	0
Survival >28 days	877	95.2	439	93.4	64	94.1	4	100.0
Delivery method								
Spontaneous vaginal	772	83.8	388	82.6	55	80.9	4	100.0
Instrumental	10	1.1	8	1.7	4	5.9	0	0
Emergency CS	74	8	40	8.5	3	4.4	0	0
Elective CS	32	3.5	16	3.4	1	1.5	0	0
Unknown	33	3.6	18	3.8	5	7.4	0	0
Hospitalization >24 hours								
Yes	71	7.7	36	7.7	2	2.9	1	25.0
No	822	89.3	414	88.1	63	92.6	3	75.0
Unknown	28	3	19	4.3	3	4.4	0	0

Abbreviations: Tuberculosis (TB), latent TB infection (LTBI), caesarean section (CS). Study participants without past or current active TB and with negative QuantiFERON TB GOLD PLUS reactivity.

1. Study participants without past or current active TB and with negative QuantiFERON TB GOLD PLUS reactivity.
2. Study participants with positive QuantiFERON TB GOLD PLUS reactivity, without past or current active TB.
3. Study participants reporting previous treatment for active TB.
4. Study participants diagnosed with active during the pregnancy or within three months of delivery.
5. Foetal demise occurring <20 weeks of gestation.
6. Foetal demise occurring from 20 weeks of gestation until and including the day of delivery.
7. Neonatal death before or at 28 days after delivery, not including the day of delivery.

Paper IV

The Swedish register material included 10 464 women with valid QFT results and at least one registered pregnancy. After exclusion of women tested outside pregnancy, who were not immigrants, who were HIV positive, as well as pregnancies occurring before the year 2000 or more than 10 years after immigration to Sweden, pregnancies occurring after or in connection to active TB disease, twin or triplet pregnancies as well as pregnancies ending in miscarriage, 7 408 women with a total of 12 443 pregnancies remained for analysis (Figure 11). For this paper, we used the more modern nomenclature “TB disease” instead of active TB, and “TB infection” (TBI) to reflect TB infection without signs or symptoms suggestive of disease, i.e. for practical purposes what was previously called LTBI [18].

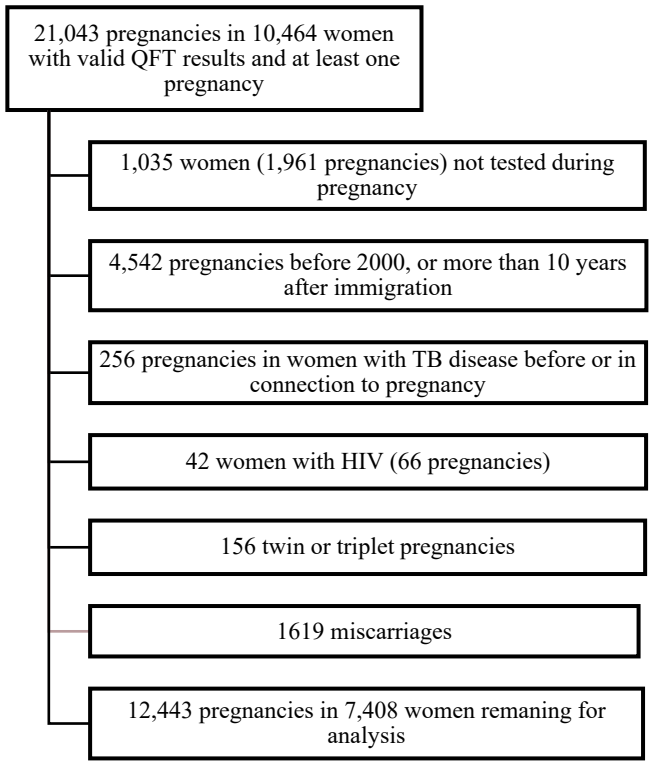

Figure 11. Flow chart of inclusion of women originating from TB-endemic countries screened for latent TB infection during pregnancy.

Of these, 1408 women (19.0%) were categorized as having TB infection, which was assumed to apply for any pregnancy irrespective of whether the QFT sampling was performed at the present or a past or subsequent pregnancy. TBI was more common in women of African (29.5%) compared to Asian (12.8%) origin; and was associated with significant but modest differences in mean age (30.0 *vs.* 29.2 years), parity (31.9 *vs.* 36.3% primipara), level of education (16.2 *vs.* 11.2% less than 9 years of formal schooling), and mean body mass index (BMI) at ANC enrolment (26.2 *vs.* 25.5 kg/m^2).

In univariable analysis, TBI was associated with stillbirth (odds ratio [OR] 2.16, 95% confidence interval [CI] 1.34-3.40), preeclampsia (OR 1.33, 95% CI 1.03-1.70), severe preeclampsia (1.68, 95% CI 1.17-2.38), low birthweight (OR 1.26, 95% CI 1.00-1.59), and emergency Caesarean section (OR 1.23, 95% CI 1.08-1.40, Figure 12). In multivariable analysis with adjustment for maternal age and African origin, as well as maternal study code as random effect to adjust for clustering between outcomes of repeated pregnancies in the same women, TBI remained significantly associated with stillbirth (adjusted odds ratio [AOR] 1.90, 95% CI

1.13-3.21, p=0.016), emergency Caesarean section (AOR 1.28, 95% CI 1.02-1.63, p=0.033) and severe forms of preeclampsia (including HELLP-syndrome and eclampsia) remained significantly associated with TBI (AOR 1.62, 95% CI 1.03-2.56, p=0.036). Finally, a sensitivity analysis additionally including parity, level of education, BMI and smoking status at ANC enrolment revealed largely similar results but wider confidence intervals due to loss of power (n=7479, 60.1%).

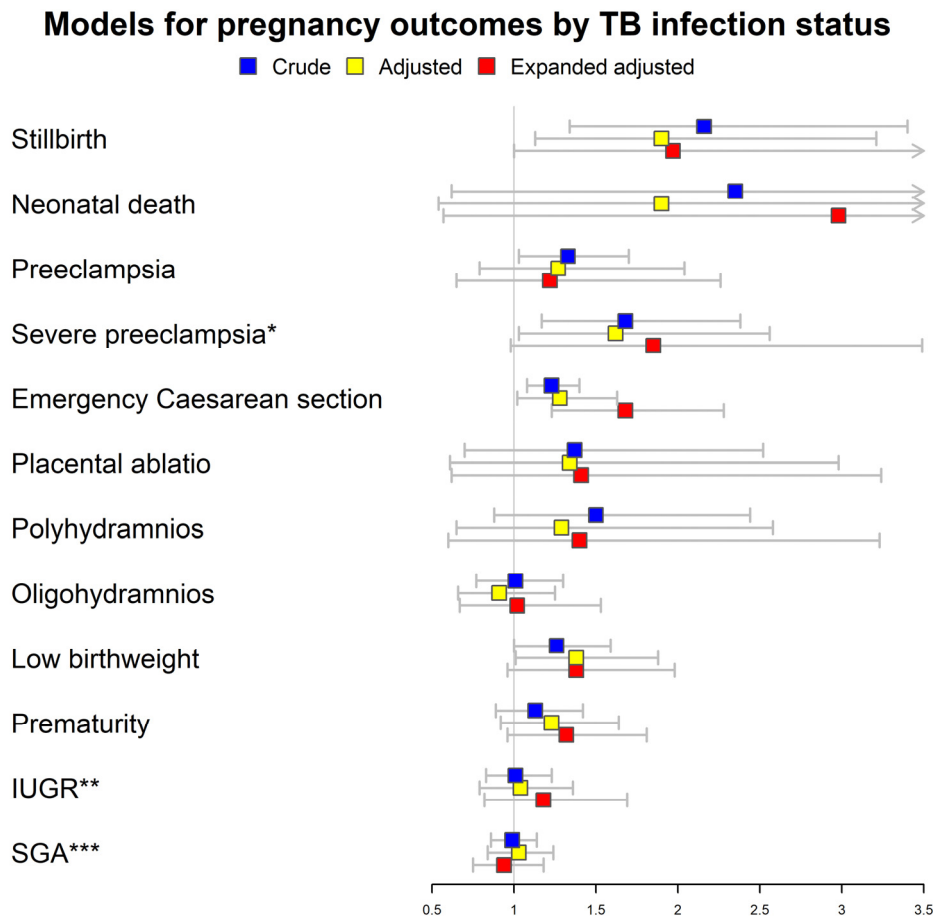

Figure 12. Forest plot depicting logistic regression models for pregnancy outcomes by tuberculosis infection. Odds ratio estimates (boxes) and 95% confidence intervals (whiskers) represent crude- (blue), adjusted- (yellow, adjusted for maternal age and origin with random effects for maternal study code) and expanded adjusted (red, sensitivity analysis additionally adjusted for parity, level of education, BMI and smoking status at ANC enrolment) models.

Discussion

Tuberculosis is among the top killers of infectious diseases, and among the leading non-obstetric causes of maternal death in high burden settings. Around 1.7 billion people are estimated to be infected by *Mycobacterium tuberculosis* [203] in an elusive and heterogenous state with respect to bacillary burden and host-pathogen interaction known as TB infection (previously LTBI), and with largely unexplored pathogenetic potential beyond the risk of progression to TB disease. The interaction between tuberculosis infection and pregnancy has been subject to a great deal of debate and controversy throughout history. This thesis work has approached epidemiological and diagnostic aspects of TB infection in pregnancy, but also the novel hypothesis that TB infection may have consequences for pregnancy outcome.

Epidemiology of TB infection in women of fertile age

Individuals in young adulthood carry the biggest burden of TB disease in sub-Saharan Africa [204–206], which is often highlighted from the perspective that direct costs and loss of income due to the disease or its treatment may have deleterious effects on individuals, their families as well as entire resource constrained high burden communities [144,207]. Several important risk factors for progression from TB exposure to infection and disease (with or without a latency phase) are enriched at one or both age extremes. Examples include immature or aged immune system, malnutrition and comorbidities like diabetes mellitus, alcoholism, liver cirrhosis, organ transplantation and haemodialysis [208–212]. This paradox may have several explanations, including diagnostic challenges such as atypical presentations and wider range of possible differential diagnoses in elderly and immunocompromised groups [213,214], but also that the risk of community exposure may differ by age category [215–217]. Even though men are diagnosed with TB disease in a larger extent than women [99,218], disease in men of economically productive age inevitably leads to community- and household exposure to women of reproductive age and young children.

Measuring TB infection in pregnancy may be a feasible way to approach the epidemiology in women of reproductive age or even the presence of contagious TB in the community in general [219]. Pregnancy is a rare occasion bringing a representative cross-section of the community in contact with healthcare, as women

from most socioeconomic and demographic circumstances often have access to free public antenatal care even in high burden settings [220].

In study II, a composite outcome (TB+) of QFT-positivity or previous or present TB disease was adopted to reflect the life-time acquisition of Mtb infection, was measured in pregnant women recruited from public antenatal care clinics in an Ethiopian city [221]. The proportion of TB+ increased from 20% at 18 years of age to 46% in women over 26 years of age, reflecting that young women in Adama are continuously exposed to contagious TB. Interestingly, this was not linked to any particular socioeconomic or demographic characteristics, and only a small minority reported known household exposure. Increasing prevalence of TB infection measured using IGRAs or TST is a consistent finding across different study populations in endemic settings [215,222–227], including pregnant women in Kenya and Uganda [59,228] and pregnant immigrant women in Sweden (Study IV and [229]). Together, these observations suggest the presence of substantial community transmission of TB in Adama as well as in other high burden settings in Africa. Among newly exposed women, several percent may progress to TB disease within two years [28]. Targeting community transmission is essential to protect vulnerable populations in high burden settings.

Ethiopia has succeeded in reaching the milestone of 20% incidence decline between 2015 and 2020 targeted by the End TB Strategy [86,88]. Still, Ethiopia has an estimated incidence of active TB of 132/100’000 person-years [88], and estimations indicate that around one in three TB cases are not diagnosed [45]. With delayed diagnosis and treatment, disease progression with development of cavernous lesions is likely, with expectoration of highly contagious aerosol [37,230]. Still, although the mortality is high, untreated individuals with smear-positive TB may remain contagious for several years until either spontaneous resolution or death [231].

Community-based active case finding directed at such groups may be effective in identifying and linking such individuals to treatment, and at reducing community transmission [232,233], but economic and logistic challenges make nationwide implementation of such strategies in TB endemic settings unfeasible. The End TB Strategy of the WHO recommends systematic screening for TB disease in certain circumstances, which were further elaborated in the 2021 Operational Handbook [86,234], aiming at better outcome for the individual as well as reduced transmission. Further studies are needed to identify sources of community transmission in endemic settings, as well as development and validation of innovative strategies that are feasible to implement and sustainable in resource constrained high endemic settings [235,236]. In addition, latent TB infection is a reservoir for TB, and considered a valid target for TB control in low burden countries [237].

However, eradication of latent TB infection in resource limited settings is limited by a range of obstacles, including the costs and technical requirements for IGRA

assays, the risk of failure to exclude atypical, mild or subclinical TB disease with development of drug resistance, the need for monitoring of side effects (mainly liver toxicity), and the risk of re-infection. Development of novel tools and strategies for diagnosis and prophylactic treatment of TB infection is a stated target of the End TB Strategy [86], but in order to have real impact on the global TB incidence and mortality this also requires consideration of feasibility for use in high-burden settings.

Diagnosis of TB infection during pregnancy

Study I investigated the performance of QFT-Plus among pregnant women recruited from antenatal care in Ethiopia. Interferon-γ levels elicited by TB2 antigen stimulation were similar to that elicited by TB1 stimulation. Although the TB2 tube alone identified 12 (4.3%) of individuals considered to be TB infected, discordance was rare and similarly distributed between TB1+/TB2- and TB1-/TB2+ types. This suggests limited additional value of the short peptides of the TB2 antigen formulation among pregnant women screened with QFT-Plus irrespective of symptoms suggestive of TB disease or reported exposure. QFT-Plus in pregnancy has since been investigated in similar settings; a recent Kenyan study similarly found very high agreement between TB1 and TB2 results irrespective or HIV status [238]. A study from Uganda reported lower concordance between TB1 and TB2 antigen formulations and a more substantial contribution to case detection of the TB2 tube (18%), as well as high numbers of indeterminate results due to low mitogen response [228].

Considering that the Tregs influence both CD4+ and CD8+ T cells, it seems plausible that the benefit of the TB2 tube may be lower in pregnancy compared to for example HIV infection which more specifically depletes the CD4+ T cells. It is also possible that the contribution may be larger in populations of pregnant women with higher prevalence of active TB, known recent exposure, or more pronounced immunosuppression (especially poorly controlled HIV infection) which were all rare in our study.

The optimal cut-off for any immune-based test for TB infection remain elusive. Conceptually, there is no gold standard against which these tests can be compared. Reactivation is in one sense the outcome which the tests aim to predict, but is a cumbersome target to study which requires vast study populations with long follow-up time and considerations of a number of potential sources of bias. Moreover, there is a within-subject biological variability upon re-testing, posing a challenge of judgement of reactivity close to the cut-off [239–242]. Several authors have recommended re-testing of subjects with near-cut-off reactivity, but the choice of range within which to re-test has also been subjected to controversy, and a number of ranges have been suggested [242–247]. Furthermore, interpretation of results also require consideration of the epidemiological likelihood for TB exposure, as well as

the immune competence of the individual. In this study, we have used a commonly cited range to define the borderline zone, 0.20-0.70 IU/mL [248]. Interferon-γ-levels within the borderline range were similarly distributed across TB1 and TB2 antigen formulations, but occurred more frequently in women with HIV infection (22.4% *vs.* 5.5%, respectively, had borderline reactivity in both formulations). In line with this, HIV positive women with TB infection (using 0.20 IU/mL as cut-off) had lower median levels of TB1- and TB2-stimulated interferon-γ levels compared to HIV negative peers, despite most being on effective antiretroviral therapy. Similar findings were reported from Kenya [238]. This indicates that a lower threshold to define positive reactivity may be motivated in HIV-positive pregnant women, especially considering that the risk of progression to disease (including disseminated disease) is substantial in this group.

Lacking a non-pregnant comparison group, Study I was not designed to study the influence of pregnancy on test performance *per se*, but longitudinal analyses suggest that the reactivity is stable or even increasing between first/second and third trimesters of gestation, despite progressive immunomodulation along the course of pregnancy [249]. Other studies have indicated the sensitivity might be negatively influenced by pregnancy [59], but utilized non-pregnant individuals recruited from hospital clinics for comparison, with risk for confounding. In our cohort, we have observed a high rate of QFT-Plus conversions between pregnancy and 9 months postpartum [249], but the extent to which this represents improved sensitivity after postpartum immune normalization *versus de novo* TB infection during the approximate year between pregnancy and postpartum re-testing is not known.

Consequences of latent TB infection in general and in pregnancy

There are ample observations indicating that TB disease is associated with adverse pregnancy outcome [144], albeit causal mechanisms still are not fully established. The contemporary view of TB infection is a dynamic state along a continuous spectre ranging from persistence of a small number of viable bacilli contained under tight immunological and mechanical control, to increasing burden of metabolically active and reproductive bacilli on the border of causing the signs and symptoms required for definition of TB disease. Here, we have explored a novel hypothesis; that women with TB infection might be at risk of pregnancy complications, in homology to that observed in women with TB disease. This has been studies in two materials, one based on a prospective cohort of women recruited form antenatal care in Ethiopia, and one based on a retrospective cohort of immigrant women screened for TB infection during Swedish routine antenatal care.

Among immigrant women screened during Swedish routine antenatal care, TB infection was independently associated with increased odds of stillbirth, severe preeclampsia, emergency caesarean section and low birthweight. In the Ethiopian cohort we found higher proportions of stillbirth in women with TB infection, but

due to a more modest effect estimate and limited sample size (n=1456) not reaching statistical significance (p=0.30), whereas mode of delivery, hospitalizations and neonatal deaths were similarly distributed between groups. A small single-centre retrospective Swedish study of immigrant and recently exposed pregnant women reported rare progression to active TB, and similar pregnancy outcome, although the sample size was limited and dichotomous pregnancy outcomes such as preeclampsia and stillbirth were not reported [250]. However, stillbirth was more common among women who reported household exposure to TB in a large Chinese register-based study, although no diagnostic tests for TB infection were reported [251].

Stillbirth is a grave and irrevocable outcome, but not specific for any particular pathophysiological process, and can result from placental disorders, foetal infection, obstructed labour, asphyxia and more. Similarly, emergency caesarean section can be motivated by a number of complications or conditions. However, the association between TB infection and severe preeclampsia may support involvement of the placenta. The placental development is dependent on carefully orchestrated modulations of the immune balance, which might be disturbed by a number of infectious diseases, which has been shown to contribute to a range of pregnancy complications including preeclampsia and stillbirth [122].

Studies from the Ethiopian cohort that are not part of this thesis work have revealed novel findings pertaining to the immune control of TB infection during pregnancy. Of importance, we have investigated the longitudinal dynamics of TB-antigen stimulated interferon-γ levels of pregnant women with TB infection. In this respect, it is worth contemplating that the appropriate null hypothesis for this experiment would not be zero change. Rather, a decline would be sensible to expect, reflecting the physiological decrease of CD4+ T cell responsivity along the course of pregnancy [118,252]. In support of this, we have observed lower mitogen response toward the end of pregnancy [253]. Instead of the expected decline in TB-antigen stimulated interferon-γ, median levels increased significantly between the first/second and third trimester, to decrease again at 9 months postpartum [249,254]. This paradoxical pattern suggests boosting of the TB-specific adaptive immune response during pregnancy.

The studies were not designed to unveil the mechanisms, but it could be speculated that a partial loss of immune control could lead to antigen presenting cells gaining access to Mtb antigens or bacilli and stimulate previously primed CD4+ T cells. This may in a sense be homologous to a “wind-up”-phenomenon which is commonly observed in TST as well as IGRAs following a TST challenge [255–257]

Principally, it can be speculated that a partial and temporary loss of control of TB infection during pregnancy could influence the developing placenta in at least two ways; (1) through triggering a systemic inflammatory response which could disturb the placental vascular development, or (2) through haematogenous dissemination to

the placenta where either the local infection or the local immune reaction could negatively influence the placental development.

Several studies have indicated that individuals considered to have TB infection may have altered systemic levels of inflammatory markers, suggesting some degree immune activation [194–199]. There is also an increasing concern that chronic low-grade inflammatory activity in individuals with TB infection may contribute to atherosclerosis and cardiovascular disease [258–261]. In this respect, it should be remembered that TB infection is a heterogenous condition, and it seems unlikely that the pattern and potential consequences of immune dysregulation should be uniformly distributed. Insights have indicated that a subset of individuals with TB infection (often labelled "LTBI outliers") have a transcriptomic phenotype resembling that of individuals with TB disease and with a high risk of progression to TB disease [67,68].

Further studies are motivated to elucidate whether TB infection is in fact causally linked to pregnancy complications.

Conclusions

- It is common that women living in TB endemic regions have acquired TB infection by the time they reach reproductive age, as we have observed in women seeking antenatal care in Ethiopia (37%) as well as immigrant women screened during antenatal care in Sweden (19%).
- Among pregnant women recruited from antenatal care in an Ethiopian city and tested using QFT-Plus irrespective of symptoms or exposure to TB, acquisition of measurable TB infection was more likely with increasing age. This reflects that living in a TB endemic setting is associated with a cumulative risk of exposure.
- Acquisition of TB infection was not confined to any socioeconomic or demographic strata, suggesting indiscriminate community transmission. HIV infection was associated with an increased burden of TB disease.
- The addition of shorter peptides in the TB2 antigen formulation in the QFT-Plus assay provided limited additional value. The correlation between absolute levels of TB antigen stimulated interferon-γ as well as agreement with respect to the recommended cut-off were very high, and TB1+/TB2- and TB1-/TB2+ discordance were both rare and symmetrically distributed.
- Among immigrant women screened for TB infection during Swedish antenatal care, TB infection was associated with increasing odds of stillbirth, severe preeclampsia, emergency caesarean section and low birthweight after adjustment for maternal origin and age, with comparable results in sensitivity models with wider adjustments. Further studies are motivated to unveil pathogenetic mechanisms and explore whether the excess risk of pregnancy complications is enriched within a particular subgroup of TB infected women, and whether TB infection is a modifiable risk factor.

Future perspectives

On a global level, the ambitious targets of the End TB are not likely to be accomplished by extrapolation from the pre-pandemic years. Study II revealed a substantial exposure of contagious TB to women of fertile age in a city in Ethiopia. The million-dollar question is – how to identify and treat those who contribute the most to community transmission? In order to shed some light on the patterns of real time transmission, we are planning to investigate TB infection acquired during a limited time period, through repeated QFT-Plus testing of mothers, children and testing of their household members. This may reveal some insights regarding the relative importance of household *vs.* community transmission, and which characteristics on individual, household and even community level (i.e. characteristics of geographic hotspots) that are associated with being exposed to contagious TB.

Provision of prophylactic treatment to individuals with TB infection at high risk of progressing to TB disease is included in the End TB Strategy, however, due to costs and required lab equipment, testing for TB infection is not performed in high-burden countries such as Ethiopia. There are also obstacles such as reliable exclusion of TB disease, and the risk of reinfection, that might make widespread use unfeasible. However, in specific contexts such as known household exposure, it is possible that prophylactic treatment guided by an immune based test might have a role to play, and we are currently evaluating a novel IGRA designed for use in low resource laboratory settings (QIAreach QuantiFERON-TB [262] [Qiagen, Hilden, Germany]) in the cohort.

Of greater importance would be better tools to discriminate TB infected individuals at high risk of progressing to TB disease, and hitherto, no signature of host inflammatory pattern has been able to accomplish this. Mtb has been recovered from peripheral blood in subjected with TB infection [263,264]. The described procedure is not feasible for clinical use, but still provides a glimmer of hope that the future may hold tools for microbe- rather than host-derived biomarkers for diagnosis and subcategorization of the spectre of TB infection.

Finally, we have observed higher risks for selected pregnancy complications in women with TB infection. Our studies have not been designed to unveil any underlying mechanisms, and there is as always in observational studies a risk of residual confounding. Provided the context that 1.7 billion people are estimated to

carry TB infection, further studies are required to attempt to reproduce our findings, and unveil any pathogenetic mechanisms.

If TB infection indeed increases the risk of severe pregnancy complications, questions such as if there is an identifiable subset of women in whom the excess risk seems to be enriched, and how this effect could be modulated arises. Still, perhaps the most effective intervention to protect mothers and neonates from the consequences of TB will be directed at ending the TB transmission.

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