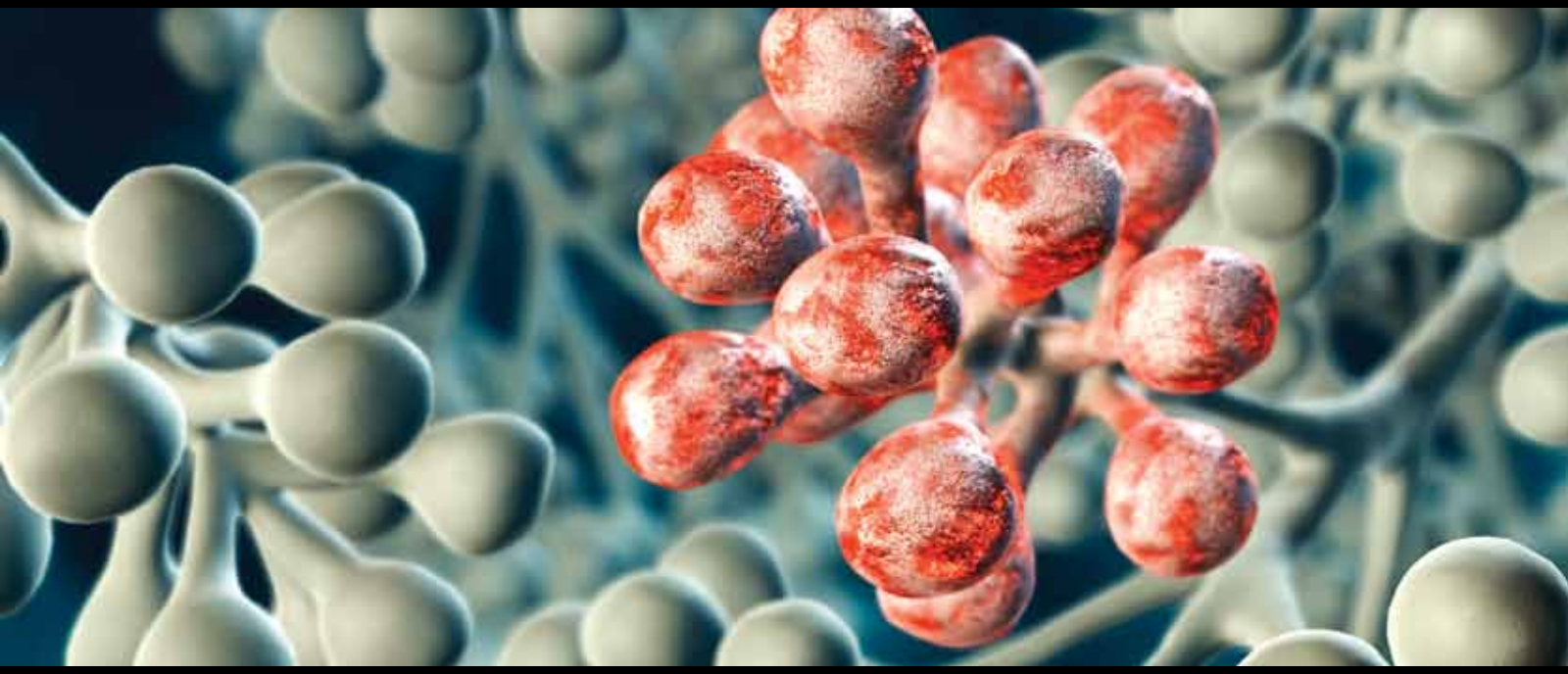
Pulmonary Tuberculosis

Guest Editors: Anete Trajman, José R. Lapa e Silva, Margareth Dalcolmo, and Jonathan E. Golub



Pulmonary Tuberculosis

Pulmonary Tuberculosis

Guest Editors: Anete Trajman, José R. Lapa e Silva, Margareth Dalcolmo, and Jonathan E. Golub

Editorial Board

Contents

Hindawi Publishing Corporation
Pulmonary Medicine
Volume 2013, Article ID 645747, 1 page
http://dx.doi.org/10.1155/2013/645747

Editorial

Pulmonary Tuberculosis

Anete Trajman,[1,2] José R. Lapa e Silva,[3,4] Margareth Dalcolmo,[5] and Jonathan E. Golub[6]

[1] *Gama Filho University, Rua Manoel Vitorino 553, Prédio AG, 5° andar, 20740-900 Rio de Janeiro, RJ, Brazil*
[2] *Chest Institute, McGill University, 3650 St. Urbain Street, Montreal, PQ, Canada H2X 2P4*
[3] *Federal University of Rio de Janeiro, Avenida Professor Rodolpho Paulo Rocco 255, 21941-913 Rio de Janeiro, RJ, Brazil*
[4] *Center for Global Health, Weill Medical College of Cornell University, New York, NY, USA*
[5] *Centro de Referência Professor Helio Fraga, FIOCRUZ, Estrada de Curicica 2000, CEP 22780-192 Rio de Janeiro, RJ, Brazil*
[6] *Johns Hopkins University School of Medicine and Bloomberg School of Public Health, Center for TB Research, 1550 Orleans Street, CRB-2, Room 1M.10, Baltimore, MD 21231, USA*

Correspondence should be addressed to Anete Trajman; atrajman@gmail.com

Received 24 February 2013; Accepted 24 February 2013

Tuberculosis (TB) is a major killer worldwide. The World Health Organization estimates that in 2011, there were 8.7 million incident cases of TB, 1.4 million deaths from TB including 0.43 million deaths from HIV-associated TB. In recent years, after a century of stagnation, new diagnostic technologies have been developed, and they are already being scaled up in some high-burden countries. Likewise, new drugs and regimens to treat TB are being evaluated, and the development of new vaccines is also progressing. However, despite the hope for reduced transmission with earlier detection and effective treatment, new cases continue to emerge from latently infected individuals.

In the present issue, P. Narasimhan et al. review risk factors for progression to active disease. Immunological aspects of TB are discussed in three papers, with J.-G. Ocejo-Vinyals et al. indicating that mannose-binding lectin 2 promoter polymorphisms and gene variants are not associated with an increased risk of pulmonary tuberculosis in a genetically conserved population in Spain; Y. V. N. Cavalcanti et al. reviewed the role of cytokines in protective immunity and susceptibility to tuberculosis; I. Takenami et al. provide evidence that blood IFN-gamma levels in tuberculin skin test positive individuals increase after six months of isoniazid treatment.

In addition, several papers evaluate the new interferon-gamma release assays (IGRAs), which have replaced or complemented the tuberculin skin test in many developed countries in the last decade. A. Trajman et al. review the current clinical uses of these tests. S. S. Shin et al. used IGRAs to investigate the effect of cigarette smoking on TB transmission, and W. Thanassi et al. explore the utility of IGRAs for serial testing in healthcare workers.

We believe that this issue can contribute to the debate of relevant topics concerning new advances on tuberculosis among pulmonologists, infectologists, clinicians, epidemiologists, and basic science researchers.

Anete Trajman
José R. Lapa e Silva
Margareth Dalcolmo
Jonathan E. Golub

Hindawi Publishing Corporation
Pulmonary Medicine
Volume 2013, Article ID 828939, 11 pages
http://dx.doi.org/10.1155/2013/828939

Review Article

Risk Factors for Tuberculosis

Padmanesan Narasimhan,[1] James Wood,[1] Chandini Raina MacIntyre,[1] and Dilip Mathai[2]

[1] *School of Public Health and Community Medicine, The University of New South Wales, Kensington, Sydney, NSW 2052, Australia*
[2] *Infectious Diseases Research and Training Centre, Department of Medicine-I and Infectious Diseases, Christian Medical College, Vellore, Tamil Nadu, India*

Correspondence should be addressed to Padmanesan Narasimhan; padmanesan@gmail.com

Received 7 October 2012; Revised 27 December 2012; Accepted 5 January 2013

Academic Editor: Anete Trajman

The risk of progression from exposure to the tuberculosis bacilli to the development of active disease is a two-stage process governed by both exogenous and endogenous risk factors. Exogenous factors play a key role in accentuating the progression from exposure to infection among which the bacillary load in the sputum and the proximity of an individual to an infectious TB case are key factors. Similarly endogenous factors lead in progression from infection to active TB disease. Along with well-established risk factors (such as human immunodeficiency virus (HIV), malnutrition, and young age), emerging variables such as diabetes, indoor air pollution, alcohol, use of immunosuppressive drugs, and tobacco smoke play a significant role at both the individual and population level. Socioeconomic and behavioral factors are also shown to increase the susceptibility to infection. Specific groups such as health care workers and indigenous population are also at an increased risk of TB infection and disease. This paper summarizes these factors along with health system issues such as the effects of delay in diagnosis of TB in the transmission of the bacilli.

1. Introduction

In addition to providing effective treatment and reducing mortality, a primary aim of tuberculosis (TB) control programs in countries of high TB incidence is to reduce the transmission from infectious TB cases. The development of TB in an exposed individual is a two-stage process following infection. In most infected persons, infection is contained by the immune system and bacteria become walled off in caseous granulomas or tubercles. In about 5% of infected cases, rapid progression to tuberculosis will occur within the first two years after infection [1]. About 10% of people with latent infection will reactivate, half within the first year, the remainder over their lifetime [2–7] mostly by reactivation of the dormant tubercle bacilli acquired from primary infection or less frequently by reinfection. Overall, about 10–15% of those infected go on to develop active disease at some stage later in life [2], but the risk of progression is much higher at about 10% per year [8, 9] in HIV-positive and other immunocompromized individuals.

The risk of progression to infection and disease is two different aspects and proper understanding of these factors is essential for planning TB control strategies [10]. The risk of infection following TB exposure is primarily governed by exogenous factors and is determined by an intrinsic combination of the infectiousness of the source case, proximity to contact and social and behavioural risk factors including smoking, alcohol, and indoor air pollution. In settings with increased chances of social mixing (together with overcrowding) transmission will be high. Similarly, conditions which prolong the length of exposure to an infectious patient include health system-related factor such as delay in diagnosis. Factors that increase the progression of infection to disease are primarily endogenous (host related). Conditions which alter the immune response increase the risk of progression to disease with HIV coinfection, the most important of these. However at the population level impact of this risk factor could vary depending on the local prevalence of the HIV. Diabetes, alcohol, malnutrition, tobacco smoke, and indoor air pollution are factors which impact a larger section of the population and accelerate progression to TB disease. This paper aims to summarize the risk factors which contribute to TB infection and disease at both individual and population level.

2. Methods

The search strategy for this paper included searching PubMed, Medline, and EMBASE databases for known risk factors. Only English language papers were included in the search, and the searches were limited to studies of risk factors influencing TB infection and disease. Factors related to TB treatment outcomes such as mortality and default were not included. Broad search terms included the following: Tuberculosis, transmission, contacts as a MeSH or heading term as well as "tuberculosis," "risk factors," and "transmission," as text words AND infectious diseases, Tuberculosis and risk factors as MeSH or subject terms and keywords. More focused searches were undertaken within specific Tuberculosis journals such as the International Journal of Tuberculosis and Lung Disease, the Indian Journal of Tuberculosis, the Bulletin of the World Health Organization, and the Indian Journal of Medical Research. Only major risk factors related to TB infection and disease were identified, relevant literature was reviewed, and factors influencing TB treatment outcomes were not included.

3. Summary of Specific Risk Factors

Figure 1 depicts the major characteristics which influence an individual's risk of contracting infection and disease, and the key risk factors are summarized below.

3.1. Factors Related to the Index Case

3.1.1. Bacillary Load. Epidemiological studies conducted during mid-20th century have shown that smear positive cases are more infectious than the others [11, 12]. An untreated sputum positive patient can infect approximately 10 individuals per year, and each smear positive case can lead to two new cases of TB, at least one of which will be infectious [2, 13].

The concentration of bacilli in the sputum from a TB case is positively correlated with the infectivity of the TB patient. Espinal and colleagues, in their prospective study of 803 household contacts of 174 index TB patients in the Dominican Republic, administered 5 TU Tubersol PPD to contacts at baseline and followed them up at 2, 8, and 14 months to study the effect of HIV on the infectiousness of *Mycobacterium tuberculosis*. In their subanalysis they showed that the odds of TST positivity for contacts with an index case sputum smear grade 1–10 (bacilli per field) and >10 (bacilli per field) compared to 0 (bacilli per field) were 1.98 (CI = 0.75–5.23) and 5.88 (CI = 1.60–21.3), which clearly shows that being a contact of an index patient with higher-grade sputum was associated with a greater likelihood of having a positive TST [14].

Smear negative patients are expected to have reduced number of bacilli than smear positive patients but can also transmit infection [15] with experimental studies confirming that the infecting dose of *M. tuberculosis* bacilli can be as few as one to ten bacilli [16, 17]. Epidemiological studies conducted in USA, UK, and India (prevalence and incidence studies) comparing infection and disease rates clearly points that prevalence of infection and disease is higher among contacts of smear positive index cases than smear negative cases, but the rates were higher among smear negative compared to general population [18–27].

Behr et al. in their molecular study in San Francisco identified 71 clusters of patients infected with identical strains, and, out of 183 secondary cases in those clusters, 17% [28] were attributed to infection by smear negative patients [29] the remainder being smear positive. Similar studies conducted by Hernández-Garduño and colleagues in the Greater Vancouver regional district showed that the episodes of transmission from smear negative clustered patients ranged from 17.3 to 22.2% in the pulmonary and 25 to 41% among extra pulmonary group [15, 30]. Tostmann from the Netherlands [31] confirmed that 13% of the secondary-cases were attributable to transmission from smear negative patients. This indicates that patients diagnosed with a sputum-positive result are more likely to be infectious [10, 12, 28, 32], but smear negative cases also remain an important source of transmission.

3.1.2. Proximity to an Infectious Case. Close contacts of infectious TB cases including household contacts and care givers/health care workers [33] are at a higher risk of becoming infected with *Mycobacterium tuberculosis* and development of primary active tuberculosis. Household contact studies among TB patients from early part of the 20th century [11, 34, 35] and large epidemiological surveys [20, 36–38] have established this effect. Morrison and colleagues performed a systematic review to determine the yield of household contact investigation [39]. Authors included 41 studies which were performed in 17 countries (49% in Africa, 29% in Asia, and 22% in Central and South America). The overall yield for all tuberculosis (bacteriologically confirmed and clinically diagnosed) was 4.5% (CI = 4.3–4.8) of contacts investigated; for cases with bacteriological confirmation the yield was 2.3% (CI = 2.1–2.5). Latent tuberculosis infection was found in 51.4% (CI = 50.6–52.2) of contacts investigated. However there was limitation, including the assumption that the transmission of infection and development of disease has occurred without biological evidence of organisms and the lack of community tuberculosis rates in the studies to see whether the findings are above the community average. TST was used in most studies for detecting LTBI, and the test is limited in its interpretation because of false positive and false negative results [40]. Subgroup analysis of sputum smear positive index cases showed that pooled yield for LTBI was 51.8% (CI = 50.9–52.8).

The risk of TB disease among individuals with LTBI (diagnosed as TST positive) relative to a person with no risk factors varies by several orders of magnitude. Several studies have asserted this finding. In two controlled clinical trials by Ferebee [41] examining the efficacy of treatment of LTBI among contacts of persons with active TB and among patients in mental hospitals, the tuberculin skin tests of 1472 participants in the placebo groups of the trials converted from negative to positive. Among persons whose tests converted, 19 developed disease in the first year of followup (12.9 cases per 1000 person-years) compared with 17 persons in the subsequent 7 years. of followup (1.6 cases per 1,000 person-years) [41]. A clear demonstration of the influence of

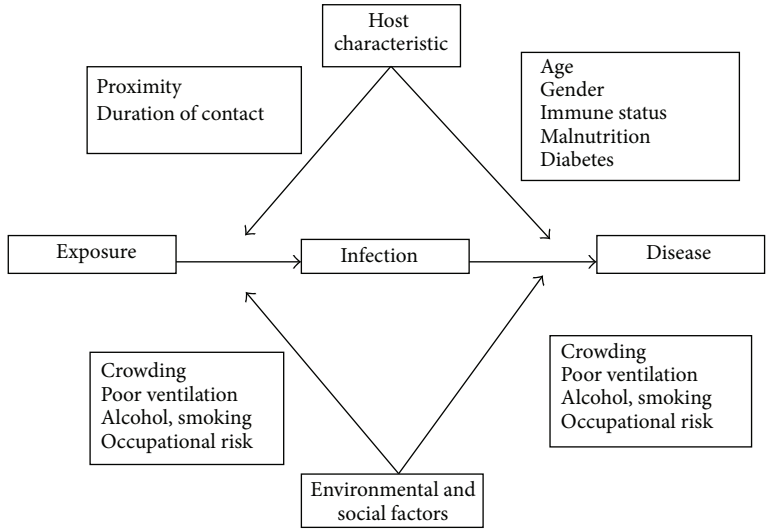


Figure 1: Risk factors for Tuberculosis infection and disease.

proximity to an infectious case was shown in an airplane outbreak investigation. Passengers seated within two rows of the index TB patient were more likely to have positive tuberculin skin test compared to those in the rest of the section (30.8% versus 3.6%, RR = 8.5, CI = 1.7–41.3) [42].

Contact tracing efforts have therefore been targeted towards household members of TB cases based on the "stone in the pond" principle, with the probability of infection increasing with the proximity [43]. But the importance of community transmission of TB has been under debate for a long time; Blomquist [44] raised the issue of difficulties in defining contacts of a case and stressed the need for extending the definition of the term "contact" to a larger number of persons associated with each patient, implying that transmission occurs beyond the households. The number of cases of infection in a particular exposure group (defined by the closeness to the source case) is the product of the risk and the number of people in the group. Thus exemplifying the Rose axiom [45] "a large number of people at small risk may give rise to more cases than a small number of people at high risk", there appears to be more cases of infection in the very large group of distant, low risk contacts than in the small group of close, high risk contacts. Conventional contact tracing generally identifies close, high risk contacts and therefore identifies only a minority of the infected contacts (20%), if higher than this, the circle of tracing needs to be widened [46].

The importance of casual contacts was noted in early epidemiological studies which showed that majority of older children with a positive TST reported no household contact with a source case and were therefore likely to have been infected in the community [19, 47–49]. Narain and colleagues in their retrospective analysis of a large household survey conducted in India [20] were able to show that, of the total persons infected in the community, only 2% belonged to case households, 7% belonged to suspect case households, and the remaining 91% of cases belonged to noncase households. The authors inferred that the zone of influence of an infectious case could extend to houses at least 10 lots distant [50]. Similar results were recorded by Radhakrishna et al. in their 15-year follow-up study of 253261 individuals in rural south India [26].

Molecular studies that identify the strain of the TB organisms have also confirmed the importance of casual transmission in both low- and high-incidence settings. In USA, Bishai and colleagues were able to show that there is an extensive transmission of TB occurring in the community. Of the 182 patients who had isolates available, 84 (46%) showed molecular clustering with 58 (32%) defined as being recently transmitted. Only 20 (24%) of 84 cases with clustered DNA fingerprints had epidemiologic evidence of recent contact. The remaining 64 (76%) cases without epidemiological links shared socioenvironmental risk factors for casual exposure to infectious TB cases (young age, homeless, alcohol, and drug use) and demographic features such as geographic aggregation in an area with inadequate housing [51]. These findings imply that TB continues to be propagated by casual recent transmission. Similar findings were found in other studies from low-incidence settings [52–56]. Similarly, Narayanan and colleagues have shown that 62% of patients (236/378) had identical strains in their large field survey in south India indicating a very high casual transmission [57]. Studies from other endemic settings like South Africa have confirmed this [58, 59].

These studies show that TB can be transmitted within a short period of contact [60], in nontraditional locations, and the opportunities for such interactions are abundant in an endemic setting with additional risk such as poverty, overcrowding, and high infection pressure [61]. Casual transmission is therefore a critical factor in TB dynamics in endemic settings [62].

3.2. Factors Related to the Individual

3.2.1. Immunosuppressive Conditions. HIV coinfection is the most potent immunosuppressive risk factor for developing active TB disease [9]. Southern Africa has the highest

prevalence of HIV infection and had the highest incidence of TB before the HIV/AIDS era. In the six southern African countries with adult HIV prevalence of more than 20%, the estimated TB case-notification rates are from 461 to 719 per 100 000 per year; by comparison, the notification rate in the USA was 5 per 100 000 per year [63]. HIV coinfection greatly increases the chances of reactivation of latent infection of TB [64] and increases the rapid TB progression following primary infection or reinfection with TB [5, 65–67]. Studies in countries with high HIV prevalence have also shown that spatial and temporal variation in TB incidence is strongly associated with the prevalence of HIV infection [9]. Individual studies conducted in both high- [68] and low-burden TB countries [69] have attributed increasing TB incidence to HIV infection.

HIV coinfection exacerbates the severity of TB disease while additionally TB coinfection accelerates HIV replication in affected organs including lungs and pleura [70]. Cell-mediated immunity is a crucial component in the host defence against *M. tuberculosis* that is weakened by HIV infection resulting in increased risks in reactivation of TB and commonly results in widespread dissemination causing EPTB. TB also accelerates HIV progression through increased systemic immune activation [71]. Therefore, coinfection leads to increases in the rate of disease progression and mortality [72, 73] among patients for multiple reasons.

Individuals with immune-mediated inflammatory disorders (IMID) are also known to be at increased risk of developing active TB, particularly after the use of tumour necrosis factor (TNF)—alpha inhibitors to treat a variety of autoimmune disease [74, 75]. Animal studies have shown that TNF is critical in host immune response in controlling a wide variety of bacterial, fungal, parasitic, and mycobacterial infection. Studies have shown that individuals are at increased risk for many of these infections, in particular for TB in areas with a high background prevalence of TB [75, 76]. Therefore screening for LTBI has been recommended before TNF-alpha inhibitor therapy is initiated. Both TST and IGRAs are being increasingly used to screen for LTBI, with IGRAs showing higher specificity. De Leon et al. evaluated TST and QFT responses in patients with RA and controls in Peru, a highly TB endemic region, where 80% of participants had a history of BCG vaccination. The proportion of patients testing positive for LTBI was significantly higher with QFT than with TST and more closely approximated that of the control group, suggesting that the IGRA was more sensitive than TST in detecting LTBI [77]. It is important to note that both the tests lack the ability to distinguish between latent TB infection and active disease; that is, none of the existing tests can accurately identify the subgroup that is at risk of progression to disease [78].

3.2.2. Malnutrition. Studies have shown that malnutrition (both micro- and macro-deficiency) increases the risk of TB because of an impaired immune response [79–82]. TB disease can itself lead to malnourishment because of decreasing appetite and changes in metabolic processes [83]. The association between malnutrition and TB has been shown with BCG vaccine trials performed in USA during the late 1960s estimating that malnourished children are twice as likely to contract TB disease as their appropriately nourished peers [84]. The first National Health and Nutrition Examination (NHANES-1) and the NHANES-1 Epidemiological Follow-up Study (NHEFS) conducted during 1982–84 from the USA among adults reported an increased adjusted hazard of TB from six- to ten-fold [85] in malnourished individuals. However, Cegielski and McMurray reviewed the relationship between malnutrition and tuberculosis with the available ecological, epidemiological, and animal studies and commented that although evidence exists to relate malnutrition and TB, the risk relative to specific levels of malnutrition still needs to be defined [79].

3.2.3. Young Age. Children are at higher risk of contracting TB infection and disease. Studies have shown that 60–80% exposed to a sputum smear-positive case became infected compared to only 30–40% who are exposed to a sputum smear-negative source case [48, 86–89]. Majority of the children less than 2 years of age get infected from the household source case, whereas, with children more than 2 years of age, majority of them became infected in the community. Household sputum positive source case is the single most important risk factor for children and remained an important contributor to infection up to 5–10 years of age [88]. Most of the disease manifestations develop within the first year following primary infection, identifying the first year following exposure as the time period of greatest risk. Children with primary infection before 2 years or after 10 years of age were at increased risk for disease development [90]. The highest risk for TB-related mortality following primary infection occurred during infancy. The risk declined to 1% between 1 and 4 years of age, before rising to more than 2% from 15 to 25 years of age [89, 90]. These findings provided the scientific basis for classical contact investigation practices, which focus on children less than 5 years of age in most developing countries and all household contacts in most industrialized countries.

3.2.4. Diabetes. Diabetes has been shown to increase the risk of active TB disease [91, 92]. It is estimated that currently 70% of people with diabetes live in low- and middle-income countries [93], and the rates are steadily increasing in areas where TB is endemic, including India and sub-Saharan Africa [94]. A systematic review comparing 13 studies examining the association between diabetes and TB found that diabetic patients had about a threefold increased risk of developing TB when compared to those without diabetes [95]. Studies have also found poorer outcomes among diabetic patients with Alisjahbana et al. in their prospective study showing that patients with TB and DM had a 22.2% smear-positive culture rate at the end of treatment compared to only 6.9% of those without diabetes [96]. Another review on treatment outcomes among patients with DM and TB found that the risk of death was 1.89 times higher compared to those without diabetes, with the risk increasing to five times higher for those with DM after adjustment for potential confounders [97].

Biological evidence supports the theory that diabetes directly impairs the innate and adaptive immune responses,

thereby accelerating the proliferation of TB. Animal studies showed a higher bacterial load among diabetic mice experimentally infected with *M. tuberculosis* [98]. Decreased production of IFN-γ and other cytokines diminished T-cell immunity [99] and reduced chemotaxis in neutrophils of diabetic patients [100] are thought to play a role in increasing the propensity of diabetic patients to developing active TB. A reverse association where TB can induce glucose intolerance and deteriorate glycaemic control in subjects with diabetes has also been identified [101]. Increasing rates of diabetes [102] in India could pose a great challenge for TB control in the future [103].

3.2.5. Healthcare Workers. Healthcare workers (HCWs) are at increased risk of exposure to TB. A review by Seidler et al. showed that, among HCWs in high-income countries, the overall incidence of TB disease in the general population and native born HCWs was less than 10 and 25 per 100 000 per year [104]. Joshi and colleagues summarized evidence on the incidence and prevalence of latent TB infection (LTBI) and disease among HCWs in low- and middle-income countries. In their review of 51 studies the authors found that the prevalence of LTBI among HCWs was on 55% (CI = 33–79), the estimates of the annual risk of LTBI ranged from 0.5 to 14.3%, and the annual incidence of TB disease ranged from 69 to 5780 per 100 000 [33].

3.3. Socioeconomic and Behavioural Factors. Rapid urbanization [105, 106] witnessed in developing countries and socioeconomic status (SES) of individuals has also been shown to have influence on a person's susceptibility to infection. The TB burden follows a strong socioeconomic gradient between and within countries with the poorest having the highest risk [107, 108]. People with low SES are exposed to several risk factors discussed above (including malnutrition, indoor air pollution, alcohol, etc.) which increases their risk for TB. People with lower SES have a higher likelihood of being exposed to crowded, less ventilated places and have limited safe cooking practicing facilities. Marginalized populations including prisoners have a higher chance of getting infected with TB [109] mostly because of crowded living conditions and coinfection with HIV and injection drug abuse [110]. While smoking rates are higher among individuals belonging to lower SES, alcohol, HIV, and diabetes are not well correlated with lower SES [107].

3.3.1. Tobacco Smoke. The association between smoking and TB has been studied in several systematic reviews [111–116]. Bates and colleagues, in their meta-analysis of 24 studies on the effects of smoking on TB, showed that the relative risk of TB disease (RR = 2.3–2.7) was high among smokers in comparison to nonsmokers and that there was clear evidence that smoking causes remained a risk factor for TB infection and disease, with additional risk of death in persons with active TB [114]. Lin et al. performed a systematic review and meta-analysis examining the role of smoking, indoor air pollution in TB from 38 studies. In their analysis of six studies specifically examining tuberculin reactivity among smokers, the pooled OR for latent TB infection (LTBI) was 2.08 (CI = 1.53–2.83) and 1.83 (1.49–2.23) at 5 and 10 mm TST cut-off points and the effect of smoking on LTBI remained even after adjustment for alcohol (OR = 1.76, CI = 1.43–2.16) [117]. The authors (with their pooled evidence showing increased risk for TB infection, disease, and deaths) commented that their data support a causal link between smoke exposure and an increased chance of acquiring TB, with the primary impact of smoking being to increase the risk of infection [117].

Biological explanations including impaired clearance of mucosal secretion [118], reduced phagocytic ability of alveolar macrophages [119, 120], and decrease in the immune response and/or CD4 + lymphopenia due to the nicotine in the cigarettes [120] have been given as reasons for increased susceptibility to pulmonary tuberculosis [112]. More recently Shang and coworkers in their animal study were able to demonstrate that exposure of mice to cigarette smoke followed by infection with *M. tuberculosis* results in a significant increase in the number of viable *M. tuberculosis* bacilli isolated from the lungs and spleen along with a decline in the adaptive immunity in the exposed mice [121].

3.3.2. Alcohol. Alcohol has been recognized as a strong risk factor for TB disease [122], and a recent meta-analysis of molecular epidemiological studies has established alcohol as a risk factor for clustering (or recent transmission of TB) in both high- (OR = 2.6, CI = 2.13–3.3) and low-incidence countries (OR = 1.4, CI = 1.1–1.9) [123]. A systematic review of 3 cohort and 18 case control studies concluded that the risk of active tuberculosis is substantially elevated (RR = 2.94, 95% CI = 1.89–4.59) among people who drink more than 40 g alcohol per day and/or have an alcohol use disorder [122]. Reasons for increased risk include alteration in the immune system, specifically in altering the signalling molecules responsible for cytokine production [124].

3.3.3. Indoor Air Pollution. In developing countries, the percentage usage of solid fuels for cooking is more than 80% [125]. Firewood or biomass smoke has been previously recognized as an independent risk factor for TB disease in case control studies conducted in India and Brazil [126–129]. Limited data on the mechanism by which biomass smoke causes chronic pulmonary diseases exists [130] however; animal studies have shown that acute wood smoke impaired macrophage phagocytic function, surface adherence [131], and bacterial clearance [132]. Also biomass combustion is shown to release large particulate matter (PM) such as carbon monoxide (CO), nitrogen oxide, formaldehyde, and polyaromatic hydrocarbons which can deposit deep into the alveoli and can cause considerable damage [133–135].

3.4. Demographic (Ethnic) Factors

3.4.1. Indigenous/Aboriginal Population. Studies from Canada and Australia have shown that indigenous or aborigines are at a higher risk of TB than the nonaborigines [136–138]. Aborigines have a higher than average prevalence of predisposing risk factors for TB such as renal failure, diabetes, alcohol abuse, and smoking. In addition, socioeconomic factors such as overcrowding and poverty are known contributors to this

Table 1: Relative risk, prevalence and population attributable risk of selected risk factors for TB.

Risk factor (reference)	Relative risk for active TB disease (range)[a]	Weighted prevalence, total population, 22 TB high burden countries[b]	Population attributable fraction (range)[c]
HIV infection	8.3 (6.1–10.8)	1.1%	7.3% (5.2–6.9)
Malnutrition	4.0 (2.0–6.0)	17.2%	34.1% (14.7–46.3)
Diabetes	3.0 (1.5–7.8)	3.4%	6.3% (1.6–18.6)
Alcohol use > 40 g/day	2.9 (1.9–4.6)	7.9%	13.1% (6.7–22.2)
Active smoking	2.6 (1.6–4.3)	18.2%	22.7% (9.9–37.4)
Indoor pollution	1.5 (1.2–3.2)	71.1%	26.2% (12.4–61.0)

[a] Range is equal to 95% confidence interval, except for malnutrition and diabetes.
[b] 22 countries that together have 80% of the estimated global TB burden.
[c] Population attributable fraction = (prevalence × (relative risk − 1))/(prevalence × (relative risk + 1)).
Source: adapted from Lönnroth and Raviglione [151].

burden [139]. A recent study showed that several aborigines in Canada had a gene deletion that may have predisposed them to developing active TB disease [140]. Clark and Vynnycky in their model predicted an increasing contribution of endogenous reactivation to total disease burden over time [138]. The high prevalence of latent infection, coupled with an increased risk of disease, may result in cases of reactivation disease in aboriginal communities.

3.5. Health System Issues. Evidences from China have demonstrated gains through strengthening health systems (by improving notification through web-based reporting), by which hospital referrals improved from 59% to 87% and the contribution of sputum positive pulmonary TB cases from hospitals doubled from 16% to 33% [141]. On the other hand, health system issues such as delays to diagnosis and treatment increase the duration in which active cases are infectious, thereby sustaining TB transmission [142]. Lin and colleagues in their cross-sectional study TB infection prevalence survey in southern China found that there was a positive association between the duration of delay to TB treatment and household infection rates [143]. The current passive case finding approach in the DOTS program is built upon the principle to treat infectious cases at the earliest to reduce the burden of infection or transmission in the community. This could be hampered by delay in diagnosis and treatment and may accelerate the transmission in the community [144, 145].

Table 1 provides summary estimates of relative risk for selected TB risk factors.

4. Conclusion

Screening for TB (to diagnose latent TB infection) and prophylactic therapy remain the most important tools to reduce the risk of progression to TB disease among high risk individuals (close contacts, HIV infected individuals, health care workers, etc.) and be considered in endemic countries to reduce the progression from infection to disease. Screening for latent TB also warrants highly sensitive and specific tools. The existing array (the newly available IGRAs) of diagnostic tests detect latent TB infection are highly specific but has reduced sensitivity [146]. Their inability to differentiate latent infection from disease and high operational costs makes them less than ideal tool for use in the developing world, where bulk of the TB infection and disease occurs.

HIV coinfection is the most important and potent risk factor for TB infection and disease. Interventions such as early HIV counselling and screening for TB patients and early diagnosis and initiation of antiretroviral therapy (ART) to coinfected individuals have all been shown to be effective in preventing TB disease [106].

In endemic countries, diagnosis and treatment (through DOTS) of smear-positive cases remains the key to TB control by reducing transmission from infectious cases. In addition to passive case-finding practices, early diagnosis of smear-positive cases can be improved through untargeted case-finding strategies in endemic countries [147]. Health system issues hampering this include a significant percentage (45% in countries like India) of TB patients accessing health care through the private sector [148]. Such patients are unaccounted for, and together with delay in diagnosis they may act as a constant reservoir for TB infection. Efforts to include private players (private practitioners, retail pharmacies, and laboratories) in TB control activities are therefore essential to curtail the epidemic.

The growing population (especially in countries like China and India) is likely to inflate the number of TB cases in future. Smoking rates are high among men in these endemic countries [143, 149], and, together with rising rates of diabetes [95], the risk of progression to TB disease will also increase. Interventions such as smoking cessation [150] and early screening for TB can be advocated, but the impact of these interventions in reducing TB risk remains negligible at population level [106].

Malnutrition and indoor air pollution are recognized risk factors which are confounded with the socioeconomic status of a setting. Rapid urbanization is shown to offset these components to an extent (by decreasing malnutrition rates and increased usage of clean fuels) [106], but increased awareness through IEC (information, education, and communication) activities should be considered. Efforts should also be made to collect risk factors data in routine surveillance for TB disease.

Conflict of Interest

The authors declared that there is no conflict of interests.

References

[1] I. Sutherland, *The Ten-Year Incidence of Clinical Tuberculosis Following "Conversion" in 2550 Individuals Aged 14 To 19 Years*, The Hague, Netherlands, 1968.

[2] D. Maher, "The natural history of Mycobacterium tuberculosis infection in adults," in *Tuberculosis: A Comprehensive Clinical Reference*, H. S. Schaaf and A. Zumla, Eds., pp. 129–132, Elsevier Health Sciences, 2009.

[3] I. Sutherland, "Recent studies in the epidemiology of tuberculosis, based on the risk of being infected with tubercle bacilli," *Advances in Tuberculosis Research*, vol. 19, pp. 1–63, 1976.

[4] E. Vynnycky and P. E. M. Fine, "The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection," *Epidemiology and Infection*, vol. 119, no. 2, pp. 183–201, 1997.

[5] P. A. Selwyn, D. Hartel, V. A. Lewis et al., "A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection," *The New England Journal of Medicine*, vol. 320, no. 9, pp. 545–550, 1989.

[6] G. Antonucci, E. Girardi, M. C. Raviglione, and G. Ippolito, "Risk factors for tuberculosis in HIV-infected persons: a prospective cohort study," *Journal of the American Medical Association*, vol. 274, no. 2, pp. 143–148, 1995.

[7] S. Lawn and L. G. Bekker, "Co pathogenesis of Tuberculosis and HIV," in *Tuberculosis: A Comprehensive Clinical Reference*, H. S. Schaaf and A. Zumla, Eds., pp. 96–106, Elsevier, 2009.

[8] E. Girardi, M. C. Raviglione, G. Antonucci, P. Godfrey-Faussett, and G. Ippolito, "Impact of the HIV epidemic on the spread of other diseases: the case of tuberculosis," *AIDS*, vol. 14, no. 3, pp. S47–S56, 2000.

[9] E. L. Corbett, C. J. Watt, N. Walker et al., "The growing burden of tuberculosis: global trends and interactions with the HIV epidemic," *Archives of Internal Medicine*, vol. 163, no. 9, pp. 1009–1021, 2003.

[10] I. Romieu and C. Trenga, "From exposure to disease: the role of environmental factors in susceptibility to and development of tuberculosis," *Epidemiologic Reviews*, vol. 23, no. 2, pp. 288–301, 2001.

[11] F. M. McPhendran and E. L. Opie, "The spread of Tuberculosis in families," *American Journal of Epidemiology*, vol. 22, no. 3, pp. 565–643, 1935.

[12] J. B. Shaw and N. Wynn-Williams, "Infectivity of pulmonary tuberculosis in relation to sputum status," *American Review of Tuberculosis*, vol. 69, no. 5, pp. 724–732, 1954.

[13] N. Ait-Khaled and D. Enarson, *Tuberculosis: A manual for medical Students*, World Health Organization, 2003.

[14] M. A. Espinal, E. N. Peréz, J. Baéz et al., "Infectiousness of Mycobacterium tuberculosis in HIV-1-infected patients with tuberculosis: a prospective study," *The Lancet*, vol. 355, no. 9200, pp. 275–280, 2000.

[15] E. Hernández-Garduño, V. Cook, D. Kunimoto, R. K. Elwood, W. A. Black, and J. M. FitzGerald, "Transmission of tuberculosis from smear negative patients: a molecular epidemiology study," *Thorax*, vol. 59, no. 4, pp. 286–290, 2004.

[16] G. L. Hobby, A. P. Holman, M. D. Iseman, and J. M. Jones, "Enumeration of tubercle bacilli in sputum of patients with pulmonary tuberculosis," *Antimicrobial Agents and Chemotherapy*, vol. 4, no. 2, pp. 94–104, 1973.

[17] A. S. Dharmadhikari and E. Nardell, "Transmission of Mycobacterium tuberculosis," in *Tuberculosis: A Comprehensive Clinical Reference*, H. S. Schaaf and A. Zumla, Eds., pp. 8–17, Elsevier Health Sciences, 2009.

[18] D. Menzies, "Issues in the management of contacts of patients with active pulmonary tuberculosis," *Canadian Journal of Public Health*, vol. 88, no. 3, pp. 197–201, 1997.

[19] S. Andersen and A. Geser, "The distribution of tuberculous infection among households in African communities," *Bulletin of the World Health Organization*, vol. 22, pp. 39–60, 1960.

[20] R. Narain, S. S. Nair, G. R. Rao, and P. Chandrasekhar, "Distribution of tuberculous infection and disease among households in a rural community," *Bulletin of the World Health Organization*, vol. 34, no. 4, pp. 639–654, 1966.

[21] A. Rouillon, S. Perdrizet, and R. Parrot, "Transmission of tubercle bacilli: the effects of chemotherapy," *Tubercle*, vol. 57, no. 4, pp. 275–299, 1976.

[22] W. Schilling, "Epidemiology and surveillance of tuberculosis in the German Democratic Republic," *Bulletin of the International Union Against Tuberculosis and Lung Disease*, vol. 65, no. 2-3, pp. 40–42, 1990.

[23] D. A. Enarson, E. A. Fanning, and E. A. Allen, "Case-finding in the elimination phase of tuberculosis: high risk groups in epidemiology and clinical practice," *Bulletin of the International Union Against Tuberculosis and Lung Disease*, vol. 65, no. 2-3, pp. 73–74, 1990.

[24] S. Etkind, "Contact tracing," in *Tuberculosis: A Comprehensive International Approach*, L. B. Reichman and E. S. Hershfield, Eds., vol. 144, pp. 275–289, Marcel Dekker, New York, NY, USA, 1993.

[25] V. K. Dhingra, S. Rajpal, N. Aggarwal, and D. K. Taneja, "Tuberculosis trend among household contacts of TB patients," *Indian Journal of Community Medicine*, vol. 29, no. 1, pp. 1–3, 2004.

[26] S. Radhakrishna, T. R. Frieden, R. Subramani, T. Santha, P. R. Narayanan, and T. R. Frieden, "Additional risk of developing TB for household members with a TB case at home at intake: a 15-year study," *The International Journal of Tuberculosis and Lung Disease*, vol. 11, no. 3, pp. 282–288, 2007.

[27] M. Singh, M. L. Mynak, L. Kumar, J. L. Mathew, and S. K. Jindal, "Prevalence and risk factors for transmission of infection among children in household contact with adults having pulmonary tuberculosis," *Archives of Disease in Childhood*, vol. 90, no. 6, pp. 624–628, 2005.

[28] H. A. van Geuns, J. Meijer, and K. Styblo, "Results of contact examination in Rotterdam, 1967–1969," *Bulletin of the International Union against Tuberculosis*, vol. 50, no. 1, pp. 107–121, 1975.

[29] M. A. Behr, S. A. Warren, H. Salamon et al., "Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli," *The Lancet*, vol. 353, no. 9151, pp. 444–449, 1999.

[30] R. K. Elwood, V. J. Cook, and E. Hernández-Garduño, "Risk of tuberculosis in children from smear-negative source cases," *International Journal of Tuberculosis and Lung Disease*, vol. 9, no. 1, pp. 49–55, 2005.

[31] A. Tostmann, S. V. Kik, N. A. Kalisvaart et al., "Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands," *Clinical Infectious Diseases*, vol. 47, no. 9, pp. 1135–1142, 2008.

[32] S. Grzybowski, G. D. Barnett, and K. Styblo, "Contacts of cases of active pulmonary tuberculosis," *Bulletin of the International Union against Tuberculosis*, vol. 50, no. 1, pp. 90–106, 1975.

[33] R. Joshi, A. L. Reingold, D. Menzies, and M. Pai, "Tuberculosis among health-care workers in low- and middle-income countries: a systematic review," *PLoS Medicine*, vol. 3, no. 12, article e494, 2006.

[34] W. H. Frost, "Risk of persons in familial contact with pulmonary tuberculosis," *American Journal of Public Health*, vol. 23, no. 5, pp. 426–432, 1933.

[35] E. L. Opie and F. M. McPhendran, "Spread of tuberculosis within families," *The Journal of the American Medical Association*, vol. 87, no. 19, pp. 1549–1551, 1926.

[36] S. Devadatta, J. J. Dawson, W. Fox et al., "Attack rate of tuberculosis in a 5-year period among close family contacts of tuberculous patients under domiciliary treatment with isoniazid plus PAS or isoniazid alone," *Bulletin of the World Health Organization*, vol. 42, no. 3, pp. 337–351, 1970.

[37] R. H. Andrews, W. Fox, S. Devadatta, and S. Radhakrishna, "Prevalence of tuberculosis among close family contacts of tuberculous patients in South India, and influence of segregation of the patient on the early attack rate," *Bulletin of the World Health Organization*, vol. 23, pp. 463–510, 1960.

[38] S. R. Kamat, J. J. Dawson, S. Devadatta et al., "A controlled study of the influence of segregation of tuberculous patients for one year on the attack rate of tuberculosis in a 5-year period in close family contacts in South India," *Bulletin of the World Health Organization*, vol. 34, no. 4, pp. 517–532, 1966.

[39] J. Morrison, M. Pai, and P. C. Hopewell, "Tuberculosis and latent tuberculosis infection in close contacts of people with pulmonary tuberculosis in low-income and middle-income countries: a systematic review and meta-analysis," *The Lancet Infectious Diseases*, vol. 8, no. 6, pp. 359–368, 2008.

[40] D. Menzies, K. Schwartzman, and M. Pai, "Immune-based tests for tuberculosis," in *Tuberculosis: A Comprehensive Clinical Reference*, H. S. Schaaf and A. Zumla, Eds., pp. 179–196, Elsevier Health Sciences, 2009.

[41] S. Ferebee, "Controlled chemoprophylaxis trials in tuberculosis. A general review," *Advances in Tuberculosis Research*, vol. 17, pp. 28–106, 1970.

[42] T. A. Kenyon, S. E. Valway, W. W. Ihle, I. M. Onorato, and K. G. Castro, "Transmission of multidrug-resistant Mycobacterium tuberculosis during a long airplane flight," *The New England Journal of Medicine*, vol. 334, no. 15, pp. 933–938, 1996.

[43] J. Veen, "Microepidemics of tuberculosis: the stone-in-the-pond principle," *Tubercle and Lung Disease*, vol. 73, no. 2, pp. 73–76, 1992.

[44] E. T. Blomquist, "Tuberculosis casefinding, 1961," *Public Health Reports*, vol. 76, no. 10, pp. 871–876, 1961.

[45] G. Rose, "Sick individuals and sick populations," *International Journal of Epidemiology*, vol. 30, no. 3, pp. 427–432, 2001.

[46] T. M. Daniel, "The history of tuberculosis: past, present, and challenges for the future," in *Tuberculosis: A Comprehensive Clinical Reference*, H. S. Schaaf and A. Zumla, Eds., pp. 1–8, Elsevier Saunders, London, UK, 1st edition, 2009.

[47] A. S. Pope, P. E. Sartwell, and D. Zacks, "Development of tuberculosis in infected children," *American Journal of Public Health*, vol. 29, no. 12, pp. 1318–1325, 1939.

[48] M. Brailey, "A study of tuberculous infection and mortality in the children of tuberculous households," *American Journal of Epidemiology*, vol. 31, no. 1, pp. 1–43, 1940.

[49] F. J. W. Miller, R. M. E. Seal, and M. D. Taylor, *Tuberculosis in Children: Evolution, Control, Treatment*, J. & A. Churchill, 1963.

[50] S. S. Nair, G. R. Rao, and P. Chandrasekhar, "Distribution of tuberculous infection and disease in clusters of rural households," *Indian Journal of Tuberculosis*, vol. 28, no. 1, pp. 3–9, 1971.

[51] W. R. Bishai, N. M. H. Graham, S. Harrington et al., "Molecular and geographic patterns of tuberculosis transmission after 15 years of directly observed therapy," *Journal of the American Medical Association*, vol. 280, no. 19, pp. 1679–1684, 1998.

[52] D. P. Chin, C. M. Crane, M. Y. Diul et al., "Spread of Mycobacterium tuberculosis in a community implementing recommended elements of tuberculosis control," *Journal of the American Medical Association*, vol. 283, no. 22, pp. 2968–2974, 2000.

[53] W. Z. Bradford, J. Koehler, H. El-Hajj et al., "Dissemination of Mycobacterium tuberculosis across the San Francisco Bay Area," *Journal of Infectious Diseases*, vol. 177, no. 4, pp. 1104–1107, 1998.

[54] J. Cacho Calvo, J. Astray Mochales, A. Pérez Meixeira, A. Ramos Martos, M. Hernando García, M. Sánchez Concheiro et al., "Ten-year population-based molecular epidemiological study of tuberculosis transmission in the metropolitan area of Madrid, Spain," *The International Journal of Tuberculosis and Lung Disease*, vol. 9, no. 11, pp. 1236–1241, 2005.

[55] R. Diel, S. Schneider, K. Meywald-Walter, C. M. Ruf, S. Rüsch-Gerdes, and S. Niemann, "Epidemiology of tuberculosis in Hamburg, Germany: long-term population-based analysis applying classical and molecular epidemiological techniques," *Journal of Clinical Microbiology*, vol. 40, no. 2, pp. 532–539, 2002.

[56] M. Ruiz Garcia, J. C. Rodríguez, J. F. Navarro, S. Samper, C. Martín, and G. Royo, "Molecular epidemiology of tuberculosis in Elche, Spain: a 7-year study," *Journal of Medical Microbiology*, vol. 51, no. 3, pp. 273–277, 2002.

[57] S. Narayanan, S. Das, R. Garg et al., "Molecular epidemiology of tuberculosis in a rural area of high prevalence in South India: implications for disease control and prevention," *Journal of Clinical Microbiology*, vol. 40, no. 12, pp. 4785–4788, 2002.

[58] C. N. Classen, R. Warren, M. Richardson et al., "Impact of social interactions in the community on the transmission of tuberculosis in a high incidence area," *Thorax*, vol. 54, no. 2, pp. 136–140, 1999.

[59] S. Verver, R. M. Warren, Z. Munch et al., "Proportion of tuberculosis transmission that takes place in households in a high-incidence area," *The Lancet*, vol. 363, no. 9404, pp. 212–214, 2004.

[60] J. E. Golub, W. A. Cronin, O. O. Obasanjo et al., "Transmission of Mycobacterium tuberculosis through casual contact with an infectious case," *Archives of Internal Medicine*, vol. 161, no. 18, pp. 2254–2258, 2001.

[61] B. J. Marais, C. C. Obihara, R. M. Warren, H. S. Schaaf, R. P. Gie, and P. R. Donald, "The burden of childhood tuberculosis: a public health perspective," *International Journal of Tuberculosis and Lung Disease*, vol. 9, no. 12, pp. 1305–1313, 2005.

[62] J. P. Aparicio, A. F. Capurro, and C. Castillo-Chavez, "Transmission and dynamics of tuberculosis on generalized households," *Journal of Theoretical Biology*, vol. 206, no. 3, pp. 327–341, 2000.

[63] E. L. Corbett, B. Marston, G. J. Churchyard, and K. M. De Cock, "Tuberculosis in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment," *The Lancet*, vol. 367, no. 9514, pp. 926–937, 2006.

[64] H. C. Bucher, L. E. Griffith, G. H. Guyatt et al., "Isoniazid prophylaxis for tuberculosis in HIV infection: a meta-analysis of randomized controlled trials," *AIDS*, vol. 13, no. 4, pp. 501–507, 1999.

[65] M. Miles Braun, N. Badi, R. W. Ryder et al., "A retrospective cohort study of the risk of tuberculosis among women of childbearing age with HIV infection in Zaire," *American Review of Respiratory Disease*, vol. 143, no. 3 I, pp. 501–504, 1991.

[66] C. L. Daley, P. M. Small, G. F. Schecter et al., "An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus: an analysis using restriction-fragment-length polymorphisms," *The New England Journal of Medicine*, vol. 326, no. 4, pp. 231–235, 1991.

[67] R. W. Shafer, S. P. Singh, C. Larkin, and P. M. Small, "Exogenous reinfection with multidrug-resistant Mycobacterium tuberculosis in an immunocompetent patient," *Tubercle and Lung Disease*, vol. 76, no. 6, pp. 575–577, 1995.

[68] S. D. Lawn, L. G. Bekker, K. Middelkoop, L. Myer, and R. Wood, "Impact of HIV infection on the epidemiology of tuberculosis in a peri-urban community in South Africa: the need for age-specific interventions," *Clinical Infectious Diseases*, vol. 42, no. 7, pp. 1040–1047, 2006.

[69] K. DeRiemer, L. M. Kawamura, P. C. Hopewell, and C. L. Daley, "Quantitative impact of human immunodeficiency virus infection on tuberculosis dynamics," *American Journal of Respiratory and Critical Care Medicine*, vol. 176, no. 9, pp. 936–944, 2007.

[70] K. R. Collins, M. E. Quiñones-Mateu, Z. Toossi, and E. J. Arts, "Impact of tuberculosis on HIV-1 replication, diversity, and disease progression," *AIDS Reviews*, vol. 4, no. 3, pp. 165–176, 2002.

[71] S. K. Sharma, A. Mohan, and T. Kadhiravan, "HIV-TB co-infection: Epidemiology, diagnosis & management," *Indian Journal of Medical Research*, vol. 121, no. 4, pp. 550–567, 2005.

[72] M. Badri, R. Ehrlich, R. Wood, T. Pulerwitz, and G. Maartens, "Association between tuberculosis and HIV disease progression in a high tuberculosis prevalence area," *International Journal of Tuberculosis and Lung Disease*, vol. 5, no. 3, pp. 225–232, 2001.

[73] C. Whalen, C. R. Horsburgh, D. Hom, C. Lahart, M. Simberkoff, and J. Ellner, "Accelerated course of human immunodeficiency virus infection after tuberculosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 151, no. 1, pp. 129–135, 1995.

[74] R. Smith, A. Cattamanchi, K. R. Steingart, C. Denkinger, K. Dheda, K. L. Winthrop et al., "Interferon-gamma release assays for diagnosis of latent tuberculosis infection: evidence in immune-mediated inflammatory disorders," *Current Opinion in Rheumatology*, vol. 23, no. 4, pp. 377–384, 2011.

[75] K. L. Winthrop, "Risk and prevention of tuberculosis and other serious opportunistic infections associated with the inhibition of tumor necrosis factor," *Nature Clinical Practice Rheumatology*, vol. 2, no. 11, pp. 602–610, 2006.

[76] K. L. Winthrop and T. Chiller, "Preventing and treating biologic-associated opportunistic infections," *Nature reviews. Rheumatology*, vol. 5, no. 7, pp. 405–410, 2009.

[77] D. P. De Leon, E. Acevedo-Vasquez, S. Alvizuri et al., "Comparison of an interferon-γ assay with tuberculin skin testing for detection of tuberculosis (TB) infection in patients with rheumatoid arthritis in a TB-endemic population," *Journal of Rheumatology*, vol. 35, no. 5, pp. 776–781, 2008.

[78] M. Pai, "Spectrum of latent tuberculosis existing tests cannot resolve the underlying phenotypes," *Nature Reviews Microbiology*, vol. 8, no. 3, p. 242, 2010.

[79] J. P. Cegielski and D. N. McMurray, "The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals," *International Journal of Tuberculosis and Lung Disease*, vol. 8, no. 3, pp. 286–298, 2004.

[80] K. Lönnroth, B. G. Williams, P. Cegielski, and C. Dye, "A consistent log-linear relationship between tuberculosis incidence and body mass index," *International Journal of Epidemiology*, vol. 39, no. 1, Article ID dyp308, pp. 149–155, 2010.

[81] R. K. Chandra, "Nutrition and the immune system: an introduction," *The American Journal of Clinical Nutrition*, vol. 66, no. 2, pp. 460S–463S, 1997.

[82] R. K. Chandra and S. Kumari, "Nutrition and immunity: an overview," *Journal of Nutrition*, vol. 124, supplement 8, pp. 1433S–1435S, 1994.

[83] K. Abba, T. D. Sudarsanam, L. Grobler, and J. Volmink, "Nutritional supplements for people being treated for active tuberculosis," *Cochrane Database of Systematic Reviews*, no. 4, Article ID CD006086, 2008.

[84] G. W. Comstock and C. E. Palmer, "Long-term results of BCG vaccination in the southern United States," *American Review of Respiratory Disease*, vol. 93, no. 2, pp. 171–183, 1966.

[85] J. P. Cegielski, L. Kohlmeier, and J. Cornoni-Huntley, "Malnutrition and tuberculosis in a nationally representative cohort of adults in the United States, 1971–1987," in *Proceedings of the 44th Annual Meeting, American Society of Tropical Medicine and Hygiene*, p. 152, American Society of Tropical Medicine and Hygiene, San Antonio, Tex, USA, 1995.

[86] P. Davies, "The natural history of tuberculosis in children," *Tubercle*, vol. 42, pp. 1–40, 1961.

[87] L. D. Zeidberg, R. S. Gass, A. Dillon, and R. H. Hutcheson, "The Williamson County Tuberculosis Study. A twenty-four-year epidemiologic study," *The American Review of Respiratory Disease*, vol. 87, article 1, 1963.

[88] B. J. Marais, R. P. Gie, H. S. Schaaf et al., "The clinical epidemiology of childhood pulmonary tuberculosis: a critical review of literature from the pre-chemotherapy era," *International Journal of Tuberculosis and Lung Disease*, vol. 8, no. 3, pp. 278–285, 2004.

[89] F. J. Bentley, S. Grzybowski, and B. Benjamin, *Tuberculosis in Childhood and Adolescence, 1954*, National Association for the Prevention of Tuberculosis, London, UK, 1954.

[90] B. J. Marais and P. R. Donald, "The natural history of tuberculosis infection and disease in children," in *Tuberculosis: A Comprehensive Clinical Reference*, H. S. Schaaf and A. Zumla, Eds., Elsevier Health Sciences, 2009.

[91] B. Alisjahbana, R. Van Crevel, E. Sahiratmadja, M. den Heijer, A. Maya, E. Istriana et al., "Diabetes mellitus is strongly associated with tuberculosis in Indonesia," *The International Journal of Tuberculosis and Lung Disease*, vol. 10, no. 6, pp. 696–700, 2006.

[92] S. J. Kim, Y. P. Hong, W. J. Lew, S. C. Yang, and E. G. Lee, "Incidence of pulmonary tuberculosis among diabetics," *Tubercle and Lung Disease*, vol. 76, no. 6, pp. 529–533, 1995.

[93] World Health Organization, "Equity, Social Determinants and Public Health Programmes," Geneva: World Health Organization, http://books.google.co.in/books?id=7JxutqCmctUC, 2010.

[94] W. Rathmann, G. Giani, S. H. Wild et al., "Global prevalence of diabetes: estimates for the year 2000 and projections for 2030," *Diabetes Care*, vol. 27, no. 10, pp. 2568–2569, 2004.

[95] C. Y. Jeon and M. B. Murray, "Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies," *PLoS Med*, vol. 5, no. 7, article e152, 2008.

[96] B. Alisjahbana, E. Sahiratmadja, E. J. Nelwan, A. M. Purwa, Y. Ahmad, T. H. M. Ottenhoff et al., "The effect of type 2

diabetes mellitus on the presentation and treatment response of pulmonary tuberculosis," *Clinical Infectious Diseases*, vol. 45, no. 4, pp. 428–435, 2007.

[97] M. Baker, A. Harries, C. Jeon, J. Hart, A. Kapur, K. Lonnroth et al., "The impact of diabetes on tuberculosis treatment outcomes: a systematic review," *BMC Medicine*, vol. 9, article 81, 2011.

[98] G. W. Martens, M. C. Arikan, J. Lee, F. Ren, D. Greiner, and H. Kornfeld, "Tuberculosis susceptibility of diabetic mice," *American Journal of Respiratory Cell and Molecular Biology*, vol. 37, no. 5, pp. 518–524, 2007.

[99] J. E. Stalenhoef, B. Alisjahbana, E. J. Nelwan et al., "The role of interferon-gamma in the increased tuberculosis risk in type 2 diabetes mellitus," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 27, no. 2, pp. 97–103, 2008.

[100] M. Delamaire, D. Maugendre, M. Moreno, M. C. Le Goff, H. Allannic, and B. Genetet, "Impaired leucocyte functions in diabetic patients," *Diabetic Medicine*, vol. 14, no. 1, pp. 29–34, 1997.

[101] K. E. Dooley and R. E. Chaisson, "Tuberculosis and diabetes mellitus: convergence of two epidemics," *The Lancet Infectious Diseases*, vol. 9, no. 12, pp. 737–746, 2009.

[102] R. M. Anjana, R. Pradeepa, M. Deepa, M. Datta, V. Sudha, R. Unnikrishnan et al., "ICMR-INDIAB Collaborative Study Group: prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: phase I results of the Indian Council of Medical Research-INdia DIABetes (ICMR-INDIAB) study," *Diabetologia*, vol. 54, pp. 3022–3027, 2011.

[103] R. Ruslami, R. E. Aarnoutse, B. Alisjahbana, A. van der ven, and R. van Crevel, "Implications of the global increase of diabetes for tuberculosis control and patient care," *Tropical Medicine & International Health*, vol. 15, no. 11, pp. 1289–1299, 2010.

[104] A. Seidler, A. Nienhaus, and R. Diel, "Review of epidemiological studies on the occupational risk of tuberculosis in low-incidence areas," *Respiration*, vol. 72, no. 4, pp. 431–446, 2005.

[105] J. N. S. Eisenberg, M. A. Desai, K. Levy et al., "Environmental determinants of infectious disease: a framework for tracking causal links and guiding public health research," *Environmental Health Perspectives*, vol. 115, no. 8, pp. 1216–1223, 2007.

[106] C. Dye and B. G. Williams, "The population dynamics and control of tuberculosis," *Science*, vol. 328, no. 5980, pp. 856–861, 2010.

[107] K. Lönnroth, E. Jaramillo, B. G. Williams, C. Dye, and M. Raviglione, "Drivers of tuberculosis epidemics: the role of risk factors and social determinants," *Social Science and Medicine*, vol. 68, no. 12, pp. 2240–2246, 2009.

[108] M. Muniyandi, R. Ramachandran, P. G. Gopi et al., "The prevalence of tuberculosis in different economic strata: a community survey from South India," *International Journal of Tuberculosis and Lung Disease*, vol. 11, no. 9, pp. 1042–1045, 2007.

[109] O. 'Grady J, M. Maeurer, R. Atun, I. Abubakar, P. Mwaba, M. Bates et al., "Tuberculosis in prisons: anatomy of global neglect," *European Respiratory Journal*, vol. 38, no. 4, pp. 752–754, 2011.

[110] C. Raina MacIntyre, N. Kendig, L. Kummer, S. Birago, and N. M. H. Graham, "Impact of tuberculosis control measures and crowding on the incidence of tuberculous infection in Maryland prisons," *Clinical Infectious Diseases*, vol. 24, no. 6, pp. 1060–1067, 1997.

[111] V. Maurya, V. K. Vijayan, and A. Shah, "Smoking and tuberculosis: an association overlooked," *International Journal of Tuberculosis and Lung Disease*, vol. 6, no. 11, pp. 942–951, 2002.

[112] L. Arcavi and N. L. Benowitz, "Cigarette smoking and infection," *Archives of Internal Medicine*, vol. 164, no. 20, pp. 2206–2216, 2004.

[113] D. G. Yanbaeva, M. A. Dentener, E. C. Creutzberg, G. Wesseling, and E. F. M. Wouters, "Systemic effects of smoking," *Chest*, vol. 131, no. 5, pp. 1557–1566, 2007.

[114] M. N. Bates, A. Khalakdina, M. Pai, L. Chang, F. Lessa, and K. R. Smith, "Risk of tuberculosis from exposure to tobacco smoke: a systematic review and meta-analysis," *Archives of Internal Medicine*, vol. 167, no. 4, pp. 335–342, 2007.

[115] K. Slama, C. Y. Chiang, D. A. Enarson et al., "Tobacco and tuberculosis: a qualitative systematic review and meta-analysis," *International Journal of Tuberculosis and Lung Disease*, vol. 11, no. 10, pp. 1049–1061, 2007.

[116] M. Pai, A. Mohan, K. Dheda et al., "Lethal interaction: the colliding epidemics of tobacco and tuberculosis," *Expert Review of Anti-Infective Therapy*, vol. 5, no. 3, pp. 385–391, 2007.

[117] H. H. Lin, M. Ezzati, and M. Murray, "Tobacco smoke, indoor air pollution and tuberculosis: a systematic review and meta-analysis," *PLoS Medicine*, vol. 4, no. 1, article e20, 2007.

[118] E. Houtmeyers, R. Gosselink, G. Gayan-Ramirez, and M. Decramer, "Regulation of mucociliary clearance in health and disease," *European Respiratory Journal*, vol. 13, no. 5, pp. 1177–1188, 1999.

[119] M. Sopori, "Effects of cigarette smoke on the immune system," *Nature Reviews Immunology*, vol. 2, no. 5, pp. 372–377, 2002.

[120] H. Wang, M. Yu, M. Ochani et al., "Nicotinic acetylcholine receptor $\alpha 7$ subunit is an essential regulator of inflammation," *Nature*, vol. 421, no. 6921, pp. 384–388, 2003.

[121] S. Shang, D. Ordway, M. Henao-Tamayo et al., "Cigarette smoke increases susceptibility to tuberculosis-evidence from in vivo and in vitro models," *Journal of Infectious Diseases*, vol. 203, no. 9, pp. 1240–1248, 2011.

[122] K. Lönnroth, B. G. Williams, S. Stadlin, E. Jaramillo, and C. Dye, "Alcohol use as a risk factor for tuberculosis-a systematic review," *BMC Public Health*, vol. 8, article 289, 2008.

[123] A. Fok, Y. Numata, M. Schulzer, and M. J. FitzGerald, "Risk factors for clustering of tuberculosis cases: a systematic review of population-based molecular epidemiology studies," *International Journal of Tuberculosis and Lung Disease*, vol. 12, no. 5, pp. 480–492, 2008.

[124] G. Szabo, "Alcohol's contribution to compromised immunity," *Alcohol Research and Health*, vol. 21, no. 1, pp. 30–38, 1997.

[125] K. R. Smith, "Indoor air pollution in developing countries: recommendations for research," *Indoor Air*, vol. 12, no. 3, pp. 198–207, 2002.

[126] V. K. Mishra, R. D. Retherford, and K. R. Smith, "Biomass cooking fuels and prevalence of tuberculosis in India," *International Journal of Infectious Diseases*, vol. 3, no. 3, pp. 119–129, 1999.

[127] R. Perez-Padilla, C. Perez-Guzman, R. Baez-Saldana, and A. Torres-Cruz, "Cooking with biomass stoves and tuberculosis: a case control study," *The International Journal of Tuberculosis and Lung Disease*, vol. 5, no. 5, pp. 441–447, 2001.

[128] C. Kolappan and R. Subramani, "Association between biomass fuel and pulmonary tuberculosis: a nested case-control study," *Thorax*, vol. 64, no. 8, pp. 705–708, 2009.

[129] A. K. Pokhrel, M. N. Bates, S. C. Verma, H. S. Joshi, C. T. Sreeramareddy, and K. R. Smith, "Tuberculosis and indoor biomass and kerosene use in Nepal: a case-control study," *Environmental Health Perspectives*, vol. 118, no. 4, pp. 558–564, 2010.

[130] J. V. Diaz, J. Koff, M. B. Gotway, S. Nishimura, and J. R. Balmes, "Case report: a case of wood-smoke-related pulmonary disease," *Environmental Health Perspectives*, vol. 114, no. 5, pp. 759–762, 2006.

[131] R. B. Fick Jr., E. S. Paul, and W. W. Merrill, "Alterations n the antibacterial properties of rabbit pulmonary macrophages exposed to wood smoke," *American Review of Respiratory Disease*, vol. 129, no. 1, pp. 76–81, 1984.

[132] J. T. Zelikoff, C. C. Lung, M. D. Cohen, and R. B. Schlesinger, "The toxicology of inhaled woodsmoke," *Journal of Toxicology and Environmental Health—part B: critical Reviews*, vol. 5, no. 3, pp. 269–282, 2002.

[133] B. C. Boman, A. B. Forsberg, and B. G. Järvholm, "Adverse health effects from ambient air pollution in relation to residential wood combustion in modern society," *Scandinavian Journal of Work, Environment and Health*, vol. 29, no. 4, pp. 251–260, 2003.

[134] N. Bruce, R. Perez-Padilla, and R. Albalak, "Indoor air pollution in developing countries: a major environmental and public health challenge," *Bulletin of the World Health Organization*, vol. 78, no. 9, pp. 1078–1092, 2000.

[135] M. Ezzati and D. M. Kammen, "The health impacts of exposure to indoor air pollution from solid fuels in developing countries: knowledge, gaps, and data needs," *Environmental Health Perspectives*, vol. 110, no. 11, pp. 1057–1068, 2002.

[136] L. Wang, "Tuberculosis among aboriginal and nonaboriginal persons in British Columbia," *Canadian Respiratory Journal*, vol. 7, no. 2, pp. 151–157, 2000.

[137] A. J. Plant, V. L. Krause, J. R. Condon, and C. Kerr, "Aborigines and tuberculosis: why they are at risk," *Australian Journal of Public Health*, vol. 19, no. 5, pp. 487–491, 1995.

[138] M. Clark and E. Vynnycky, "The use of maximum likelihood methods to estimate the risk of tuberculous infection and disease in a Canadian First Nations population," *International Journal of Epidemiology*, vol. 33, no. 3, pp. 477–484, 2004.

[139] M. Clark, P. Riben, and E. Nowgesic, "The association of housing density, isolation and tuberculosis in Canadian First Nations communities," *International Journal of Epidemiology*, vol. 31, no. 5, pp. 940–945, 2002.

[140] C. M. T. Greenwood, T. M. Fujiwara, L. J. Boothroyd et al., "Linkage of tuberculosis to chromosome 2q35 loci, including NRAMP1, in a large Aboriginal Canadian family," *American Journal of Human Genetics*, vol. 67, no. 2, pp. 405–416, 2000.

[141] World Health Organization, "Global Tuberculosis Control," http://www.who.int/tb/country/en/index.html, 2010.

[142] J. Golub, S. Bur, W. A. Cronin et al., "Delayed tuberculosis diagnosis and tuberculosis transmission," *International Journal of Tuberculosis and Lung Disease*, vol. 10, no. 1, pp. 24–30, 2006.

[143] H. H. Lin, M. Murray, T. Cohen, C. Colijn, and M. Ezzati, "Effects of smoking and solid-fuel use on COPD, lung cancer, and tuberculosis in China: a time-based, multiple risk factor, modelling study," *The Lancet*, vol. 372, no. 9648, pp. 1473–1483, 2008.

[144] M. Demissie, B. Lindtjorn, and Y. Berhane, "Patient and health service delay in the diagnosis of pulmonary tuberculosis in Ethiopia," *BMC Public Health*, vol. 2, no. 1, article 23, 2002.

[145] C. Lienhardt, J. Rowley, K. Manneh et al., "Factors affecting time delay to treatment in a tuberculosis control programme in a sub-Saharan African country: the experience of The Gambia," *International Journal of Tuberculosis and Lung Disease*, vol. 5, no. 3, pp. 233–239, 2001.

[146] M. Pai, A. Zwerling, and D. Menzies, "Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update," *Annals of Internal Medicine*, vol. 149, no. 3, pp. 177–184, 2008.

[147] E. L. Corbett, T. Bandason, T. Duong et al., "Comparison of two active case-finding strategies for community-based diagnosis of symptomatic smear-positive tuberculosis and control of infectious tuberculosis in Harare, Zimbabwe (DETECTB): a cluster-randomised trial," *The Lancet*, vol. 376, no. 9748, pp. 1244–1253, 2010.

[148] S. Satyanarayana, S. A. Nair, S. S. Chadha, R. Shivashankar, G. Sharma, S. Yadav et al., "From where are tuberculosis patients accessing treatment in India? Results from a cross-sectional community based survey of 30 districts," *PLoS ONE*, vol. 6, no. 9, Article ID e24160, 2011.

[149] V. Gajalakshmi, R. Peto, T. S. Kanaka, and P. Jha, "Smoking and mortality from tuberculosis and other diseases in India: retrospective study of 43 000 adult male deaths and 35000 controls," *The Lancet*, vol. 362, no. 9383, pp. 507–515, 2003.

[150] C. P. Wen, T. C. Chan, H. T. Chan, M. K. Tsai, T. Y. Cheng, and S. P. Tsai, "The reduction of tuberculosis risks by smoking cessation," *BMC Infectious Diseases*, vol. 10, article 156, 2010.

[151] K. Lönnroth and M. C. Raviglione, "Global epidemiology of tuberculosis: prospects for control," *Seminars in Respiratory and Critical Care Medicine*, vol. 29, no. 5, pp. 481–491, 2008.

Hindawi Publishing Corporation
Pulmonary Medicine
Volume 2013, Article ID 601737, 11 pages
http://dx.doi.org/10.1155/2013/601737

Review Article

Interferon-Gamma Release Assays versus Tuberculin Skin Testing for the Diagnosis of Latent Tuberculosis Infection: An Overview of the Evidence

A. Trajman,[1,2] R. E. Steffen,[3] and D. Menzies[2]

[1] *Gama Filho University, 20740-900 Rio de Janeiro, RJ, Brazil*
[2] *Montreal Chest Institute, McGill University, Montreal, QC, Canada H2X 2P4*
[3] *Federal University of Rio de Janeiro, 21941-913 Rio de Janeiro, RJ, Brazil*

Correspondence should be addressed to A. Trajman; atrajman@gmail.com

Received 5 October 2012; Accepted 10 January 2013

Academic Editor: Jonathan Golub

A profusion of articles have been published on the accuracy and uses of interferon-gamma releasing assays. Here we review the clinical applications, advantages, and limitations of the tuberculin skin test and interferon-gamma release assays and provide an overview of the most recent systematic reviews conducted for different indications for the use of these tests. We conclude that both tests are accurate to detect latent tuberculosis, although interferon-gamma release assays have higher specificity than tuberculin skin testing in BCG-vaccinated populations, particularly if BCG is received after infancy. However, both tests perform poorly to predict risk for progression to active tuberculosis. Interferon-gamma release assays have significant limitations in serial testing because of spontaneous variability and lack of a validated definition of conversion and reversion, making it difficult for clinicians to interpret changes in category (conversions and reversions). So far, the most important clinical evidence, that is, that isoniazid preventive therapy reduces the risk for progression to disease, has been produced only in tuberculin skin test-positive individuals.

1. Introduction

Tuberculosis (TB) is an important cause of morbidity and mortality worldwide [1]. Governmental and non-governmental organization efforts and investments in the last decades to control the epidemic have resulted in a steady decline in disease incidence and mortality [2]. One third of the world population, however, has latent tuberculosis (TB) infection (LTBI), and to reach the United Nations Millennium Goals of eliminating the disease by 2050, it is its necessary to couple diagnosis and treatment of active disease with new approaches to reduce this vast reservoir of LTBI, sufficient for generating new TB cases for many decades even if transmission was suppressed [3]. Thus, in addition to rapid, accurate, and inexpensive detection of active TB, the detection—and treatment—of LTBI is also an important strategy for TB control [1]. In the present paper, we summarize the advantages and limitations of tuberculin skin testing (TST) and overview the evidence for the use of the newer interferon-gamma release assays (IGRA) for the diagnosis of LTBI (Table 1).

2. Tuberculin Skin Testing

Until the beginning of this century, TST was the only diagnostic method for detecting LTBI. The test is based on a delayed-type hypersensitivity reaction that occurs when those infected with *M. tuberculosis* are exposed to certain antigenic components present in extracts of culture filtrates, the "tuberculins." In this type of reaction, T cells, sensitized by prior infection, are recruited to the skin where the tuberculin was injected and release lymphokines. The result is local induration of the skin through local vasodilatation, edema, fibrin deposition, and recruitment of other inflammatory cells to the area [4]. An induration greater than 5 mm is widely accepted as a positive reaction. Different cut-off sizes can be considered. Although widely used, TST has limitations. TST sensitivity may be reduced by malnutrition, severe

TB diseases and immunodeficiency, such as that related to HIV [5, 6]. Decreased TST specificity might occur in settings where nontuberculous mycobacteria (NTM) are prevalent and in populations who have received BCG vaccination after infancy, although the effect of BCG vaccination on TST reactions is very modest after 10 years or more if vaccination is given in infancy [7]. Additionally, completing the TST requires two health care visits, for tuberculin injection and induration measurement, which results in loss of reading in approximately 10% of cases [8]. Also, measurement of reaction size is subject to interobserver variability, although this is greatly reduced with adequate training [9–11]. In addition, one positive TST result does not distinguish recent from remote infection, which has a lower risk of progression to disease [12, 13]. Although TST reversions are shown to occur, they are more common in older adults, estimated at 8% per year [14]. When a tuberculin reaction reaches 10 mm or greater, the additional tests becomes uninterpretable [15], meaning that serial TST has no place in monitoring treatment response. Moreover, repeated injections of tuberculin are known to elicit the booster phenomenon [15]. For this reason, in situations where serial testing is indicated, a two-step tuberculin test is recommended at the time of first testing [16]. Finally, in low- and medium-income countries with a high-TB incidence, diagnosis of LTBI is reported to be difficult because of the extra workload brought by TST [17]. In those countries, detection and treatment of active TB is the priority, although some could invest in detection and treatment of LTBI if an easier test was available [18].

Yet, TST has many advantages. It is a century-old test that has been widely used, and its clinical applications are very well studied. Cut-off points have been established for indication of isoniazid preventive therapy (IPT) for different ages and risk groups [19]. More importantly, the benefit of IPT in groups with a positive TST has been widely proven, as has been the lack of benefit of IPT in TST-negative subjects [19, 20]. Finally, there are very clear definitions of conversion and boosting [15]. Conversion is defined as an induration over 10 mm with an increase of at least 6 mm over the previous result. This is because the standard deviation of repeated readings (intra- and interobserver variation and within-subject variation) is 3 mm. It is thus expected that 95% of subjects will have an increase of less than 6 mm (two standard deviations) not related to TB exposure. In summary, the main application of TST is to detect LTBI, but its role alone for evaluating the risk for progression to disease is limited. It must be interpreted in light of the clinical situation and epidemiological history to allow for evaluation of risk of disease.

3. Interferon-Gamma Release Assays (IGRAs)

IGRAs were designed to detect the immune response to specific *M. tuberculosis* antigens, which are not present in BCG or certain nontuberculous mycobacteria. Two tests are commercially available; one is based on the Elispot (T-SPOT.TB, Oxford Immunotec, UK) and the other on the enzyme-linked immunosorbent assays (ELISA) technique (QuantiFERON-TB Gold-in-Tube, Cellestis, Australia, QFT-GIT). Both tests are based on the principle that the T cells of an individual who have acquired TB infection will respond by secreting the cytokine interferon-gamma (IFN-γ) when restimulated with *M. tuberculosis* antigens [21]. To avoid cross-reactivity, these tests use antigens encoded in the region of difference 1 (RD1), a portion of the *Mycobacterium tuberculosis* genome that is absent from the genome of BCG and many NTM [21]. The QFT-GIT uses the patient's whole-blood and is based on the ELISA measurement of IFN-γ produced in response to three *M. tuberculosis* antigens (ESAT-6, CFP-10, and TB7.7). The second test, T-SPOT.TB, is based on the enzyme-linked immunospot measurement of the number of peripheral mononuclear cells that produce IFN-γ after stimulation with two mycobacterial antigens: ESAT-6 and CFP-10.

There have been several publications on IGRAs' accuracy, predictive value, cost-effectiveness, and indications since they became available commercially. Many high-income countries have incorporated these tests in their national guidelines [32]. However, the World Health Organization (WHO) has recently published recommendations against their use in low- and medium-income countries (LMIC) [33], since most IGRA studies have been done in high-income countries and mere extrapolation to low- and middle-income settings with high background TB infection rates being not appropriate. In addition, systematic reviews have suggested that IGRA performance differs in high- versus low-TB and HIV incidence settings, with relatively lower sensitivity in high-burden settings [33]. One other important aspect against their use in LMICs is the cost. Given similar performance but higher costs, IGRAs replacing TSTs as a public health intervention in settings with limited budget being not recommended.

4. Accuracy of IGRAs to Diagnose LTBI

The evaluation of the accuracy of these tests to diagnose LTBI has been hampered by the fact that there is no gold standard. Evaluation of new techniques, therefore, relies on the use of proxies for LTBI such as active TB, TB exposure gradients, rates of TB incidence in untreated cohorts and results of randomized clinical trials comparing the benefit of preventive treatment in patients who test positive and negative. Here, we briefly review the evidence using each of these approaches.

4.1. Active TB. Most of the studies on sensitivity were performed in patients with active TB, relying on the principle that patients with active TB are necessarily infected. The main drawback of this approach is that patients with active TB usually have immunosuppression due to the disease itself or to malnutrition, hampering the ability to respond both to tuberculin and to specific *M. tuberculosis* antigens present in IGRA tests [22]. Thus, sensitivity of both TST and IGRA tests for LTBI is likely to be underestimated in these studies. Conversely, specificity has been evaluated in subjects believed not to have been exposed to TB antigens, which is

Table 1: Comparison of TST and IGRA regarding several tests' characteristics.

Test characteristics	TST	QFT	T-SPOT.TB
Overall sensitivity [22]	77%	78%	92%
HIV uninfected [9]		84%	88%
HIV infected [18]		65%	68%
Overall specificity [22]	97%	98%	93%
HIV uninfected		NA	NA
HIV infected [18]		52%	61%
Decreased specificity if BCG vaccinated?	Yes, particularly if done after infancy or repeatedly [22]	No effect [22]	
Prone to nonspecific variations in test results?	Yes, from reader variability [10]	Yes, from innumerous factors [23–28]	
Thresholds for conversions evidence based?	Yes [15]	No	No
Prone to conversions?	Yes, mostly due to TB exposure	Yes (high when only negative-positive definitions are used) [29]	
Prone to reversions?	Yes [29]	High in individuals with weak IGRA positivity and discordance with TST [29]	
Boosting?	Yes [15]	Yes, possibly if given 3 days after performing TST [24]	
Conversion associated with risk of progression to active TB?	Yes (strong evidence)	No direct evidence	
Ability to detect those at high risk of developing active TB	Weak [30, 31]	Weak [30, 31]	
Preventive treatment of individuals with conversion reduces risk of progression to active TB?	Yes [19, 20]	No evidence	

difficult to prove. In a metaanalysis, Pai et al. [22] assessed the accuracy of IGRAs for the diagnosis of LTBI. Sensitivity was determined by using patients with newly diagnosed active TB as a surrogate of latent infection. This meta-analysis included studies performed in low- and high TB-burden countries and found a pooled sensitivity of 78% (95% confidence interval (CI), 73% to 82%) for QuantiFERON-TB Gold and 92% (CI, 90% to 93%) for T-SPOT.TB. Sensitivities of both TST and Quanti-FERON were highly heterogeneous, possibly due to HIV coinfection, advanced disease, and malnourishment, which reduce immune response. Indeed, in a study of mostly smear negative active TB patients, that is, with probably early disease, 10% were TST negative [34], while in a study in Japan, 43% of patients with advanced TB were TST negative [35]. The specificity studies were all from countries with low rate of TB incidence with varying BCG policies and found a pooled specificity of 98% (CI, 96% to 99%) for all QFT studies, with little difference among BCG and non-BCG-vaccinated populations (96% versus 99%, resp.) and 93% (CI, 86% to 100%) for T-SPOT.TB (only one study among BCG-vaccinated subjects). The TST sensitivity was similar, with a pooled estimate of 77% (CI, 71% to 82%). Specificity, however, showed a more marked difference between non-BCG and BCG-vaccinated participants. In non-BCG-vaccinated subjects, the pooled estimate was consistently high, 97% (CI, 95% to 99%) but in the BCG vaccinated population specificity was only 59% (CI, 46% to 73%). Four of the 6 studies, however, were from countries where BCG vaccination is done after infancy or done multiple times [36]. No studies have directly compared TST and IGRAs in populations that were BCG vaccinated only at birth, where specificity of TST is much higher.

4.2. Exposure Gradients. Usually, TB exposure is captured either as a dichotomous variable (exposed and unexposed) or as a gradient (degree of exposure is graded according to duration, or proximity). In exposure gradient studies, contacts are assigned to 3 or more categories from most to least exposed. The proportion of positive results should parallel the exposure gradient. The best test should demonstrate the closest correlation of proportion positive to this gradient. The main challenge with these methods is the difficulty in quantifying the exact degree of exposure of contacts. In addition, sometimes the criteria for exposure are different among studies, making pooled estimates difficult. A total of 10 studies in pediatric populations used this method [37–46]. There was great variation in the definition of the exposure groups. For TST, most studies used a 10 mm cut-off point, while 2 studies used 5 mm and one study 15 mm. The overall pooled odds ratio (OR) was 1.34 (CI, 0.66–2.72), 1.93 (CI, 0.98–3.77), and 1.83 (CI, 0.67–5.02) for TST when

5 mm, 10 mm, or 15 mm were used, respectively, as the cut-off value [47]. For QuantiFERON, the pooled OR was 3.51 (CI, 1.85–6.66), and for T-SPOT it was 1.31 (CI, 0.76–2.27) [47]. All confidence intervals overlapped, indicating similar performance for all tests [47].

4.3. Incidence of TB in Cohorts. More important than identifying individuals with LTBI is the recognition of those who will progress to active disease, because those are the subjects who will benefit from isoniazid preventive therapy (IPT). So far, IGRAs have been shown to have a low predictive ability for progression from LTBI to active disease although individuals with positive IGRA have slightly higher risk than those with positive TST results [30, 48–51].

Two recent meta-analyses have reviewed the predictive value of TST and QFT [30, 31]. One review included fifteen studies comparing the incidence of active TB in IGRA-positive and TST-positive subjects [31], including studies in children, healthcare workers, and PLWHA [47, 52, 53]. Among these studies, 5 looked at the risk for progression to TB in PLWHA [54–58]. Three showed a higher risk in IGRA-positive compared to IGRA-negative subjects [54–56], but 2 did not [57, 58]. By contrast, the risk of developing active TB in TST-positive PLWHA is well documented. Moreover, the benefit of IPT in TST-positive PLWHA has been confirmed in many studies [20, 59]. Overall, the incidence rate of TB over a median time of 4 years was very low in both TST- and IGRA-positive test subjects (3.7 to 4.8 and 1.0 to 4.5 per 1000 persons-year, resp.). These low incidence rates show that both tests have a low predictive value for active disease.

The second review on the predictive value of IGRAs [30] included more studies, since it did not exclude those in which IGRA results were not blinded to the treating physician, ignoring the risk of "incorporation bias" of these studies. Although the authors found that progression rates for commercial IGRAs were significantly higher than those for the TST (2.7% versus 1.5%), they also concluded that they are both low in absolute numbers [30].

4.4. Evidence of Benefit from Isoniazid Preventive Therapy. More important than establishing the accuracy and predictive value of these tests is to evaluate the benefit from IPT in reducing the risk for progression to active disease in subjects with a positive result. While there is very strong evidence of the protection from IPT in TST-positive subjects [19, 20], so far there are no studies showing direct evidence of the efficacy of IPT based on IGRA results. Indirect evidence of this benefit is based on isoniazid protection against TB in patients who have a positive TST. A recent study in Botswana has shown that long-term IPT in TST-positive persons living with HIV/AIDS (PLWHA) has a strong impact on survival. The study has also shown that isoniazid has little benefit in PLWHA TST-negative individuals [20, 59].

TST conversion usually reflects recent infection. The risk of progression to disease in recent converters is still higher, thus, IPT is recommended, regardless of HIV status or background of TB incidence. However, for PLWHA who become TST positive after initiating highly active antiretroviral therapy (HAART), conversion should be interpreted with caution, as it may be due to immune reconstitution and not recent infection.

Given the similarity of TST and IGRAs in cohort studies, it is reasonable to believe that IPT in IGRA-positive individuals should provide at least the same benefit as in TST-positive individuals [60], but the lack of benefit in IGRA-negative individuals is yet to be evaluated.

5. IGRA as a Marker of Cure Following Treatment of LTBI

As previously remarked, TST reversion is not associated with successful treatment of LTBI. To test the usefulness of IGRAs to monitor TB and LTBI treatment, IGRA tests have been applied serially during and after treatment of patients with active TB and of healthy subjects undergoing LTBI treatment [61–76]. Overall, results were highly contradictory, with increases or decreases of interferon-gamma levels being observed randomly, regardless of clinical response to treatment [77]. Chee et al. [63] compared QFT-GIT and the T-SPOT.TB at baseline, after treatment completion and after 6 months in sputum-positive, immunocompetent patients. Although there was a significant rate of reversions (13.9% for T-SPOT.TB versus 39.2% for QFT-IT), a substantial proportion of patients remained test positive 6 months after TB treatment (79% and 46% with T-SPOT.TB and QFT-GIT, resp.). There are no long-term studies of IGRA in the followup of subjects treated for LTBI, but a few short-term studies have shown that a decrease in quantitative results can occur after IPT, regardless of reversion. Dyrhol-Riise et al. [71] tested the performance of QFT-GIT for monitoring LTBI treatment in patients before and three and 15 months after the beginning of isoniazid and rifampicin treatment. The IFN-γ responses were comparable at the three time points, with no statistically significant change during the course of treatment. A recent meta-analysis supports these findings [77]. In summary, IGRAs have, at present, no place in monitoring response to treatment of TB or LTBI.

6. LTBI in Special Populations

The accuracy of IGRA tests have been tested in distinct subpopulations such as children [38, 47, 48, 56, 78–84], PLWHA [23, 29, 39, 40, 47, 54, 55, 85–103], other immunosuppressed patients (those with renal disease or in use of tumor necrosis factor-alpha inhibitors), [104–106] and healthcare workers [53].

6.1. Immunosuppressed Patients. As in immunocompetent populations, the sensitivity of IGRA tests in PLWHA has also been analyzed in patients with active TB. In 18 studies, 16 of them in LMIC [52], sensitivities of both TST and IGRA tests were lower in PLWHA because both require an adequate immune response. Pooled sensitivity of QFT-GIT was 60% (CI, 34 to 82%). T-SPOT.TB seemed slightly less affected by immunosuppression, with a pooled sensitivity of 76%

(CI, 45% to 92%) [18], but only five studies compared their sensitivity head to head [93, 107–110], with only one study showing QFT-GIT with a greater sensitivity than T-SPOT.TB in PLWHA [110]. Likewise, 14 studies have analyzed the role of IGRA in patients with autoimmune disease [111–124], 10 of them in countries where BCG vaccination is not routinely done [111–113, 116–118, 120, 121, 123, 124]. Most studies included a small number of patients, immunosuppressive therapy was variable or not yet started, and very few were conducted in countries with high-TB prevalence. Above all, no longitudinal study to assess the risk of progression to disease was carried out in this population [106]. Finally, there are no studies on the role of IGRA tests among transplanted patients under immunosuppressive therapy.

As mentioned above, special caution is needed when interpreting conversion and reversions in PLWHA. Reversion can be a consequence of declining immunity and should alert the physician. In contrast, LTBI/TB treatment of naive TST-negative individuals with advanced HIV infection (CD4+ cell count <200/μL) started on HAART should be retested for LTBI once they achieve CD4+ cell counts >200/μL [125].

6.2. Children. Regarding LTBI in the youngest, although a few studies have shown a lower sensitivity of IGRA tests in very young children [39, 40, 47, 87, 126–128], the sensitivity and specificity of IGRA in older children exposed to index cases are similar than in adults, as long as they are not infected by HIV [47]. Although BCG is not expected to interfere with IGRAs, the lower sensitivity found both in TST and IGRAs among very young children might be explained by immune immaturity or by capturing a population with underlying conditions that may interfere with immunity, such as coinfections with helminths and malnutrition [129]. Overall, the sensitivity and specificity among all children with active TB were similar for TST, QFT-G/QFT-GIT (QFT), and T-SPOT.TB, with a pooled sensitivity of 80% (CI, 70% to 90%), 83% (CI, 75% to 92%), and 84% (63% to 100%), respectively. Pooled specificity for TST, QFT, and T-SPOT.TB was 85% (CI, 63% to 100%), 91% (CI, 78% to 100%), and 94% (87% to 100%), respectively [47].

6.3. Highly Exposed Populations and Serial Testing. Highly exposed groups, such as health care workers and prisoners, as well as patients at high risk for disease, such as PLWHA, are screened for LTBI every 6 to 12 months. Although it has been described that tuberculin injection may also result in boosting of IGRA responses, this effect is not seen if QFT-GIT or T-SPOT.TB is done within 3 days of performing the TST [24, 130].

Unlike TST, which should only be repeated if previously negative, IGRA tests may be repeated, regardless of their previous results. However, serial testing subjects not undergoing treatment indicate that there are high rates of spontaneous reversions and conversions, although it is always harder to know if conversion is spontaneous or a consequence of real TB infections [23, 131–135]. Other possible explanations for a conversion would be nonspecific infections and vaccines, laboratorial technique variability, such as blood withdrawal preparation, delay to and time of incubation, and lot of the kit. Biological variables might also interfere. Although there is some evidence that QFT-GIT conversion is related to a greater risk of progression to TB [136], the predictive value of IGRA (QFT) conversion for the development of TB disease is still controversial, and fluctuations in IFN-γ responses among serially tested individuals reported in longitudinal studies remain unexplained and nonspecific.

In addition, spontaneous reversions are more likely to occur in subjects with borderline results, close to the cut-off value, are not related to treatment, and are more likely if TST negative [24, 137–140]. In such cases, it has been interpreted as self-clearance of infection, but there is not enough evidence for such a conclusion [71, 125, 141]. For these reasons, serial testing with QFT-GIT and T.SPOT-TB should be considered unreliable and is not recommended at least until the conversion and reversion phenomena are better understood.

7. Conclusions and Recommendations

There is still a lot to be learned about IGRAs. Until then, IGRAs can be interpreted similarly to TST for the diagnosis of LTBI; with the exception of populations BCG vaccinated after infancy, IGRAs have a better positive predictive value because of their higher specificity.

More importantly, although both TST and IGRAs are useful for detecting LTBI, neither test is all that accurate for predicting risk for disease alone, and positive tests must be interpreted in light of other clinical risk factors. LTBI testing screening is recommended only for those with a known risk factor for progression to disease, such as recent infection, young age, and immune suppression. Clinicians can be confronted with discordant TST and IGRA results and be unsure of how to manage these patients. Because protection from IPT is well established only among TST+ subjects, and because spontaneous reversion of positive IGRA is very common if the TST is negative, the authors recommend not treating TST−/IGRA+ individuals, unless they have very high risk for disease, as in HIV-positive individuals, and are clearly exposed to TB, in which case treatment for LTBI should be considered [142].

Physicians still need to rely on clinical and epidemiological grounds in order to decide if IPT is indicated in an individual patient with a positive TST or IGRA. This decision should take into account the presence of risk factors for development of disease and the risk for hepatotoxicity from IPT, which increases with age. Patients' preferences should also be considered. To help physicians decide, an interesting tool has been developed and is accessible at http://www.tstin3d.com [12].

As for treatment monitoring and other uses of serial testing, more knowledge is needed before we can incorporate IGRAs in clinical practice.

Acknowledgments

This review was supported by CNPq (558383/2009-2), FAPERJ (E-26/102.712/2008 and E-26/110.637/2012), and

ICOHRTA (5U 2R TW006883-02). None of the funding agencies is responsible for the statements in this paper. The authors declare no conflict of interests.

References

[1] World Health Organization, *Global Tuberculosis Control: WHO Report 2011*, World Health Organization, Geneva, Switzerland, 2011.

[2] C. Dye, C. J. Watt, D. M. Bleed, S. M. Hosseini, and M. C. Raviglione, "Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally," *Journal of the American Medical Association*, vol. 293, no. 22, pp. 2767–2775, 2005.

[3] C. Dye and B. G. Williams, "Eliminating human tuberculosis in the twenty-first century," *Journal of the Royal Society Interface*, vol. 5, no. 23, pp. 653–662, 2008.

[4] R. E. Huebner, M. F. Schein, and J. B. Bass, "The tuberculin skin test," *Clinical Infectious Diseases*, vol. 17, no. 6, pp. 968–975, 1993.

[5] E. L. Pesanti, "The negative tuberculin test: tuberculin, HIV, and anergy panels," *American Journal of Respiratory and Critical Care Medicine*, vol. 149, no. 6, pp. 1699–1709, 1994.

[6] R. E. Huebner, M. E. Villarino, and D. E. Snider, "Tuberculin skin testing and the HIV epidemic," *Journal of the American Medical Association*, vol. 267, no. 3, pp. 409–410, 1992.

[7] M. Farhat, C. Greenaway, M. Pai, and D. Menzies, "False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria?" *International Journal of Tuberculosis and Lung Disease*, vol. 10, no. 11, pp. 1192–1204, 2006.

[8] R. Diel, A. Nienhaus, C. Lange, and T. Schaberg, "Cost-optimisation of screening for latent tuberculosis in close contacts," *European Respiratory Journal*, vol. 28, no. 1, pp. 35–44, 2006.

[9] J. E. Bearman, H. Kleinman, V. V. Glyer, and O. M. Lacroix, "A study of variability in tuberculin test reading," *American Review of Respiratory Disease*, vol. 90, pp. 913–919, 1964.

[10] S. D. Chaparas, H. M. Vandiviere, and I. Melvin, "Tuberculin test. Variability with the Mantoux procedure," *American Review of Respiratory Disease*, vol. 132, no. 1, pp. 175–177, 1985.

[11] E. Perez-Stable and G. Slutkin, "A demonstration of lack of variability among six tuberculin skin test readers," *American Journal of Public Health*, vol. 75, no. 11, pp. 1341–1343, 1985.

[12] D. Menzies, G. Gardiner, M. Farhat, C. Greenaway, and M. Pai, "Thinking in three dimensions: a web-based algorithm to aid the interpretation of tuberculin skin test results," *International Journal of Tuberculosis and Lung Disease*, vol. 12, no. 5, pp. 498–505, 2008.

[13] E. Vynnycky and P. E. M. Fine, "The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection," *Epidemiology and Infection*, vol. 119, no. 2, pp. 183–201, 1997.

[14] S. Grzybowski and E. A. Allen, "The challenge of tuberculosis in decline. A study based on the epidemiology of tuberculosis in ontario, Canada," *The American Review of Respiratory Disease*, vol. 90, pp. 707–720, 1964.

[15] D. Menzies, "Interpretation of repeated tuberculin tests: boosting, conversion, and reversion," *American Journal of Respiratory and Critical Care Medicine*, vol. 159, no. 1, pp. 15–21, 1999.

[16] J. B. Bass Jr. and R. A. Serio, "The use of repeat skin tests to eliminate the booster phenomenon in serial tuberculin testing," *American Review of Respiratory Disease*, vol. 123, no. 4, pp. 394–396, 1981.

[17] D. Menzies, H. Al Jahdali, and B. Al Otaibi, "Recent developments in treatment of latent tuberculosis infection," *Indian Journal of Medical Research*, vol. 133, no. 3, pp. 257–266, 2011.

[18] J. Z. Metcalfe, C. K. Everett, K. R. Steingart, A. Cattamanchi, L. Huang, P. C. Hopewell et al., "Interferon-γ release assays for active pulmonary tuberculosis diagnosis in adults in low- and middle-income countries: systematic review and meta-analysis," *Journal of Infectious Diseases*, vol. 204, supplement 4, pp. S1120–S1129, 2011.

[19] H. C. Bucher, L. E. Griffith, G. H. Guyatt et al., "Isoniazid prophylaxis for tuberculosis in HIV infection: a meta-analysis of randomized controlled trials," *AIDS*, vol. 13, no. 4, pp. 501–507, 1999.

[20] T. Samandari, T. B. Agizew, S. Nyirenda et al., "6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial," *The Lancet*, vol. 377, no. 9777, pp. 1588–1598, 2011.

[21] P. Andersen, M. E. Munk, J. M. Pollock, and T. M. Doherty, "Specific immune-based diagnosis of tuberculosis," *The Lancet*, vol. 356, no. 9235, pp. 1099–1104, 2000.

[22] M. Pai, A. Zwerling, and D. Menzies, "Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update," *Annals of Internal Medicine*, vol. 149, no. 3, pp. 177–184, 2008.

[23] M. Pai, R. Joshi, S. Dogra et al., "T-cell assay conversions and reversions among household contacts of tuberculosis patients in rural India," *International Journal of Tuberculosis and Lung Disease*, vol. 13, no. 1, pp. 84–92, 2009.

[24] K. Dheda, R. N. Van Zyl-Smit, L. A. Sechi et al., "Utility of quantitative T-cell responses versus unstimulated interferon-γ for the diagnosis of pleural tuberculosis," *European Respiratory Journal*, vol. 34, no. 5, pp. 1118–1126, 2009.

[25] A. K. Detjen, L. Loebenberg, H. M. S. Grewal et al., "Short-term reproducibility of a commercial interferon gamma release assay," *Clinical and Vaccine Immunology*, vol. 16, no. 8, pp. 1170–1175, 2009.

[26] D. Doberne, R. L. Gaur, and N. Banaei, "Preanalytical delay reduces sensitivity of QuantiFERON-TB gold in-tube assay for detection of latent tuberculosis infection," *Journal of Clinical Microbiology*, vol. 49, no. 8, pp. 3061–3064, 2011.

[27] F. C. Ringshausen, A. Nienhaus, J. Torres Costa, H. Knoop, S. Schlosser, G. Schultze-Werninghaus et al., "Within-subject variability of *Mycobacterium tuberculosis*-specific gamma interferon responses in German health care workers," *Clinical and Vaccine Immunology*, vol. 18, no. 7, pp. 1176–1182, 2011.

[28] M. Slater, J. Parsonnet, and N. Banaei, "Investigation of false-positive results given by the QuantiFERON-TB gold in-tube assay," *Journal of Clinical Microbiology*, vol. 50, no. 9, pp. 3105–3107, 2012.

[29] M. Pai and R. O'Brien, "Serial testing for tuberculosis: can we make sense of T cell assay conversions and reversions?" *PLoS Medicine*, vol. 4, no. 6, Article ID e208, 2007.

[30] R. Diel, R. Loddenkemper, and A. Nienhaus, "Predictive value of interferon-gamma release assays and tuberculin skin testing for predicting progression from latent TB infection to disease state: a meta-analysis," *Chest*, vol. 142, no. 1, pp. 63–75, 2012.

[31] M. X. Rangaka, K. A. Wilkinson, J. R. Glynn, D. Ling, D. Menzies, J. Mwansa-Kambafwile et al., "Predictive value of interferon-gamma release assays for incident active tuberculosis: a systematic review and meta-analysis," *The Lancet Infectious Diseases*, vol. 12, no. 1, pp. 45–55, 2012.

[32] C. M. Denkinger, K. Dheda, and M. Pai, "Guidelines on interferon-γ release assays for tuberculosis infection: concordance, discordance or confusion?" *Clinical Microbiology and Infection*, vol. 17, no. 6, pp. 806–814, 2011.

[33] World Health Organization, *Use of Tuberculosis Interferon-Gamma Release Assays (IGRAs) in Low- and Middle-Income Countries: Policy Statement*, World Health Organization, Geneva, Switzerland, 2011.

[34] S. J. Tsiouris, D. Coetzee, P. L. Toro, J. Austin, Z. Stein, and W. El-Sadr, "Sensitivity analysis and potential uses of a novel gamma interferon release assay for diagnosis of tuberculosis," *Journal of Clinical Microbiology*, vol. 44, no. 8, pp. 2844–2850, 2006.

[35] Y. Kobashi, K. Mouri, S. Yagi et al., "Usefulness of the QuantiFERON TB-2G test for the differential diagnosis of pulmonary tuberculosis," *Internal Medicine*, vol. 47, no. 4, pp. 237–243, 2008.

[36] A. Zwerling, M. A. Behr, A. Verma, T. F. Brewer, D. Menzies, and M. Pai, "The BCG world atlas: a database of global BCG vaccination policies and practices," *PLoS Medicine*, vol. 8, no. 3, Article ID e1001012, 2011.

[37] J. K. Chun, C. K. Kim, H. S. Kim et al., "The role of a whole blood interferon-γ assay for the detection of latent tuberculosis infection in Bacille Calmette-Guérin vaccinated children," *Diagnostic Microbiology and Infectious Disease*, vol. 62, no. 4, pp. 389–394, 2008.

[38] L. Bianchi, L. Galli, M. Moriondo et al., "Interferon-gamma release assay improves the diagnosis of tuberculosis in children," *Pediatric Infectious Disease Journal*, vol. 28, no. 6, pp. 510–514, 2009.

[39] E. Hansted, A. Andriuskeviciene, R. Sakalauskas, R. Kevalas, and B. Sitkauskiene, "T-cell-based diagnosis of tuberculosis infection in children in Lithuania: a country of high incidence despite a high coverage with bacille Calmette-Guerin vaccination," *BMC Pulmonary Medicine*, vol. 9, article 41, 2009.

[40] A. C. Hesseling, A. M. Mandalakas, H. L. Kirchner et al., "Highly discordant T cell responses in individuals with recent exposure to household tuberculosis," *Thorax*, vol. 64, no. 10, pp. 840–846, 2009.

[41] K. Higuchi, Y. Kawabe, S. Mitarai, T. Yoshiyama, N. Harada, and T. Mori, "Comparison of performance in two diagnostic methods for tuberculosis infection," *Medical Microbiology and Immunology*, vol. 198, no. 1, pp. 33–37, 2009.

[42] J. Lighter, M. Rigaud, R. Eduardo, C. H. Peng, and H. Pollack, "Latent tuberculosis diagnosis in children by using the QuantiFERON-TB gold in-tube test," *Pediatrics*, vol. 123, no. 1, pp. 30–37, 2009.

[43] M. Lucas, P. Nicol, E. McKinnon et al., "A prospective large-scale study of methods for the detection of latent *Mycobacterium tuberculosis* infection in refugee children," *Thorax*, vol. 65, no. 5, pp. 442–448, 2010.

[44] D. C. Stefan, A. Dippenaar, A. K. Detjen et al., "Interferon-gamma release assays for the detection of *Mycobacterium tuberculosis* infection in children with cancer," *International Journal of Tuberculosis and Lung Disease*, vol. 14, no. 6, pp. 689–694, 2010.

[45] A. M. Mandalakas, A. C. Hesseling, N. N. Chegou et al., "High level of discordant IGRA results in HIV-infected adults and children," *International Journal of Tuberculosis and Lung Disease*, vol. 12, no. 4, pp. 417–423, 2008.

[46] J. Domínguez, J. Ruiz-Manzano, M. De Souza-Galvão et al., "Comparison of two commercially available gamma interferon blood tests for immunodiagnosis of tuberculosis," *Clinical and Vaccine Immunology*, vol. 15, no. 1, pp. 168–171, 2008.

[47] A. M. Mandalakas, A. K. Detjen, A. C. Hesseling, A. Benedetti, and D. Menzies, "Interferon-gamma release assays and childhood tuberculosis: systematic review and meta-analysis," *International Journal of Tuberculosis and Lung Disease*, vol. 15, no. 8, pp. 1018–1032, 2011.

[48] M. Bakir, K. A. Millington, A. Soysal et al., "Prognostic value of a T-cell-based, interferon-γ biomarker in children with tuberculosis contact," *Annals of Internal Medicine*, vol. 149, no. 11, pp. 777–786, 2008.

[49] H. del Corral, S. C. Paris, N. D. Marin, D. M. Marin, L. Lopez, H. M. Henao et al., "IFNgamma response to *Mycobacterium tuberculosis*, risk of infection and disease in household contacts of tuberculosis patients in Colombia," *PLoS ONE*, vol. 4, no. 12, Article ID e8257, 2009.

[50] P. C. Hill, D. J. Jackson-Sillah, A. Fox et al., "Incidence of tuberculosis and the predictive value of ELISPOT and Mantoux tests in Gambian case contacts," *PLoS ONE*, vol. 3, no. 1, Article ID e1379, 2008.

[51] S. V. Kik, W. P. J. Franken, M. Mensen et al., "Predictive value for progression to tuberculosis by IGRA and TST in immigrant contacts," *European Respiratory Journal*, vol. 35, no. 6, pp. 1346–1353, 2010.

[52] A. Cattamanchi, R. Smith, K. R. Steingart et al., "Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals: a systematic review and meta-analysis," *Journal of Acquired Immune Deficiency Syndromes*, vol. 56, no. 3, pp. 230–238, 2011.

[53] A. Zwerling, S. van den Hof, J. Scholten, F. Cobelens, D. Menzies, and M. Pai, "Interferon-gamma release assays for tuberculosis screening of healthcare workers: a systematic review," *Thorax*, vol. 67, no. 1, pp. 62–70, 2011.

[54] M. C. Aichelburg, A. Rieger, F. Breitenecker et al., "Detection and prediction of active tuberculosis disease by a whole-blood interferon-γ release assay in HIV-l-infected individuals," *Clinical Infectious Diseases*, vol. 48, no. 7, pp. 954–962, 2009.

[55] S. A. Clark, S. L. Martin, A. Pozniak et al., "Tuberculosis antigen-specific immune responses can be detected using enzyme-linked immunospot technology in human immunodeficiency virus (HIV)-1 patients with advanced disease," *Clinical and Experimental Immunology*, vol. 150, no. 2, pp. 238–244, 2007.

[56] S. Jonnalagadda, B. L. Payne, E. Brown et al., "Latent tuberculosis detection by interferon γ release assay during pregnancy predicts active tuberculosis and mortality in human immunodeficiency virus type 1-infected women and their children," *Journal of Infectious Diseases*, vol. 202, no. 12, pp. 1826–1835, 2010.

[57] M. Santin, S. Casas, M. Saumoy et al., "Detection of latent tuberculosis by the tuberculin skin test and a whole-blood interferon-γ release assay, and the development of active tuberculosis in HIV-seropositive persons," *Diagnostic Microbiology and Infectious Disease*, vol. 69, no. 1, pp. 59–65, 2011.

[58] L. F. Zhang, X. Q. Liu, L. Y. Zuo, T. S. Li, G. H. Deng, and A. X. Wang, "Longitudinal observation of an interferon gamma-released assay (T-SPOT.TB) for *Mycobacterium tuberculosis* infection in AIDS patients on highly active antiretroviral therapy," *Chinese Medical Journal*, vol. 123, no. 9, pp. 1117–1121, 2010.

[59] C. Akolo, I. Adetifa, S. Shepperd, and J. Volmink, "Treatment of latent tuberculosis infection in HIV infected persons," *Cochrane Database of Systematic Reviews*, no. 1, Article ID CD000171, 2010.

[60] D. W. Dowdy and J. E. Golub, "Tests for latent tuberculosis infection and isoniazid preventive therapy," *The Lancet Infectious Diseases*, vol. 12, no. 11, pp. 827–828, 2012.

[61] S. Ribeiro, K. Dooley, J. Hackman et al., "T-SPOT.TB responses during treatment of pulmonary tuberculosis," *BMC Infectious Diseases*, vol. 9, article 23, 2009.

[62] V. Bosshard, P. Roux-Lombard, T. Perneger et al., "Do results of the T-SPOT.TB interferon-γ release assay change after treatment of tuberculosis?" *Respiratory Medicine*, vol. 103, no. 1, pp. 30–34, 2009.

[63] C. B. Chee, K. W. KhinMar, S. H. Gan, T. M. Barkham, C. K. Koh, L. Shen et al., "Tuberculosis treatment effect on T-cell interferon-gamma responses to *Mycobacterium tuberculosis*-specific antigens," *European Respiratory Journal*, vol. 36, no. 2, pp. 355–361, 2010.

[64] I. Latorre, N. Altet, M. de Souza-Galvao, J. Ruiz-Manzano, A. Lacoma, C. Prat et al., "Specific *Mycobacterium tuberculosis* T cell responses to RD1-selected peptides for the monitoring of anti-tuberculosis therapy," *Scandinavian Journal of Infectious Diseases*, vol. 44, no. 3, pp. 161–167, 2012.

[65] J. Domínguez, M. D. Souza-Galvão, J. Ruiz-Manzano et al., "T-cell responses to the *Mycobacterium tuberculosis*-specific antigens in active tuberculosis patients at the beginning, during, and after antituberculosis treatment," *Diagnostic Microbiology and Infectious Disease*, vol. 63, no. 1, pp. 43–51, 2009.

[66] K. Dheda, A. Pooran, M. Pai et al., "Interpretation of *Mycobacterium tuberculosis* antigen-specific IFN-γ release assays (T-SPOT.TB) and factors that may modulate test results," *Journal of Infection*, vol. 55, no. 2, pp. 169–173, 2007.

[67] W. P. J. Franken, S. M. Arend, S. F. T. Thijsen et al., "Interferon-gamma release assays during follow-up of tuberculin skin test-positive contacts," *International Journal of Tuberculosis and Lung Disease*, vol. 12, no. 11, pp. 1286–1294, 2008.

[68] I. Sauzullo, F. Mengoni, M. Lichtner et al., "In vivo and in vitro effects of antituberculosis treatment on mycobacterial interferon-γ T cell response," *PLoS ONE*, vol. 4, no. 4, Article ID e5187, 2009.

[69] Y. Kobashi, T. Sugiu, Y. Ohue et al., "Long-term follow-up of the QuantiFERON TB-2G test for active tuberculosis disease," *Internal Medicine*, vol. 47, no. 22, pp. 1957–1961, 2008.

[70] D. Goletti, M. P. Parracino, O. Butera et al., "Isoniazid prophylaxis differently modulates T-cell responses to RD1-epitopes in contacts recently exposed to *Mycobacterium tuberculosis*: a pilot study," *Respiratory Research*, vol. 8, article 5, 2007.

[71] A. M. Dyrhol-Riise, G. Gran, T. Wenzel-Larsen, B. Blomberg, C. G. Haanshuus, and O. Mørkve, "Diagnosis and follow-up of treatment of latent tuberculosis; the utility of the QuantiFERON-TB Gold In-tube assay in outpatients from a tuberculosis low-endemic country," *BMC Infectious Diseases*, vol. 10, article 57, 2010.

[72] S. H. Lee, W. J. Lew, H. J. Kim et al., "Serial interferon-gamma release assays after rifampicin prophylaxis in a tuberculosis outbreak," *Respiratory Medicine*, vol. 104, no. 3, pp. 448–453, 2010.

[73] S. W. Lee, C. T. Lee, and J. J. Yim, "Serial interferon-gamma release assays during treatment of active tuberculosis in young adults," *BMC Infectious Diseases*, vol. 10, article 300, 2010.

[74] M. Bocchino, P. Chairadonna, A. Matarese et al., "Limited usefulness of QuantiFERON-TB GOLD in-tube for monitoring anti-tuberculosis therapy," *Respiratory Medicine*, vol. 104, no. 10, pp. 1551–1556, 2010.

[75] J. L. Herrmann, M. Belloy, R. Porcher et al., "Temporal dynamics of interferon gamma responses in children evaluated for tuberculosis," *PLoS ONE*, vol. 4, no. 1, Article ID e4130, 2009.

[76] N. Nenadic, B. K. Kirin, I. Z. Letoja, D. Plavec, R. Z. Topic, and S. Dodig, "Serial interferon-gamma release assay in children with latent tuberculosis infection and children with tuberculosis," *Pediatric Pulmonology*, vol. 47, no. 4, pp. 401–408, 2012.

[77] E. Chiappini, F. Fossi, F. Bonsignori, S. Sollai, L. Galli, and M. de Martino, "Utility of interferon-gamma release assay results to monitor anti-tubercular treatment in adults and children," *Clinical Therapeutics*, vol. 34, no. 5, pp. 1041–1048, 2012.

[78] A. R. J. Bamford, A. M. Crook, J. E. Clark et al., "Comparison of interferon-γ release assays and tuberculin skin test in predicting active tuberculosis (TB) in children in the UK: a paediatric TB network study," *Archives of Disease in Childhood*, vol. 95, no. 3, pp. 180–186, 2010.

[79] T. G. Connell, M. A. Davies, C. Johannisen et al., "Reversion and conversion of *Mycobacterium tuberculosis* IFN-γ ELISpot results during anti-tuberculous treatment in HIV-infected children," *BMC Infectious Diseases*, vol. 10, article 138, 2010.

[80] B. Kampmann, E. Whittaker, A. Williams et al., "Interferon-γ release assays do not identify more children with active tuberculosis than the tuberculin skin test," *European Respiratory Journal*, vol. 33, no. 6, pp. 1374–1382, 2009.

[81] D. A. Lewinsohn, M. N. Lobato, and J. A. Jereb, "Interferon-γ release assays: new diagnostic tests for *Mycobacterium tuberculosis* infection, and their use in children," *Current Opinion in Pediatrics*, vol. 22, no. 1, pp. 71–76, 2010.

[82] S. MacHingaidze, C. S. Wiysonge, Y. Gonzalez-Angulo et al., "The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children: a systematic review and meta-analysis," *Pediatric Infectious Disease Journal*, vol. 30, no. 8, pp. 694–700, 2011.

[83] N. Nenadic, B. K. Kirin, I. Z. Letoja, D. Plavec, R. Z. Topic, and S. Dodig, "Serial interferon-γ release assay in children with latent tuberculosis infection and children with tuberculosis," *Pediatric Pulmonology*, vol. 47, no. 4, pp. 401–408, 2012.

[84] M. P. Nicol, M. A. Davies, K. Wood et al., "Comparison of T-SPOT. TB assay and tuberculin skin test for the evaluation of young children at high risk for tuberculosis in a community setting," *Pediatrics*, vol. 123, no. 1, pp. 38–43, 2009.

[85] M. G. Aabye, P. Ravn, G. PrayGod et al., "The impact of HIV infection and CD4 cell count on the performance of an interferon gamma release assay in patients with pulmonary tuberculosis," *PLoS ONE*, vol. 4, no. 1, Article ID e4220, 2009.

[86] I. M. O. Adetifa, M. D. Lugos, A. Hammond et al., "Comparison of two interferon gamma release assays in the diagnosis of *Mycobacterium tuberculosis* infection and disease in The Gambia," *BMC Infectious Diseases*, vol. 7, article 122, 2007.

[87] I. M. O. Adetifa, M. O. C. Ota, D. J. Jeffries et al., "Commercial interferon gamma release assays compared to the tuberculin skin test for diagnosis of latent *Mycobacterium tuberculosis* infection in childhood contacts in the gambia," *Pediatric Infectious Disease Journal*, vol. 29, no. 5, pp. 439–443, 2010.

[88] K. Baba, S. Sørnes, A. A. Hoosen et al., "Evaluation of immune responses in HIV infected patients with pleural tuberculosis by the QuantiFERON TB-Gold interferon-gamma assay," *BMC Infectious Diseases*, vol. 8, article 35, 2008.

[89] I. Brock, M. Ruhwald, B. Lundgren, H. Westh, L. R. Mathiesen, and P. Ravn, "Latent tuberculosis in HIV positive, diagnosed by the M. tuberculosis specific interferon-γ test," *Respiratory Research*, vol. 7, article 56, 2006.

[90] A. Cattamanchi, I. Ssewenyana, J. L. Davis et al., "Role of interferon-gamma release assays in the diagnosis of pulmonary tuberculosis in patients with advanced HIV infection," *BMC Infectious Diseases*, vol. 10, article 75, 2010.

[91] K. Dheda, A. Lalvani, R. F. Miller et al., "Performance of a T-cell-based diagnostic test for tuberculosis infection in HIV-infected individuals is independent of CD4 cell count," *AIDS*, vol. 19, no. 17, pp. 2038–2041, 2005.

[92] K. Dheda, R. Van Zyl Smit, M. Badri, and M. Pai, "T-cell interferon-γ release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings," *Current Opinion in Pulmonary Medicine*, vol. 15, no. 3, pp. 188–200, 2009.

[93] K. Dheda, R. N. van Zyl-Smit, R. Meldau et al., "Quantitative lung T cell responses aid the rapid diagnosis of pulmonary tuberculosis," *Thorax*, vol. 64, no. 10, pp. 847–853, 2009.

[94] R. S. Garfein, R. Lozada, L. Liu et al., "High prevalence of latent tuberculosis infection among injection drug users in Tijuana, Mexico," *International Journal of Tuberculosis and Lung Disease*, vol. 13, no. 5, pp. 626–632, 2009.

[95] W. Jiang, L. Shao, Y. Zhang et al., "High-sensitive and rapid detection of *Mycobacterium tuberculosis* infection by IFN-γ release assay among HIV-infected individuals in BCG-vaccinated area," *BMC Immunology*, vol. 10, article 31, 2009.

[96] S. S. J. Lee, Y. C. Liu, T. S. Huang et al., "Comparison of the interferon-gamma; release assay and the tuberculin skin test for contact investigation of tuberculosis in BCG-vaccinated health care workers," *Scandinavian Journal of Infectious Diseases*, vol. 40, no. 5, pp. 373–380, 2008.

[97] A. Machado Jr., K. Emodi, I. Takenami et al., "Analysis of discordance between the tuberculin skin test and the interferon-gamma release assay," *International Journal of Tuberculosis and Lung Disease*, vol. 13, no. 4, pp. 446–453, 2009.

[98] M. X. Rangaka, K. A. Wilkinson, R. Seldon et al., "Effect of HIV-1 infection on T-cell-based and skin test detection of tuberculosis infection," *American Journal of Respiratory and Critical Care Medicine*, vol. 175, no. 5, pp. 514–520, 2007.

[99] L. Richeldi, M. Losi, R. D'Amico et al., "Performance of tests for latent tuberculosis in different groups of immunocompromised patients," *Chest*, vol. 136, no. 1, pp. 198–204, 2009.

[100] I. Rivas, I. Latorre, A. Sanvisens et al., "Prospective evaluation of latent tuberculosis with interferon-γ release assays in drug and alcohol abusers," *Epidemiology and Infection*, vol. 137, no. 9, pp. 1342–1347, 2009.

[101] M. Ruhwald, J. Petersen, K. Kofoed et al., "Improving T-cell assays for the diagnosis of latent TB infection: potential of a diagnostic test based on IP-10," *PLoS ONE*, vol. 3, no. 8, Article ID e2858, 2008.

[102] C. Stephan, T. Wolf, U. Goetsch et al., "Comparing QuantiFERON-tuberculosis gold, T-SPOT tuberculosis and tuberculin skin test in HIV-infected individuals from a low prevalence tuberculosis country," *AIDS*, vol. 22, no. 18, pp. 2471–2479, 2008.

[103] N. J. Talati, U. Seybold, B. Humphrey et al., "Poor concordance between interferon-γ release assays and tuberculin skin tests in diagnosis of latent tuberculosis infection among HIV-infected individuals," *BMC Infectious Diseases*, vol. 9, article 15, 2009.

[104] B. Chang, H. Y. Park, K. Jeon, J. K. Ahn, H. S. Cha, E. M. Koh et al., "Interferon-gamma release assay in the diagnosis of latent tuberculosis infection in arthritis patients treated with tumor necrosis factor antagonists in Korea," *Clinical Rheumatology*, vol. 30, no. 12, pp. 1535–1541, 2011.

[105] E. Tavast, T. Tuuminen, S. H. Pakkanen, M. Eriksson, A. Kantele, A. Jarvinen et al., "Immunosuppression adversely affects TST but Not IGRAs in patients with psoriasis or inflammatory musculoskeletal diseases," *International Journal of Rheumatology*, vol. 2012, Article ID 381929, 8 pages, 2012.

[106] R. Smith, A. Cattamanchi, K. R. Steingart, C. Denkinger, K. Dheda, K. L. Winthrop et al., "Interferon-gamma release assays for diagnosis of latent tuberculosis infection: evidence in immune-mediated inflammatory disorders," *Current Opinion in Rheumatology*, vol. 23, no. 4, pp. 377–384, 2011.

[107] D. I. Ling, M. Pai, V. Davids, L. Brunet, L. Lenders, R. Meldau et al., "Are interferon-gamma release assays useful for diagnosing active tuberculosis in a high-burden setting?" *European Respiratory Journal*, vol. 38, no. 3, pp. 649–656, 2011.

[108] C. B. E. Chee, S. H. Gan, K. W. KhinMar et al., "Comparison of sensitivities of two commercial gamma interferon release assays for pulmonary tuberculosis," *Journal of Clinical Microbiology*, vol. 46, no. 6, pp. 1935–1940, 2008.

[109] L. Leidl, H. Mayanja-Kizza, G. Sotgiu et al., "Relationship of immunodiagnostic assays for tuberculosis and numbers of circulating $CD4^+$ T-cells in HIV infection," *European Respiratory Journal*, vol. 35, no. 3, pp. 619–626, 2010.

[110] R. Markova, Y. Todorova, R. Drenska, I. Elenkov, M. Yankova, and D. Stefanova, "Usefulness of interferon-gamma release assays in the diagnosis of tuberculosis infection in HIV-infected patients in Bulgaria," *Biotechnology and Biotechnological Equipment*, vol. 23, no. 1, pp. 1103–1108, 2009.

[111] F. Bartalesi, S. Vicidomini, D. Goletti et al., "QuantiFERON-TB Gold and the TST are both useful for latent tuberculosis infection screening in autoimmune diseases," *European Respiratory Journal*, vol. 33, no. 3, pp. 586–593, 2009.

[112] S. M. Behar, D. S. Shin, A. Maier, J. Coblyn, S. Helfgott, and M. E. Weinblatt, "Use of the T-SPOT.TB assay to detect latent tuberculosis infection among rheumatic disease patients on immunosuppressive therapy," *Journal of Rheumatology*, vol. 36, no. 3, pp. 546–551, 2009.

[113] M. Bocchino, A. Matarese, B. Bellofiore et al., "Performance of two commercial blood IFN-γ release assays for the detection of *Mycobacterium tuberculosis* infection in patient candidates for anti-TNF-α treatment," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 27, no. 10, pp. 907–913, 2008.

[114] N. Cobanoglu, U. Ozcelik, U. Kalyoncu et al., "Interferon-gamma assays for the diagnosis of tuberculosis infection before using tumour necrosis factor-alpha blockers," *International Journal of Tuberculosis and Lung Disease*, vol. 11, no. 11, pp. 1177–1182, 2007.

[115] F. Gogus, Z. Günendi, R. Karakus, Z. Erdogan, K. Hizel, and F. Atalay, "Comparison of tuberculin skin test and QuantiFERON-TB gold in tube test in patients with chronic inflammatory diseases living in a tuberculosis endemic population," *Clinical and Experimental Medicine*, vol. 10, no. 3, pp. 173–177, 2010.

[116] S. Kleinert, O. Kurzai, J. Elias et al., "Comparison of two interferon-γ release assays and tuberculin skin test for detecting latent tuberculosis in patients with immune-mediated inflammatory diseases," *Annals of the Rheumatic Diseases*, vol. 69, no. 4, pp. 782–784, 2010.

[117] A. J. Kwakernaak, P. M. Houtman, J. F. L. Weel, J. P. L. Spoorenberg, and T. L. T. A. Jansen, "A comparison of an interferon-gamma release assay and tuberculin skin test in refractory inflammatory disease patients screened for latent tuberculosis prior to the initiation of a first tumor necrosis factor α inhibitor," *Clinical Rheumatology*, vol. 30, no. 4, pp. 505–510, 2011.

[118] E. Laffitte, J. P. Janssens, P. Roux-Lombard et al., "Tuberculosis screening in patients with psoriasis before antitumour necrosis factor therapy: comparison of an interferon-γ release assay vs. tuberculin skin test," *British Journal of Dermatology*, vol. 161, no. 4, pp. 797–800, 2009.

[119] C. D. L. Marques, A. L. B. P. Duarte, V. M. B. De Lorena et al., "Evaluation of an interferon gamma assay in the diagnosis of latent tuberculosis infection in patients with rheumatoid arthritis," *Rheumatology International*, vol. 30, no. 1, pp. 57–62, 2009.

[120] J. Martin, C. Walsh, A. Gibbs et al., "Comparison of interferon γ release assays and conventional screening tests before tumour necrosis factor α blockade in patients with inflammatory arthritis," *Annals of the Rheumatic Diseases*, vol. 69, no. 1, pp. 181–185, 2010.

[121] G. Matulis, P. Jüni, P. M. Villiger, and S. D. Gadola, "Detection of latent tuberculosis in immunosuppressed patients with autoimmune diseases: performance of a *Mycobacterium tuberculosis* antigen-specific interferon γ assay," *Annals of the Rheumatic Diseases*, vol. 67, no. 1, pp. 84–90, 2008.

[122] D. P. De Leon, E. Acevedo-Vasquez, S. Alvizuri et al., "Comparison of an interferon-γ assay with tuberculin skin testing for detection of tuberculosis (TB) infection in patients with rheumatoid arthritis in a TB-endemic population," *Journal of Rheumatology*, vol. 35, no. 5, pp. 776–781, 2008.

[123] A. M. Schoepfer, B. Flogerzi, S. Fallegger et al., "Comparison of interferon-gamma release assay versus tuberculin skin test for tuberculosis screening in inflammatory bowel disease," *American Journal of Gastroenterology*, vol. 103, no. 11, pp. 2799–2806, 2008.

[124] D. Vassilopoulos, N. Stamoulis, E. Hadziyannis, and A. J. Archimandritis, "Usefulness of enzyme-linked immunosorbent assay (Elispot) compared to tuberculin skin testing for latent tuberculosis screening in rheumatic patients scheduled for anti-tumor necrosis factor treatment. Addendum," *Journal of Rheumatology*, vol. 35, no. 7, article 1464, 2008.

[125] G. H. Mazurek, J. Jereb, A. Vernon, P. LoBue, S. Goldberg, and K. Castros, "Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection—United States, 2010," *Morbidity and Mortality Weekly Report*, vol. 59, no. RR05, pp. 1–25, 2010.

[126] H. Nakaoka, L. Lawson, S. B. Squire et al., "Risk for tuberculosis among children," *Emerging Infectious Diseases*, vol. 12, no. 9, pp. 1383–1388, 2006.

[127] K. Okada, T. E. Mao, T. Mori et al., "Performance of an interferon-gamma release assay for diagnosing latent tuberculosis infection in children," *Epidemiology and Infection*, vol. 136, no. 9, pp. 1179–1187, 2008.

[128] R. Petrucci, N. A. Amer, R. Q. Gurgel et al., "Interferon gamma, interferon-gamma-induced-protein 10, and tuberculin responses of children at high risk of tuberculosis infection," *Pediatric Infectious Disease Journal*, vol. 27, no. 12, pp. 1073–1077, 2008.

[129] T. A. Thomas, D. Mondal, Z. Noor et al., "Malnutrition and helminth infection affect performance of an interferon γ-release assay," *Pediatrics*, vol. 126, no. 6, pp. e1522–e1529, 2010.

[130] J. C. Choi, J. W. Shin, J. Y. Kim, I. W. Park, B. W. Choi, and M. K. Lee, "The effect of previous tuberculin skin test on the follow-up examination of whole-blood interferon-γ assay in the screening for latent tuberculosis infection," *Chest*, vol. 133, no. 6, pp. 1415–1420, 2008.

[131] K. Ewer, K. A. Millington, J. J. Deeks, L. Alvarez, G. Bryant, and A. Lalvani, "Dynamic antigen-specific T-cell responses after point-source exposure to *Mycobacterium tuberculosis*," *American Journal of Respiratory and Critical Care Medicine*, vol. 174, no. 7, pp. 831–839, 2006.

[132] P. C. Hill, R. H. Brookes, A. Fox et al., "Longitudinal assessment of an ELISPOT test for *Mycobacterium tuberculosis* infection," *PLoS Medicine*, vol. 4, no. 6, pp. 1061–1070, 2007.

[133] M. Pai, R. Joshi, S. Dogra et al., "Serial testing of health care workers for tuberculosis using interferon-γ assay," *American Journal of Respiratory and Critical Care Medicine*, vol. 174, no. 3, pp. 349–355, 2006.

[134] R. N. van Zyl-Smit, A. Zwerling, K. Dheda, and M. Pai, "Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review," *PLoS ONE*, vol. 4, no. 12, Article ID e8517, 2009.

[135] T. Yoshiyama, N. Harada, K. Higuchi, Y. Nakajima, and H. Ogata, "Estimation of incidence of tuberculosis infection in health-care workers using repeated interferon-γ assays," *Epidemiology and Infection*, vol. 137, no. 12, pp. 1691–1698, 2009.

[136] S. Machingaidze, S. Verver, H. Mulenga, D. A. Abrahams, M. Hatherill, W. Hanekom et al., "Predictive value of recent quantiferon conversion for tuberculosis disease in adolescents," *American Journal of Respiratory and Critical Care Medicine*, vol. 186, no. 10, pp. 1051–1056, 2012.

[137] S. Perry, L. Sanchez, S. Yang, Z. Agarwal, P. Hurst, and J. Parsonnet, "Reproducibility of QuantiFERON-TB gold in-tube assay," *Clinical and Vaccine Immunology*, vol. 15, no. 3, pp. 425–432, 2008.

[138] A. Schablon, M. Harling, R. Diel, F. C. Ringshausen, J. Torres Costa, and A. Nienhaus, "Serial testing with an interferon-gamma release assay in German healthcare workers," *GMS Krankenhaushygiene Interdisziplinär*, vol. 5, no. 2, 2010.

[139] D. Tripodi, B. Brunet-Courtois, V. Nael et al., "Evaluation of the tuberculin skin test and the interferon-release assay for TB screening in French healthcare workers," *Journal of Occupational Medicine and Toxicology*, vol. 4, no. 1, article 30, 2009.

[140] S. E. Van Brummelen, A. M. Bauwens, N. J. Schlösser, and S. M. Arend, "Kinetics of a tuberculosis-specific gamma interferon release assay in military personnel with a positive tuberculin skin test," *Clinical and Vaccine Immunology*, vol. 17, no. 6, pp. 937–943, 2010.

[141] K. Higuchi, N. Harada, and T. Mori, "Interferon-γ responses after isoniazid chemotherapy for latent tuberculosis," *Respirology*, vol. 13, no. 3, pp. 468–472, 2008.

[142] J. E. Kaplan, C. Benson, K. H. Holmes, J. T. Brooks, A. Pau, and H. Masur, "Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America," *Morbidity and Mortality Weekly Report. Recommendations and Reports*, vol. 58, no. 4, Article ID CE1, p. 1, 2009.

Hindawi Publishing Corporation
Pulmonary Medicine
Volume 2012, Article ID 291294, 7 pages
doi:10.1155/2012/291294

Research Article

Delineating a Retesting Zone Using Receiver Operating Characteristic Analysis on Serial QuantiFERON Tuberculosis Test Results in US Healthcare Workers

Wendy Thanassi,[1,2,3,4] Art Noda,[4,5] Beatriz Hernandez,[4,5] Jeffery Newell,[4] Paul Terpeluk,[6] David Marder,[7] and Jerome A. Yesavage[4,5]

[1] *Department of Medicine, Veterans Affairs Palo Alto Health Care System, 3801 Miranda Avenue MC-, Palo Alto, CA 94304-1207, USA*
[2] *Occupational Health Strategic Health Care Group, Office of Public Health, Veterans Health Administration, Washington, DC 20006, USA*
[3] *Division of Emergency Medicine, Stanford University School of Medicine, Stanford, CA 94304, USA*
[4] *War Related Illness and Injury Study Center (WRIISC) and Mental Illness Research Education and Clinical Center (MIRECC), Department of Veterans Affairs, Palo Alto, CA 94304, USA*
[5] *Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94304, USA*
[6] *Department of Occupational Health, The Cleveland Clinic, Cleveland, OH 44195, USA*
[7] *University Health Services, University of Illinois Chicago, Chicago, IL 60612, USA*

Correspondence should be addressed to Wendy Thanassi, wendy.thanassi@gmail.com

Received 5 October 2012; Revised 29 November 2012; Accepted 30 November 2012

Academic Editor: Anete Trajman

Objective. To find a statistically significant separation point for the QuantiFERON Gold In-Tube (QFT) interferon gamma release assay that could define an optimal "retesting zone" for use in serially tested low-risk populations who have test "reversions" from initially positive to subsequently negative results. *Method*. Using receiver operating characteristic analysis (ROC) to analyze retrospective data collected from 3 major hospitals, we searched for predictors of reversion until statistically significant separation points were revealed. A confirmatory regression analysis was performed on an additional sample. *Results*. In 575 initially positive US healthcare workers (HCWs), 300 (52.2%) had reversions, while 275 (47.8%) had two sequential positive tests. The most statistically significant (Kappa = 0.48, chi-square = 131.0, $P < 0.001$) separation point identified by the ROC for predicting reversion was the tuberculosis antigen minus-nil (TBag-nil) value at 1.11 International Units per milliliter (IU/mL). The second separation point was found at TBag-nil at 0.72 IU/mL (Kappa = 0.16, chi-square = 8.2, $P < 0.01$). The model was validated by the regression analysis of 287 HCWs. *Conclusion*. Reversion likelihood increases as the TBag-nil approaches the manufacturer's cut-point of 0.35 IU/mL. The most statistically significant separation point between those who test repeatedly positive and those who revert is 1.11 IU/mL. Clinicians should retest low-risk individuals with initial QFT results < 1.11 IU/mL.

1. Introduction

We report the findings of a multisite study of United States healthcare workers (HCWs) that began as a quality control initiative in the Veterans Administration Palo Alto Health Care System (VAPAHCS) when QuantiFERON Gold In-Tube (QFT) serial screening tests were observed to be initially positive and were subsequently negative in those low-risk individuals. This seemingly spontaneous "reversion" has been reported around the world in the literature, and the variability that occurs mostly around the baseline is recognized [1–6].

This study design was driven by the clinical experience: when an HCW presents with a positive QFT result, what can the clinician do to discern whether the next test is likely to remain positive or become negative?

The foundation of the problem lies in the dichotomous nature of the results reported. Currently, a QuantiFERON tuberculosis antigen minus-nil (TBag-nil) ≥ 0.35 International Units per milliliter (IU/mL) is reported as "positive." At that point the provider has a decision to make, one that is generally to investigate further with a chest radiograph, seek specialty consultation, and/or recommend medical treatment. Whereas positive tuberculin skin tests (TSTs) were often felt to be erroneous due to prior BCG vaccination, and compliance and treatment rates were low; studies are showing that positive interferon-gamma release assay (IGRA) results are more likely to lead to both the recommendation and the acceptance of chemotherapy [7–10]. Chemotherapy puts the patient at risk for side effects including hepatotoxicity [11], as well as social stigma or workplace discrimination [12]. The presumptive diagnosis of tuberculosis infection in HCWs, particularly when interpreted as an occupational conversion, can trigger Occupational Safety and Health Administration, National Institute for Occupational Safety, and Health or hospital infection control contact investigations that are both time consuming and costly. Thus the presence of spontaneous "reversions" implies that clinicians and patients are experiencing unnecessary concern, action, or expense and potentially placing patients in harm's way for transiently positive results which are forced by the binary nature of the current reporting structure. There is a need for increased accuracy and efficiency in the screening process to reduce the burdens to the patient and the system, and utilization of this predictive tool may lend some assistance.

In response to the persistent concerns regarding reversions near the cut-point of 0.35 IU/mL, a 2010 Morbidity and Mortality Weekly Report published by the Centers for Disease Control and Prevention (CDC) recommended that quantitative QFT results should be reported. The CDC did not, however, provide guidance for either the interpretation or the use of these values [13]. We investigated reversions in US HCWs in order to develop a validated model, using receiver operating characteristic analysis, to define the range of results that best predicts a transiently positive result. With the ability to predict the likelihood of reversion, clinicians and patients could choose to retest rather than to pursue costly and time-consuming consultations and therapies.

2. Materials and Methods

2.1. Participants and Variables. Data were obtained from a retrospective review of available clinical laboratory records from three different sites: (1) Veterans Administration Palo Alto Health Care System (VAPAHCS), California, (2) University of Illinois Chicago (UIC) Il, and the (3) Cleveland Clinic (CC), Ohio, where each HCW undergoes preemployment and annual QFT testing irrespective of previous results. All subjects are US HCWs who were serially tested by QFT Gold-in-Tube in their hospital's laboratory. All HCWs at least 18 years of age with available records were included. The study's date range was January 2009 through June 2011 at VAPAHCS, from August 2008 through June 2011 at UIC, and from October 2009 through December 2011 at the Cleveland Clinic.

Table 1: Test results for analyzed HCWs from VA Palo Alto Health Care System (VAPAHCS), University of Illinois Chicago (UIC) and the Cleveland Clinic (CC).

Test results	VAPAHCS	UIC	CC	Total (n)
Repeat positive result	113	338	25	476
Reversion	73	275	38	386
Total (n)	186	613	63	862

Note: HCWs were excluded if their only positive test result was their last test taken or if data for only one test result was available.

HCWs who tested consistently negative and those with only a single test result were excluded. Results reported without the QFT TBag-nil numerical value, as well as HCWs with negative-to-positive discordance/conversion at the conclusion of their testing series were removed from the dataset (22/195 from VAPAHCS, 124/742 from UIC, and 61/127 from CC). To be included in the analysis, at least two QFT tests were required, one of which was a positive result that was followed by either a positive or a negative result. This reproduces the clinician's actionable decision point; that is, when a patient presents with a positive result, the action to test further, to refer, or to treat is initiated. Patients were only included once (see Table 1).

2.2. Participant Sites. The VAPAHCS is a suburban teaching hospital located in Palo Alto, California. The county in which it resides, Santa Clara, has the 3rd highest tuberculosis (TB) rate in California [14] at 11.4% from 2006–2011 [15], and California is ranked 3rd in USA for TB cases behind Alaska and Hawaii [16]. All VAPAHCS HCWs are United States citizens. The HCW population is approximately 3,500. The lab performed over 16,000 QFT-GIT tests (including patient testing) during this period. Of the 4,019 HCWs who were tested between January 1, 2009 and June 30, 2011, 2,706 (67%) tested negative one time and 293 (7%) tested positive one time, without repeat testing. (Note that VAPA also tests researchers, students, volunteers, and Peace Corps personnel, most of whom are on campus for only one testing cycle). Of the 4,019 unique HCWs, 781 (19%) tested negative more than once and never tested positive. Thus the overall negative rate at VAPA is (67% + 19%) = 86%, while 14% of personnel tested positive at least once in their series. The indeterminate rate in this lab is 0.4%.

The University of Illinois, Chicago (UIC) is a public, urban academic teaching hospital. The HCW population is approximately 5,000. Their laboratory performed over 50,000 QFT-GIT tests by June 2011; 20,543 of these were on HCWs. Annual HCW TB screening is mandated and compliance is 99%, with most HCWs tested annually, but some who are on surveillance are tested every six months. UIC reports a HCW QFT negative rate of 89.5%, with 1.1% indeterminate and 9.4% positive at some point in their series. Illinois ranks 21st for tuberculosis cases in the nation [16], and Chicago itself had a TB incidence rate recorded at 7.4% during 2006–2010 [17].

The Cleveland Clinic Foundation (Cleveland, OH, USA) is an urban teaching hospital. The laboratory had performed over 10,000 QFT-GIT tests by June 2011. This includes patient and HCW testing. Cleveland Clinic hires approximately 2,500 HCWs annually. The HCW population is 98.5% negative with 0.5% indeterminate and 1% positive at some point in their series. Ohio ranks 35th in the nation for tuberculosis cases [16] with Cleveland itself having a 6.4% case rate from 2006–2010 [18].

2.3. QuantiFERON Gold In-Tube Blood Assay (Qiagen, Inc). The interferon gamma released was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol though with an 8-point standard curve for each microplate. The results were read at 450 nm by the Diamedix DS2 Automated ELISA System (Diamedix Corporation, Miami, FL) at VAPAHCS, by the Diamedix DSX Automated ELISA System (Diamedix Corporation, Miami, FL) at UIC, and by a BioTek ELx800 Absorbance Microplate Reader (BioTek Instruments, Winooski, VT) at CC. All tests in this series met the nil, mitogen, and the equation criteria for test validity delineated in the manufacturer's package insert [19].

2.4. Measures. In the absence of a gold standard against which to evaluate latent tuberculosis infection, the expected probability of two consecutive positive tests was employed as a proxy for corroboration of the test result in question, which is the implied presence of latent tuberculosis disease. In seeking what would best predict whether an individual was likely to be a "reverter", the initial TBag-nil value in the series of two sequential tests was evaluated as a possible predictor variable. Note that the QFT result was considered positive if the TBag-nil was ≥0.35 IU/mL, so all TBag-nil values were at least 0.35 IU/mL in this analysis.

2.5. Data Analytic Approach. We used a two-step data analytic approach. First, we employed a receiver operating characteristic analysis (ROC) [20, 21] on two-thirds (the Exploratory Group) of the 862 HCW sample to identify characteristics that might significantly differentiate reversions from those with two consecutively positive results. An ROC analysis is an exploratory process that searches every value of every predictor variable entered to identify the variable and value that results in the highest sensitivity and specificity (using the weighted kappa statistic) for identifying the targeted criterion. The targeted criterion in this case is reversion. Second, because ROC is an exploratory technique, we conducted a confirmatory logistic regression analysis and chi-square tests using the remaining one-third of the HCWs (the Confirmatory Group) to examine whether the predictor that had been identified in the first step did in fact predict reversion in an independent sample.

Regarding the details of the ROC analysis, once the optimal variable and associated separation point are identified, the association with the success criterion is tested against a stopping rule. Stopping rules include a subgroup sample size too small for further analysis ($n < 20$) and/or when no further variables are selected because the P value associated with the Chi-square statistic is ≥0.01. If the association does not meet the criteria for the stopping rule, the sample is divided into two groups based on the optimal variable and identified separation point. The ROC analysis is then restarted, separately, for each of these two subgroups. The result is a decision tree identifying the HCW characteristics and associated separation points that best predict reversions, with P values, chi-square, and Kappa values calculated and reported. The ROC software developed by Drs. Yesavage and Kraemer is publicly available [21], and the logistic regression and Chi-square tests were performed using SAS software (Version 9.3, Cary, NC, USA).

3. Results and Discussion

3.1. Results. HCWs from each site had undergone between 2 and 9 tests in series. The most recent positive test that was followed by either a positive (no reversion) or negative (reversion) result defined the two test results in the series that were analyzed (see Table 1). The mean number of days between tests was 434 for VAPAHCS, 261 for UIC, and 235 for CC.

The 862 HCWs who met inclusion criteria were randomly assigned to one of two groups: the Exploratory Group ($n = 575$) or the Confirmatory Group ($n = 287$). The Exploratory Group of tested HCWs had a 52.2% (300/575) reversion rate. The results of the ROC analysis performed on the Exploratory Group are presented as a decision tree shown in Figure 1. TBag-nil in IU/mL was most statistically significant for predicting reversion at the separation point 1.11 IU/mL (Kappa = 0.48, chi-Square = 131.0, $P < 0.001$). Two groups of HCWs were identified:

group 1: 75% reversions: 225/300 HCWs with TBag-nil <1.11 IU/mL;

group 2: 27% reversions: 75/275 HCWs with Tbag-nil ≥1.11 IU/mL.

The ROC analysis further identified two subgroups of HCWs derived from group 1 above with a TBag-nil at 0.72 IU/mL (Kappa = 0.16, chi-square = 8.2, $P < 0.01$):

group a: 80% reversions: 163/204 HCWs with TBag-nil <0.72 IU/mL;

group b: 65% reversions: 62/96 HCWs with TBag-nil ≥0.72 and <1.11 IU/mL.

Two subgroups of HCWs were also identified from group 2 above with TBag-nil at 2.17 IU/mL (Kappa = 0.27, chi-Square = 20.4, $P < 0.001$):

group c: 43% reversions: 43/99 HCWs with TBag-nil ≥ 1.11 and <2.17 IU/mL;

group d: 18% reversions: 32/176 HCWs with TBag-nil ≥ 2.17 IU/mL.

Figure 2 contains a decision tree classifying the 575 HCWs in the Exploratory Group as Reversions or No Reversions and by TBag-nil values using the ROC selected

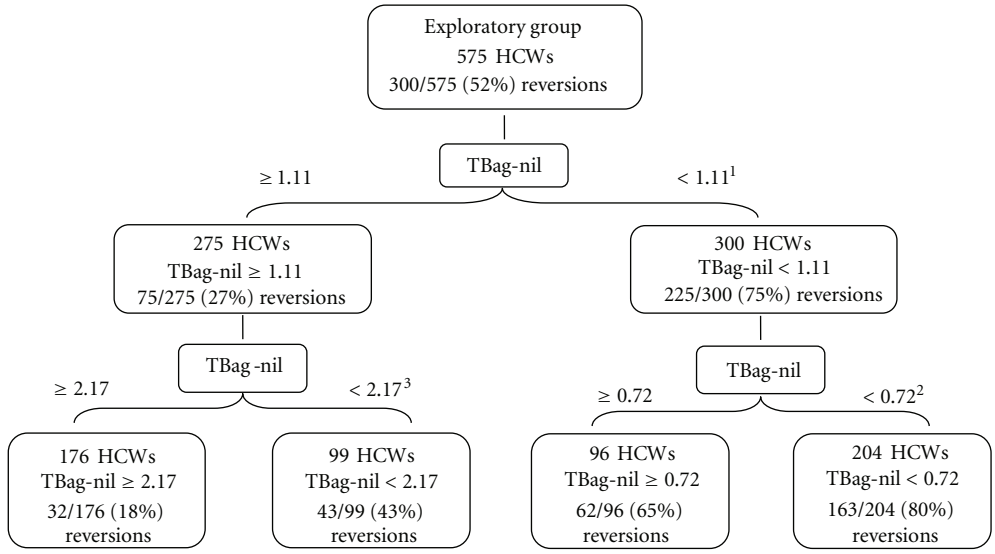


Figure 1: Receiver operating characteristic (ROC) decision tree identifying statistically significant TBag-nil (in IU/mL) separation points which predict those HCWs with a positive TB test result at time one who retest negative at time two. Logistic regression analysis on a separate Confirmatory sample of 287 HCWs validated all 3 separation points at 0.72, 1.11, and 2.17 IU/mL and remained statistically significant for all subgroups by chi-square ($P < 0.001$). 1 Kappa = 0.48, chi-square = 131.0, $P < 0.001$, 2 Kappa = 0.16, chi-square = 8.2, $P < 0.01$, 3 Kappa = 0.27, chi-square = 20.4, $P < 0.001$.

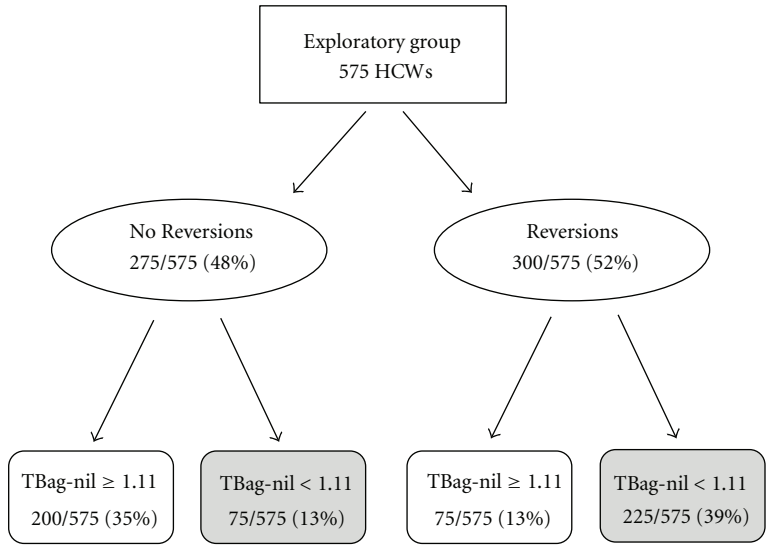


Figure 2: Exploratory group with 575 HCWs classified as No Reversions (those with two positive tests) or Reversions (with a positive TB test result at time one and who retest negative at time two). The two groups are further classified by TBag-nil values using the ROC selected separation point of 1.11 IU/mL (Kappa = 0.48, chi-square = 131.0, $P < 0.001$). Highlighted boxes emphasize the difference in number of No Reversions versus Reversions when TBag-nil < 1.11 IU/mL, the identified retesting zone.

separation point of 1.11 IU/mL. Note that 225 of the 300 "reverters" are identified at this separation point.

A logistic regression analysis was conducted in the Confirmatory Group ($n = 287$) using the same dependent measure (reversion) and predictor variable (TBag-nil) identified in the primary ROC analysis. The relationship remained statistically significant ($P < 0.001$). All three separation points at 0.72, 1.11, and 2.17 IU/mL (4 subgroups) identified in the ROC analysis also remained statistically significant for all subgroups by chi-square ($P < 0.001$).

4. Discussion

Multiple papers have reported within-subject variability in serial QFT results [1, 3, 6, 22, 23], and much work has been done to unmask a retesting zone by suggesting alternative

separation points of 0.5, 0.7 [6], or 1.0 IU/mL [23]. In Europe, employing a borderline zone between 0.2–0.7 IU/mL decreased conversions and reversions from 1.9 to 0.6% and from 6.1 to 2.6%, respectively, with no active tuberculosis cases occurring in the "positive" population in a 2-year follow-up period [24].

Further, it is both observed and understood that QFT reversions are much more common than conversions. Among the many studies published and reviewed on this topic [25], Schablon et al. [22] reports a conversion to reversion ratio of 6.1 versus 32.6% in 287 German healthcare workers, which is the same range as studies conducted in the United States (6.3 versus 33%) [26].

The predominance of reversions is likely explained in part by the statistical phenomenon of regression to the mean [24]. Regression to the mean (RTM) is the tendency of observations to move closer to the mean when repeated. When measurements are repeated in individuals, and measures are selected based on exceeding an absolute threshold in an inherently continuous range of values, influence by RTM should be considered. Examples of RTM are common in clinical medicine. In this case, since the observed mean result in these US HCWs is <0.35 IU/mL, retesting a population subset that is initially above that mean will likely yield values that are closer to the population mean (in this case, in the negative range). The population "conversion" rate will be a mix of both true incident disease (proportional to the epidemiology of TB in the US HCWs) and false positives that will likely have reversions. The challenge is to identify a retesting zone with an upper value that minimizes noise while still identifying clinically significant cases for followup in a cost-effective manner.

As for the reliability of that negative result, the QFT Gold In-Tube has a specificity of 99% [19], reflecting the measurement of persons correctly identified as not having the condition (in this case tuberculosis). Further, the prevalence of disease in this population is low, making the pretest probability of positive results low. Additionally, Diel et al. conducted a study of 954 persons exposed to active tuberculosis and report a negative predictive value of 99.7% after 5 years [27]. With all of this in mind, the authors conclude that while the decision on how to act upon a test result lays with the clinician and never purely with numerical data, a negative QFT result is significantly more reliable than a low positive result in its ability to predict disease or the lack thereof.

To help clarify a practice algorithm, there is a call in the literature for a statistically based, data-driven retesting zone. Zwerling et al. in a 2011 review article in *Thorax* concluded that "the use of IGRAs for serial testing is complicated by lack of data on optimum cut-offs for serial testing …" [28], and a 2012 editorial in *Chest* stated that "it is quite arbitrary to limit true conversion to those with a QFT-GIT of >1.0 IU/mL, since that value, though a nice round figure, has not been validated" [29]. Here we offer that a statistically driven optimal separation point between consistently positive serially tested US healthcare workers and healthcare workers who are likely to revert is 1.11 IU/mL.

We focus on 1.11 IU/mL as the border of a retesting zone because it was determined by the Kappa statistic in the ROC software as the optimally sensitive and specific separation point between the "reversions" from those who did not "revert" in this multisite cohort. At a separation point of 1.11 IU/mL, sensitivity is 0.75 and specificity is 0.73, whereas at a separation point of 0.72 IU/mL sensitivity is 0.54 and specificity is 0.85. The respective Kappas are 0.48 versus 0.39. As is the case by lowering the retesting separation point to 0.72 IU/mL, further lowering it to 0.50 IU/mL would increase the specificity of the measure to 0.93 and capture 84/103 (82%) of reversions in this range, but this would include only 103/575 (18%) of the total population and 84/300 (28%) of the "reverters." The sensitivity of this separation point would be only 0.28 and its Kappa 0.21. Thus with the ROC there are trade-offs in sensitivity versus specificity, depending upon the separation points selected. The 1.11 IU/mL measurement was chosen by the ROC analysis for this population because that value maximizes the percentage of "reverters" while optimizing sensitivity and specificity.

5. Conclusion

We present a validated model on a sample of 862 US healthcare workers from three major US hospitals that could be used to define a QuantiFERON Gold In-Tube retesting zone between 0.35 and 1.11 IU/mL. The upper value was selected by a receiver operating characteristic analysis to maximize separation between HCWs who have two consecutive positive tests and those who have reversions ($P < 0.001$). Our sample of HCWs had a 75% risk for reversion if their initial positive test fell within this range. While 0.35–1.11 IU/mL is therefore the optimal retesting zone identified here, 0.35–0.72 IU/mL (80% reversion; $P < 0.01$) is another possible separation point also selected by the ROC and could be reasonably employed by providers based on the clinical situation much like the 5, 10, and 15 mm tuberculin skin test cut-off points that are used in different settings. Acceptance of TBag-nil values reported above as the delineators of a QFT retesting zone could lessen patient anxiety, decrease unnecessary radiographs, prevent unnecessary exposure investigations, and possibly spare patients from inappropriate medical treatment due to transiently "positive" QFT test results.

6. Limitations and Future Directions

Limitations of the study include that while the current analyses incorporated over 850 positive HCW records, these data are from only three facilities. Furthermore, results in this study are weighted towards UIC, since their data comprise the majority of the sample group. While there could be selection bias among those HCWs who present for serial testing, it is not clear how that could influence these results. Finally, it should also be noted that prospective long-term followup would be required to provide thorough validation of the results.

Future analyses using the same statistical methods could include additional data from other institutions in USA, Europe, or from countries with higher risk for HCWs. There is a possibility that there could be local variation based on biologic or regional laboratory differences that would be exposed when more data are analyzed.

Conflict of Interests

The authors declare that they have no Conflict of interests.

Acknowledgments

The authors thank Leah Friedman, Senior Research Associate, Stanford University School of Medicine, for editorial assistance and Professor Helena Kraemer, Stanford University (Emerita) and University of Pittsburgh, for her writing of the data analysis software and advice regarding methodology. Additional thanks are to Dr. Drew Levy for methodological review and Dr. George Todaro for his original data review and insights. This work was supported in part by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development and by the Department of Veterans Affairs Sierra-Pacific Mental Illness Research, Education, and the War Related Illness and Injury Study Center (MIRECC).

References

[1] R. Belknap, J. Kelaher, K. Wall et al., "Diagnosis of latent tuberculosis infection in U.S. health care workers: reproducibility, repeatability and 6 month follow-up with interferon-γ release assays (IGRAs)," *American Journal of Respiratory and Critical Care Medicine*, vol. 179, Article ID A4101, 2009.

[2] A. K. Detjen, L. Loebenberg, H. M. S. Grewal et al., "Short-term reproducibility of a commercial interferon γ release assay," *Clinical and Vaccine Immunology*, vol. 16, no. 8, pp. 1170–1175, 2009.

[3] M. Pai, R. Joshi, S. Dogra et al., "Serial testing of health care workers for tuberculosis using interferon-γ assay," *American Journal of Respiratory and Critical Care Medicine*, vol. 174, no. 3, pp. 349–355, 2006.

[4] S. Rafiza and K. G. Rampal, "Serial testing of Malaysian health care workers with QuantiFERON(R)-TB gold in-tube," *The International Journal of Tuberculosis and Lung Disease*, vol. 16, no. 2, pp. 163–168, 2012.

[5] F. C. Ringshausen, A. Nienhaus, J. T. Costa et al., "Within-subject variability of *Mycobacterium tuberculosis*-specific γ interferon responses in German health care workers," *Clinical and Vaccine Immunology*, vol. 18, no. 7, pp. 1176–1182, 2011.

[6] J. T. Costa, R. Silva, R. Sá, M. J. Cardoso, and A. Nienhaus, "Serial testing with the interferon-γ release assay in Portuguese healthcare workers," *International Archives of Occupational and Environmental Health*, vol. 84, no. 4, pp. 461–469, 2011.

[7] R. Sahni, C. Miranda, B. Yen-Lieberman et al., "Does the implementation of an interferon-γ release assay in lieu of a tuberculin skin test increase acceptance of preventive therapy for latent tuberculosis among healthcare workers?" *Infection Control and Hospital Epidemiology*, vol. 30, no. 2, pp. 197–199, 2009.

[8] R. Diel, R. Loaddenkemper, and A. Nienhaus, "Evidence-based comparison of commercial Interferon-γ release assays for detecting active TB: a metaanalysis," *Chest*, vol. 137, no. 4, pp. 952–968, 2010.

[9] Y. Hirsch-Moverman, A. Daftary, J. Franks, and P. W. Colson, "Adherence to treatment for latent tuberculosis infection: systematic review of studies in the US and Canada," *International Journal of Tuberculosis and Lung Disease*, vol. 12, no. 11, pp. 1235–1254, 2008.

[10] C. R. Horsburgh Jr., "Priorities for the treatment of latent tuberculosis infection in the United States," *The New England Journal of Medicine*, vol. 350, no. 20, pp. 2060–2067, 2004.

[11] F. F. Fountain, E. Tolley, C. R. Chrisman, and T. H. Self, "Isoniazid hepatotoxicity associated with treatment of latent tuberculosis infection: a 7-year evaluation from a public health tuberculosis clinic," *Chest*, vol. 128, no. 1, pp. 116–123, 2005.

[12] U.S. Equal Employment Opportunity Commission, *Health Partners, Inc. To Pay $25, 000 to Resolve EEOC Disability Discrimination Case*, 2012, http://www.eeoc.gov/eeoc/newsroom/release/5-30-12.cfm.

[13] G. H. Mazurek, J. Jereb, A. Vernon, P. LoBue, S. Goldberg, and K. Castros, "Updated guidelines for using interferon γ release assays to detect *Mycobacterium tuberculosis* infection—United States, 2010," *Morbidity and Mortality Weekly Report*, vol. 59, no. 5, pp. 1–25, 2010.

[14] Tuberculosis Control Branch, California Department of Public Health, *California Tuberculosis Data, 2011*, 2011, http://www.cdph.ca.gov/programs/tb/Documents/TBCB-CA-TB-2011-Data-Tables.pdf.

[15] Tuberculosis Control Branch, California Department of Public Health, *Tuberculosis Cases and Case Rates Per 100, 000 Population: California, 1985–2011*, 2011, http://www.cdph.ca.gov/programs/tb/Documents/TBCB-CA-TB-2011-Data-Tables.pdf.

[16] CDC, *Reported Tuberculosis in the United States, 2011*, 2011, http://www.cdc.gov/tb/statistics/reports/2011/table19.htm.

[17] Health and Human Services Unit, Chicago Department of Innovation and Technology, *Tuberculosis Cases and Average Annual Incidence Rate, Chicago, 2007–2011*, 2011, https://data.cityofchicago.org/Health-Human-Services/Public-Health-Statistics-Tuberculosis-cases-and-av/ndk3-zftj.

[18] Center for Public Statisitics and Informatics, Ohio Department of Health, *Ohio TB Morbidity by County and Seven Major Cities, 2006–2011*, 2012, http://www.odh.ohio.gov/~/media/ODH/ASSETS/Files/hst/tuberculosis surveillance/countyratetable2011.ashx.

[19] Cellestis Inc, QuantiFERON-TB Gold (In-Tube Method) [package insert], Note: the above is the official onlinxe package insert from the manufacturer, not a patient product/counseling notice, The date (July 2011) is the last modification date, normally found at the end of the package insert file, Doc. No. US05990301K, http://www.cellestis.com.

[20] H. C. Kraemer, *Evaluating Medical Tests: Objective and Quantitative Guidelines*, Sage, Newbury Park, Calif, USA, 1992.

[21] J. A. Yesavage and H. C. Kraemer, "Signal detection software for receiver operator characteristics," Version 4. 22 [Computer software], 2007, http://www.stanford.edu/~yesavage/ROC.html.

[22] A. Schablon, M. Harling, R. Diel et al., "Serial testing with an interferon-γ release assay in German healthcare workers," *GMS Krankenhhygiene Interdisziplinär*, vol. 5, no. 2, Article ID Doc05, 2010.

[23] K. S. Fong, J. W. Tomford, L. Teixeira et al., "Challenges of interferon-γ release assay conversions in serial testing of health

care workers in a tuberculosis control program," *Chest*, vol. 142, no. 1, pp. 55–62, 2012.

[24] F. C. Ringshausen, A. Nienhaus, A. Schablon, S. Schlösser, G. Schultze-Werninghaus, and G. Rohde, "Predictors of persistently positive Mycobacterium-tuberculosis-specific interferon-γ responses in the serial testing of health care workers," *BMC Infectious Diseases*, vol. 10, article 220, 2010.

[25] F. C. Ringshausen, A. Schablon, and A. Nienhaus, "Interferon-γ release assays for the tuberculosis serial testing of health care workers: a systematic review," *Journal of Occupational Medicine and Toxicology*, vol. 7, no. 1, article 6, 2012.

[26] S. Perry, L. Sanchez, S. Yang, Z. Agarwal, P. Hurst, and J. Parsonnet, "Reproducibility of QuantiFERON-TB gold in-tube assay," *Clinical and Vaccine Immunology*, vol. 15, no. 3, pp. 425–432, 2008.

[27] R. Diel, R. Loddenkemper, S. Niemann, K. Meywald-Walter, and A. Nienhaus, "Negative and positive predictive value of a whole-blood interferon-γ release assay for developing active tuberculosis: an update," *American Journal of Respiratory and Critical Care Medicine*, vol. 183, no. 1, pp. 88–95, 2011.

[28] A. Zwerling, S. van den Hof, J. Scholten et al., "Interferon-γ release assays for tuberculosis screening of healthcare workers: a systematic review," *Thorax*, vol. 67, no. 1, pp. 62–70, 2012.

[29] R. Loddenkemper, R. Diel, and A. Nienhaus, "To repeat or not to repeat-that is the question!: serial testing of health-care workers for TB infection," *Chest*, vol. 142, no. 1, pp. 10–11, 2012.

Hindawi Publishing Corporation
Pulmonary Medicine
Volume 2012, Article ID 757152, 6 pages
doi:10.1155/2012/757152

Clinical Study

Levels of Interferon-Gamma Increase after Treatment for Latent Tuberculosis Infection in a High-Transmission Setting

Iukary Takenami,[1] Brook Finkmoore,[2] Almério Machado Jr.,[3,4] Krisztina Emodi,[2] Lee W. Riley,[2] and Sérgio Arruda[1,3]

[1] *Advanced Laboratory of Public Health, Oswaldo Cruz Foundation, Gonçalo Moniz Research Center, Salvador, Bahia 40296 710, Brazil*
[2] *Division of Infectious Diseases & Vaccinology, School of Public Health, University of California, Berkeley, CA 94720, USA*
[3] *Bahia School of Medicine and Public Health, Salvador, Bahia 40290 000, Brazil*
[4] *Hospital Especializado Octávio Mangabeira, Secretary of Health of Bahia State, Salvador, Bahia 40320 350, Brazil*

Correspondence should be addressed to I. Takenami, iukary@yahoo.com.br

Received 7 September 2012; Accepted 7 November 2012

Academic Editor: José R. Lapa e Silva

Objectives. We investigated IFN-γ levels before and after a six month course of isoniazid among individuals with latent tuberculosis infection (LTBI) in a high-transmission setting. *Design*. A total of 26 household contacts of pulmonary tuberculosis patients who were positive for LTBI by tuberculin skin test completed six months of treatment and submitted a blood sample for a follow-up examination. The IFN-γ response to *Mycobacterium tuberculosis*-specific antigens was measured, and the results before and after the completion of LTBI treatment were compared. *Results*. Of the 26 study participants, 25 (96%) showed an IFN-γ level higher than their baseline level before treatment ($P \leq 0.001$). Only one individual had a decreased IFN-γ level after treatment but remained positive for LTBI. *Conclusion*. In a high-transmission setting, the IFN-γ level has increased after LTBI treatment. Further studies must be undertaken to understand if this elevation is transient.

1. Introduction

Commercially available interferon-gamma release assays (IGRAs) diagnose *Mycobacterium tuberculosis (M. tb)* infection by measuring interferon-gamma (IFN-γ) released by cells of whole blood after *in vitro* stimulation with *M. tb*-specific antigens, early secreted antigenic target 6 (ESAT-6), and culture filtrate protein 10 (CFP-10) [1]. These diagnostic tests are more specific than the tuberculin skin test (TST) because they include *M. tb*-specific antigens encoded by the region of difference 1 (RD1) which is absent from *Bacillus* Calmette-Guérin (BCG) and most nontuberculous mycobacteria (NTM) [1–3]. A recent meta-analysis concluded that the commercially available IGRAs have excellent specificity to diagnose latent tuberculosis infection (LTBI) and are unaffected by BCG vaccination [4].

The capacity of IGRAs to monitor the treatment response tuberculosis (TB) and LTBI is under investigation. This study was motivated by studies which have found that the level of IFN-γ released by cells of whole blood after *in vitro* stimulation with *M. tb*-specific antigens in commercial IGRAs declines in patients treated with multidrug regimens for active TB [5–7]. Despite these results, some investigators have concluded that IGRAs may not be helpful in monitoring TB treatment because of high intersubject variability and because test reversion is rare [8, 9].

In contrast to treatment of active TB, previous studies on T-cell response before and after treatment for LTBI have shown conflicting results [10–12]. A study from Japan found that the levels of IFN-γ decreased after LTBI treatment although the commercial IGRA result did not revert to negative [11]. Another study in health care workers in India

found that IFN-γ levels remain high after LTBI treatment [10]. In Singapore, a study found that LTBI treatment had a differential effect on T-cell responses depending on which RD1 antigen the T-cells were exposed to [12]. It is unclear if the amount of IFN-γ released by T-cells stimulated with the *M.tb*-specific antigens in commercial IGRAs follow a specific pattern after completion of LTBI treatment. Moreover, it is unknown if specific T-cell responses after LTBI treatment are different in settings where the transmission of *M.tb* in the community is low compared to setting where transmission is high [10, 13–16]. This study compares the IFN-γ levels, measured by a commercial IGRA, of household contacts (HHCs) of pulmonary TB patients before and after six months of isoniazid (H) for the treatment of LTBI in a high-transmission setting.

2. Study Population and Methods

2.1. Setting. Participants were recruited from Hospital Especializado Octávio Mangabeira (HEOM) a 217-bed public chest-disease hospital in Salvador, Brazil. In 2007, Salvador had a TB incidence of approximately 79 per 100 000 population [17].

2.2. Study Participants. Study participants were HHCs of patients hospitalized with pulmonary TB at HEOM who tested positive for LBTI by TST and completed a six-month course of H [18]. None of the index cases or HHC were taking medication for the management of HIV. The characteristics of the index cases and all HHC are described elsewhere [19]. The characteristics of the HHC who initiated LTBI treatment and their adherence to the six-month regimen are described elsewhere [20]. It is well documented in the literature that HHC with LTBI are at high risk of developing active disease during the two years after infection [21, 22].

The study was approved by the human subjects committees of the Oswaldo Cruz Foundation in Salvador, Brazil, and the University of California at Berkeley, USA. Informed consent was obtained for all study participants.

2.3. Data Collection. Study participants underwent a baseline examination (TST, blood test, and interview) as part of another study [19]. LTBI could only be diagnosed in those who returned to have their TST read. Those eligible for LTBI treatment were offered the six-month supply of H free of charge [20]. Among the HHC who initiated LTBI treatment, HHCs considered to have completed treatment were those who collected six supplies of 30 H tablets from HEOM. A member of the study team (IT) called all HHC who completed LTBI treatment and invited them to return to HEOM for a follow-up examination and blood draw. HHCs were called and asked to participate in the follow-up examination a maximum of four times.

2.4. Treatment. Study participants were given six months of daily H treatment (5 mg/kg, up to 300 mg daily); this is the standard treatment regimen in Brazil [18].

2.5. Laboratory Tests. The TST was administered according to the Mantoux method, by injecting intradermally 2 tuberculin units (in 0.1 mL) of purified protein derivate (RT23 PPD; Staten Serum Institute, Copenhagen, Denmark). TST reaction was 72 hours after an administration by the chest physician on the study team (AMJ). The cut-off point for a positive reaction was $\geq$10 mm induration because this was the cutoff used in the decision to initiate treatment in Brazil at the time of the study, according to the Brazilian Society of Thoracic and Phthisiology [18].

The blood was examined with a commercially available IGRA, the QuantiFERON-TB Gold In Tube (QFT-IT; Cellestis Limited, Carnegie, VIC, Australia). QFT-IT includes the following *M.tb*-specific proteins: secreted antigenic protein 6 kDa (ESAT-6), culture filtrate protein 10 kDa (CFP-10), and TB7.7 (Rv 2654). The test was performed according to the manufacturers instructions at the immunology laboratory at Gonçalo Moniz Research Center in Salvador, Bahia, Brazil. The cut-off value for a positive response was 0.35 IU/mL. Samples that gave indeterminate results were reprocessed. Blood was drawn for the baseline IGRA before the TST was administered; both were conducted on the same day.

2.6. Statistical Analysis. Data were analyzed using GraphPad Prism v.5.0 (GraphPad Inc., San Diego, CA, USA). Wilcoxon signed rank test was used to compare the median IFN-γ levels before and after H treatment. The difference between the median values at the two time points was considered statistically significant when the P value $\leq$0.05.

3. Results

Of the 101 HHC of pulmonary TB patients who tested positive for LTBI by TST and initiated on LTBI treatment between January 2007 and February 2008, 55 (54.5%) completed six months of therapy with H. Of the 55 HHC, 26 (47.3%) returned to HEOM for the follow-up examination and submitted a second blood sample for a second test. The second blood sample was submitted for follow-up examination between four and 14 months after the completion of LTBI treatment (Figure 1). None of the 26 HHC who returned for the follow-up examination sought medical attention at HEOM for symptoms consistent with TB between the time they concluded LTBI treatment and the follow-up examination.

The median age of the 26 HHC who returned for the follow-up examination was 27 years (IQR 12.0–37.5). Sixteen (61.5%) were women, and 23 (88.5%) had BCG vaccination scars. At baseline examination, the median value of the TST induration was 13 mm (IQR 12.0–16.7) among the 26 HHC who returned for follow-up examination. Of the 29 HHC who did not return for follow-up examination, the median age was 19 years (IQR 11.5–39.0); 15 (51.7%) were male and 28 (96.5%) had a BCG vaccination scar, and the median value of the TST induration was 16 mm (IQR 11.0–19.5) at baseline examination. The sociodemographic, clinical, and laboratory profile of the HHC who did and did not return

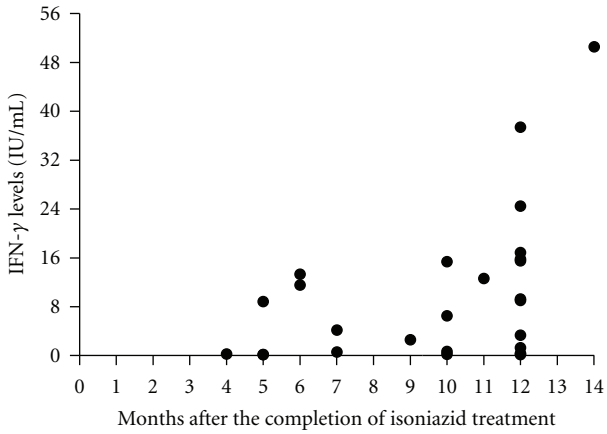


Figure 1: The difference between the baseline and follow-up IGRA result according to the number of months elapsed between the completion of H treatment and the follow-up examination. IFN-γ levels were measured by QFT-IT and plotted for each study participant ($n = 26$). H = isoniazid; IFN-γ = interferon-gamma; QFT-IT = QuantiFERON TB Gold in Tube.

for follow-up examinations were not significantly different (data not shown).

Of the 26 HHC who completed H treatment and returned for follow-up examination, 16 (61.5%) were QFT-IT positive, and 10 (38.5%) were QFT-IT negative at baseline examination. All 16 HHC who were QFT-IT positive at baseline tested positive by QFT-IT in the follow-up examination. Of the 10 HHC who were QFT-IT negative at baseline, five (50%) tested negative and five (50%) tested positive by QFT-IT in the follow-up examination. Not one HHC who tested positive by QFT-IT at baseline tested negative after completing H treatment (Figure 2).

Among the 16 HHC who tested positive by QFT-IT before LTBI treatment, the median IFN-γ level to the *M. tb*-specific antigens at baseline evaluation was 5.55 IU/mL; the median IFN-γ level of this group after six-months of H treatment was 12.97 IU/mL (P value = 0.0007) (Figure 3(a)). One individual who tested QFT-IT positive before LTBI treatment had a decreased IFN-γ level after treatment but remained QFT-IT positive (IFN-$\gamma \geq 0.35$ IU/mL) (Figure 3(a)). Among the 10 HHC who tested negative by QFT-IT before LTBI treatment, the median IFN-γ level to the *M. tb*-specific antigens at baseline evaluation was 0.02 IU/mL; the median IFN-γ level of this group after six months of H treatment was 0.32 IU/mL (P value = 0.008) (Figure 3(b)).

The difference in the median value of IFN-γ before and after treatment was 7.42 IU/mL and 0.30 IU/mL in HHC who were QFT-IT positive and those negative at baseline, respectively (P value = 0.0012).

4. Discussion

The treatment of LTBI is a basic strategy for TB control. A six-month course of a single drug, H, is effective in preventing individuals with LTBI from progressing to active disease [23]. It is difficult, however, to monitor LTBI treatment in individual patients. It is unknown if the results from commercial IGRAs that are approved for the diagnosis of TB and LTBI correlate with clinical outcomes or follow a specific pattern after antibiotic treatment for *M. tb* infection.

The current published data on the effects of LTBI treatment on IFN-γ levels are inconsistent. Studies from low-transmission settings where repeat exposure to *M. tb* is unlikely have found that T-cell IFN-γ levels decline after treatment. One such study in the United Kingdom followed the T-cell responses in students exposed to *M. tb* in a point-source school TB outbreak. Students meeting the UK guideline for the treatment of LTBI by indication from TST results were given both rifampin (R) and H; after the completion of therapy, the levels of IFN-γ response in the students substantially decreased. However, the levels of IFN-γ response also decreased in the students who tested negative by TST and did not undergo treatment [24]. Another study in recent immigrants to the UK showed that T cells produced higher levels of IFN-γ one month after the initiation of treatment for LTBI but towards the end of the LTBI treatment course, the IFN-γ level decreased. This study found that the T-cell response did not change in those with LTBI who were not initiated on treatment. Also, the author showed that peripheral blood mononuclear cells infected with *M. tb* and treated *in vitro* with H, but not R, led to an increase in the number of IFN-γ producing cells. These results suggest that H acts by actively destroying the bacilli through the disruption of the cell wall. Such a process may contribute to the increased release of cell wall-associated antigens, resulting in an increased number of antigens-specific T cells being detected during treatment [15].

Pai et al. (2006) have demonstrated that the baseline of T-cell IFN-γ response among Indian health care workers in a high-transmission setting was high even ten months after the completion of treatment for LTBI [10]. On the other hand, a study by Higuchi et al. (2008) of individuals who had contact with TB patients in Tokyo showed a decline in IFN-γ levels six months after the completion of treatment for LTBI without test reversion [11]. A study conducted in Rome, also an area of low transmission, found a significant decrease in IFN-γ levels in patients after 1-2 months of LTBI treatment and found that response of the majority of the patients became undetectable after six months [16].

Here we found that the IFN-γ levels of individuals with documented LTBI increased after H treatment. Regardless of baseline QFT-IT, 25 of 26 (96%) HHC had a higher IFN-γ level between six and 14 months after the completion of H treatment. Individuals who tested QFT-IT positive at baseline had a greater increase in IFN-γ levels after treatment than did individuals who tested QFT-IT negative at baseline (Figures 3(a) and 3(b)).

Increased production of IFN-γ by T cells may be the result of a massive release of antigens when the *M. tb* bacilli are killed by H treatment [24]. This theory is supported by our finding that HHC who tested positive by QFT-IT at baseline and presumably had a higher bacillary load experienced a greater increase in IFN-γ levels after H therapy than those who tested negative by QFT-IT.

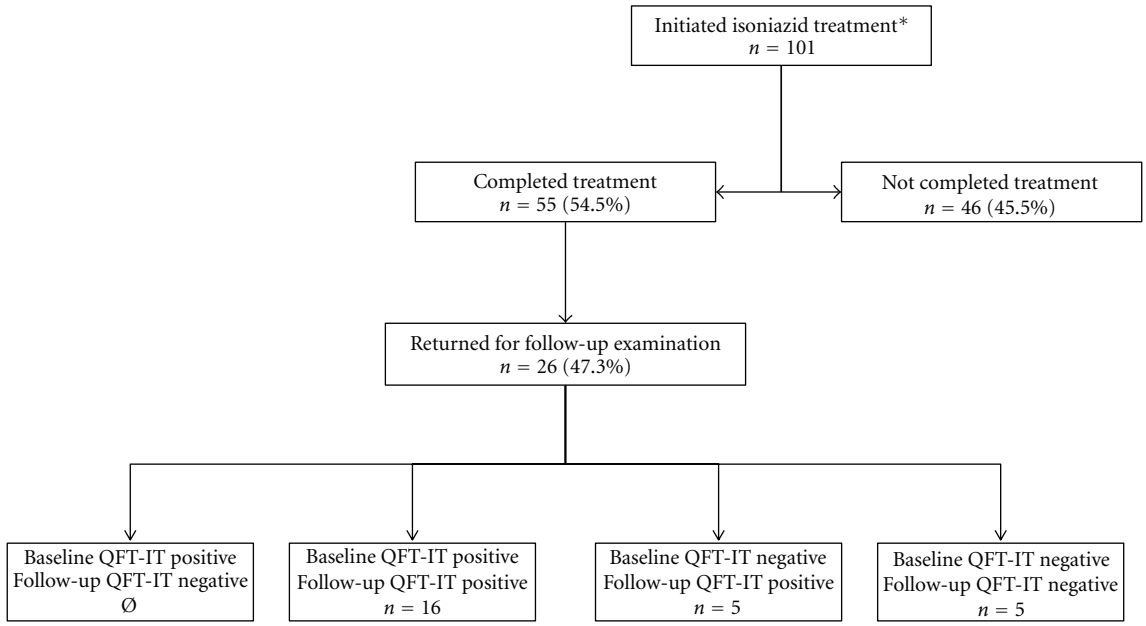


Figure 2: Flow chart of the study population and the study participants. *TST ≥ 10 mm. H: isoniazid; QFT-IT: QuantiFERON TB Gold in Tube; TST: tuberculin skin test.

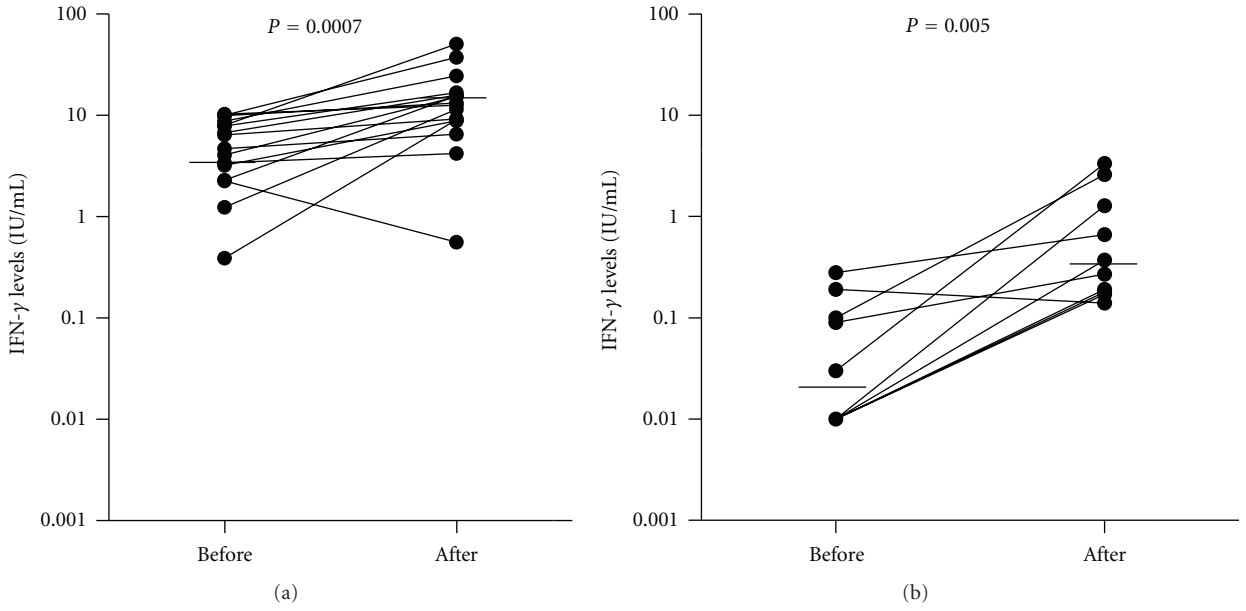


Figure 3: IFN-γ levels before and after H treatment for LTBI measured by QFT-IT stratified by baseline QFT-IT result; (a) positive QFT-IT and (b) negative QFT-IT. The solid line represents the median value of IFN-γ levels at the two time points. The differences between the median values in each group before and after H treatment are significant by the Wilconxon's signed rank test ($P = 0.0007$ and $P = 0.005$, resp.). IFN-γ: interferon-gamma; H: isoniazid; QFT-IT: QuantiFERON-TB Gold in Tube.

In combination, effector memory T cells can also upregulate IFN-γ production [25].

TST was unlikely to influence the baseline IGRA, since the HHC submitted the blood sample for baseline testing before the TST was administered. However, the TST given at the baseline evaluation may have interfered with the follow-up IGRA result due to TST-mediated boosting of IGRA responses which has been reported elsewhere [26, 27]. The 26 HHC in this study submitted blood for follow-up IGRA testing between 4 and 14 months after the baseline evaluation that included TST (Figure 1). A recent systematic review suggests that TST affects IGRA responses 3 days after the TST is administered, but its influence may persist for several months [28].

This study was conducted in a high-transmission setting; Salvador has one of the highest incidence rates of TB in Brazil. Therefore, it is possible that some of the HHCs were reinfected with *M. tb* after the completion of treatment and before they submitted blood samples for the follow-up examination. Moreover, follow-up examinations occurred between four and 14 months after the completion of H treatment. This long period of time increases the possibility that elevated IFN-γ levels are due to reinfection. It is also possible that study participants were exposed to environmental mycobacteria which share the ESAT-6 and CFP-10 genes (e.g., *M. kansasii*), and this contributed to elevated production of IFN-γ by T cells. On the other hand, an important aspect that should also be considered is the genetic factors, which has a key role in human phenotypic variability. Several polymorphisms have been described in genes associated with cytokine expression. These polymorphisms could also influence interindividual variability and direct IFN-γ production [29, 30].

IGRAs are not able to accurately estimate IFN-γ production by T cells beyond 10 IU/mL, and two of 26 HHC had IGRA results above this level in the baseline examination, and 10 of 26 HHC had IGRA results above this level in the follow-up examination. These values are likely to overestimate the IFN-γ production and should not be considered to be different than 10 IU/mL.

One of this study's limitations is that the medications were self-administered [20]. We assume that the HHCs who returned to the hospital each month for six months and received H refills were motivated to take the medication. However, we cannot exclude the possibility that some of the 26 HHCs included in this study did not complete the LTBI treatment according the protocol. Another limitation of this study is that less than half of the HHCs who completed H treatment submitted blood for follow-up examination by QFT-IT. There were not substantial epidemiologic differences between those HHCs who returned for the follow-up exam and those who did not. Therefore, it is unlikely that the 26 HHCs who were satisfactorily tested by two IGRAs were a biased group. Another limitation is that the single QFT-IT test for each study participant before and after the completion of H treatment were not taken at the same time. Finally, this study did not have a control group of untreated HHC with LTBI in whom the T-cell responses were monitored at the same time. Limitations in the study design and the lack of active contact-tracing programs through which to monitor individuals at high risk for progressing to active disease precluded data from being collected.

5. Conclusion

The data presented in this study suggest that IGRA results increase in individuals after the completion of LTBI treatment; however, conflicting data from other studies and the limitations of our study do not allow us to exclude the potential role of IGRAs in monitoring LTBI treatment. To further investigate this, larger studies in high-transmission settings must be conducted, which collect data on the T-cell responses of people with LTBI before, during, and after LTBI treatment over a longer time period.

Conflict of Interests

None of the authors of this paper have any conflict of interests.

Acknowledgments

The authors acknowledge the director of the HOEM, the clinical laboratory of the hospital, and the National Institute of Science and Technology in Tuberculosis (INCT-TB) by supporting given to master' student Iukary Takenami. they also thank EBMSP students by their contribution with medical questionnaire interviews. Financial support was provided by NIH Fogarty International Center 5U2RTW 006885.

References

[1] P. Andersen, M. E. Munk, J. M. Pollock, and T. M. Doherty, "Specific immune-based diagnosis of tuberculosis," *The Lancet*, vol. 356, no. 9235, pp. 1099–1104, 2000.

[2] M. Pai, L. W. Riley, and J. M. Colford, "Interferon-γ assays in the immunodiagnosis of tuberculosis: a systematic review," *Lancet Infectious Diseases*, vol. 4, no. 12, pp. 761–766, 2004.

[3] S. Y. Eum, Y. J. Lee, H. K. Kwak et al., "Evaluation of the diagnostic utility of a whole-blood interferon-γ assay for determining the risk of exposure to *Mycobacterium tuberculosis* in Bacille Calmette-Guerin (BCG)-vaccinated individuals," *Diagnostic Microbiology and Infectious Disease*, vol. 61, no. 2, pp. 181–186, 2008.

[4] M. Pai, A. Zwerling, and D. Menzies, "Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update," *Annals of Internal Medicine*, vol. 149, no. 3, pp. 177–184, 2008.

[5] A. M. Aiken, P. C. Hill, A. Fox et al., "Reversion of the ELISPOT test after treatment in Gambian tuberculosis cases," *BMC Infectious Diseases*, vol. 6, article 66, 2006.

[6] Y. Kobashi, K. Mouri, S. Yagi, Y. Obase, N. Miyashita, and M. Oka, "Transitional changes in T-cell responses to *Mycobacterium tuberculosis*-specific antigens during treatment," *Journal of Infection*, vol. 58, no. 3, pp. 197–204, 2009.

[7] I. Sauzullo, F. Mengoni, M. Lichtner et al., "In vivo and in vitro effects of antituberculosis treatment on mycobacterial interferon-γ T cell response," *PLoS ONE*, vol. 4, no. 4, Article ID e5187, 2009.

[8] V. Bosshard, P. Roux-Lombard, T. Perneger et al., "Do results of the T-SPOT.TB interferon-γ release assay change after treatment of tuberculosis?" *Respiratory Medicine*, vol. 103, no. 1, pp. 30–34, 2009.

[9] S. Ribeiro, K. Dooley, J. Hackman et al., "T-SPOT.TB responses during treatment of pulmonary tuberculosis," *BMC Infectious Diseases*, vol. 9, article 23, 2009.

[10] M. Pai, R. Joshi, S. Dogra et al., "Persistently elevated T cell interferon-γ responses after treatment for latent tuberculosis infection among health care workers in India: a preliminary report," *Journal of Occupational Medicine and Toxicology*, vol. 1, no. 1, article 7, 2006.

[11] K. Higuchi, N. Harada, and T. Mori, "Interferon-γ responses after isoniazid chemotherapy for latent tuberculosis," *Respirology*, vol. 13, no. 3, pp. 468–472, 2008.

[12] C. B. E. Chee, T. M. S. Barkham, K. W. KhinMar, S. H. Gan, and Y. T. Wang, "Quantitative T-cell interferon-gamma responses to *Mycobacterium tuberculosis*-specific antigens in active and latent tuberculosis," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 28, no. 6, pp. 667–670, 2009.

[13] S. Carrara, D. Vincenti, N. Petrosillo, M. Amicosante, E. Girardi, and D. Goletti, "Use of a T cell-based assay for monitoring efficacy of antituberculosis therapy," *Clinical Infectious Diseases*, vol. 38, no. 5, pp. 754–756, 2004.

[14] A. Lalvani, "Counting antigen-specific T cells: a new approach for monitoring response to tuberculosis treatment?" *Clinical Infectious Diseases*, vol. 38, no. 5, pp. 757–759, 2004.

[15] K. A. Wilkinson, O. M. Kon, S. M. Newton et al., "Effect of treatment of latent tuberculosis infection on the T cell response to *Mycobacterium tuberculosis* antigens," *Journal of Infectious Diseases*, vol. 193, no. 3, pp. 354–359, 2006.

[16] D. Goletti, M. P. Parracino, O. Butera et al., "Isoniazid prophylaxis differently modulates T-cell responses to RD1-epitopes in contacts recently exposed to *Mycobacterium tuberculosis*: a pilot study," *Respiratory Research*, vol. 8, article 5, 2007, American Thoracic Society, Targeted tuberculin testing and treatment of latent tuberculosis infection, Morbidity and Mortality Weekly Report, vol. 49, pp. 1–51, 2000.

[17] MS—Ministério da Saúde, Brasil, Fundação Nacional de Saúde, Centro Nacional de Epidemiologia, Informe epidemiológico do DATASUS, Brasília, 2009, http://tabnet.datasus.gov.br.

[18] MS—Ministério da Saúde, "II Consenso Brasileiro de Tuberculose—Diretrizes Brasileiras para Tuberculose 2004," *Jornal Brasileiro de Pneumologia*, vol. 30, supplement 1, pp. S2–S56, 2004.

[19] A. Machado, K. Emodi, I. Takenami et al., "Analysis of discordance between the tuberculin skin test and the interferon-gamma release assay," *International Journal of Tuberculosis and Lung Disease*, vol. 13, no. 4, pp. 446–453, 2009.

[20] A. Machado, B. Finkmoore, K. Emodi et al., "Risk factors for failure to complete a course of latent tuberculosis infection treatment in Salvador, Brazil," *International Journal of Tuberculosis and Lung Disease*, vol. 13, no. 6, pp. 719–725, 2009.

[21] N. M. Parrish, J. D. Dick, and W. R. Bishai, "Mechanisms of latency in *Mycobacterium tuberculosis*," *Trends in Microbiology*, vol. 6, no. 3, pp. 107–112, 1998.

[22] S. Radhakrishna, T. R. Frieden, R. Subramani, T. Santha, and P. R. Narayanan, "Additional risk of developing TB for household members with a TB case at home at intake: a 15-year study," *The International Journal of Tuberculosis and Lung Disease*, vol. 11, no. 3, pp. 282–288, 2007.

[23] B. L. Johnston and J. M. Conly, "Re-examining treatment of latent tuberculosis infection," *Canadian Journal of Infectious Diseases*, vol. 12, no. 4, pp. 211–214, 2001.

[24] K. Ewer, K. A. Millington, J. J. Deeks, L. Alvarez, G. Bryant, and A. Lalvani, "Dynamic antigen-specific T-cell responses after point-source exposure to *Mycobacterium tuberculosis*," *American Journal of Respiratory and Critical Care Medicine*, vol. 174, no. 7, pp. 831–839, 2006.

[25] J. Geginat, F. Sallusto, and A. Lanzavecchia, "Cytokine-driven proliferation and differentiation of human naïve, central memory and effector memory $CD4^+$ T cells," *Pathologie Biologie*, vol. 51, no. 2, pp. 64–66, 2003.

[26] E. M. S. Leyten, C. Prins, A. W. J. Bossink et al., "Effect of tuberculin skin testing on a *Mycobacterium tuberculosis*-specific interferon-γ assay," *European Respiratory Journal*, vol. 29, no. 6, pp. 1212–1216, 2007.

[27] R. N. Van Zyl-Smit, M. Pai, K. Peprah et al., "Within-subject variability and boosting of t-cell interferon-γ responses after tuberculin skin testing," *American Journal of Respiratory and Critical Care Medicine*, vol. 180, no. 1, pp. 49–58, 2009.

[28] R. N. Van Zyl-Smit, A. Zwerling, K. Dheda, and M. Pai, "Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review," *PLoS ONE*, vol. 4, no. 12, Article ID e8517, 2009.

[29] P. Selvaraj, K. Alagarasu, M. Harishankar, M. Vidyarani, D. Nisha Rajeswari, and P. R. Narayanan, "Cytokine gene polymorphisms and cytokine levels in pulmonary tuberculosis," *Cytokine*, vol. 43, no. 1, pp. 26–33, 2008.

[30] D. López-Maderuelo, F. Arnalich, R. Serantes et al., "Interferon-γ and interleukin-10 gene polymorphisms in pulmonary tuberculosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 167, no. 7, pp. 970–975, 2003.

Hindawi Publishing Corporation
Pulmonary Medicine
Volume 2012, Article ID 828106, 7 pages
doi:10.1155/2012/828106

Research Article

Cigarette-Smoking Intensity and Interferon-Gamma Release Assay Conversion among Persons Who Inject Drugs: A Cohort Study

Sanghyuk S. Shin,[1,2] Manuel Gallardo,[3] Remedios Lozada,[3] Daniela Abramovitz,[2] Jose Luis Burgos,[2] Rafael Laniado-Laborin,[4] Timothy C. Rodwell,[2] Thomas E. Novotny,[1] Steffanie A. Strathdee,[2] and Richard S. Garfein[2]

[1] *San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-4162, USA*
[2] *Division of Global Public Health, Department of Medicine, School of Medicine, University of California San Diego, 9500 Gilman Drive, MC-0507, San Diego, CA 92093-0507, USA*
[3] *Patronato Pro-COMUSIDA, Ninos Heroes No. 697, Zona Centro, Tijuana, BC, Mexico*
[4] *Parque Industrial Internacional, Universidad Autonoma de Baja California, Calzada Universidad 14418, Tijuana, BC, Mexico*

Correspondence should be addressed to Richard S. Garfein, rgarfein@ucsd.edu

Received 18 September 2012; Accepted 15 November 2012

Academic Editor: Jonathan Golub

We analyzed data from a longitudinal cohort study of persons who inject drugs (PWID) in Tijuana, Mexico, to explore whether cigarette smoking increases the risk of interferon gamma release assay (IGRA) conversion. PWID were recruited using respondent driven sampling (RDS). QuantiFERON-TB Gold In-Tube (QFT) assay conversion was defined as interferon-gamma concentrations <0.35 IU/mL at baseline and ≥0.7 IU/mL at 18 months. We used multivariable Poisson regression adjusted for RDS weights to estimate risk ratios (RRs). Of 129 eligible participants, 125 (96.9%) smoked at least one cigarette during followup with a median of 11 cigarettes smoked daily, and 52 (40.3%) had QFT conversion. In bivariate analysis, QFT conversion was not associated with the number of cigarettes smoked daily ($P = 0.716$). Controlling for age, gender, education, and alcohol use, the RRs of QFT conversion for smoking 6–10, 11–15, and ≥16 cigarettes daily compared to smoking 0–5 cigarettes daily were 0.9 (95% confidence interval (CI), 0.5–1.6), 0.5 (95% CI, 0.3–1.2), and 0.7 (95% CI, 0.3–1.6), respectively. Although this study did not find an association between self-reported smoking intensity and QFT conversion, it was not powered sufficiently to negate such an association. Larger longitudinal studies are needed to fully explore this relationship.

1. Introduction

Evidence has accumulated over the years which demonstrates a causal relationship between tobacco use and increased tuberculosis (TB) morbidity and mortality [1–6]. However, the strength of evidence for this relationship varies by TB outcome [3]. For example, while high-quality longitudinal cohort studies provide strong evidence that tobacco use increases the risk of TB disease, the evidence for the relationship between tobacco use and the risk of *Mycobacterium tuberculosis* infection is relatively weak [3, 7, 8]. Previous studies exploring this relationship utilized cross-sectional or case-control methodologies to determine the association between "ever" or "current" smoking and lifetime infection with *M. tuberculosis* as determined by a single tuberculin skin test (TST) result [9–14]. Therefore, these studies were not able to assess the temporality between tobacco use and *M. tuberculosis* infection. For example, a participant infected with *M. tuberculosis* as a child who subsequently began smoking years later would contribute to the positive association between smoking and TST positivity.

An improved understanding of the relationship between cigarette smoking and *M. tuberculosis* infection would help inform the implementation of tobacco control efforts as a

part of global TB interventions. However, due to the low incidence of *M. tuberculosis* infection in most populations, conducting longitudinal cohort studies to strengthen the evidence regarding this relationship would necessitate the enrollment and long-term followup of a large number of participants. Furthermore, while interferon gamma release assays (IGRAs) have been shown to have higher specificity than TSTs for the diagnosis of latent TB infection (LTBI), no study has explored the effect of tobacco use on serial IGRA test results [15, 16].

The objective of the present study was to investigate the association between level of cigarette smoking and IGRA conversion among persons who inject drugs (PWID) in Tijuana, Mexico, a population at high risk for *M. tuberculosis* infection. Previous studies using this cohort showed a high baseline LTBI prevalence LTBI of 67% and an 18-month IGRA conversion rate of 28.7% to 51.9%, depending on the definition of conversion used [17, 18]. We hypothesized that higher levels of cigarette smoking in this population would be associated with increased risk for IGRA conversion in a dose-response relationship.

2. Materials and Methods

2.1. Study Design and Population. We analyzed data from a longitudinal cohort study of PWID in Tijuana, Mexico that sought to determine risk factors for incident HIV, TB, and syphilis [19]. Study recruitment and data collection methods have been described in detail previously [19]. Briefly, eligible study participants were ages 18 years or older, had injected illicit drugs within the previous month, and had no plans to move from Tijuana during the followup period. Participants were recruited through respondent-driven sampling (RDS), which relies on recruiting participants through referrals from previously enrolled participants [20, 21]. RDS allows for the derivation of population-representative estimates of prevalence and risk factors by adjusting for the information collected on the participants' social networks during analysis [20, 21]. Enrolled participants made study visits at baseline and at 6, 12, and 18 months. To increase retention, community outreach workers actively contacted participants to remind them of their followup appointments. Participants were also provided with $10 at baseline and $5 at followup visits as compensation for their time and travel expenses. Only participants who tested IGRA negative at baseline and who had IGRA results available at 18 months were included in the present analysis. Institutional Review Boards at University of California, San Diego and the Tijuana General Hospital reviewed and approved the study protocol, and informed consent was obtained from all participants.

2.2. Measures. An in-depth questionnaire was administered via person-to-person interview at each visit. The questionnaire contained items on demographic characteristics and substance use behavior, including injection and noninjection use of illicit drugs, alcohol consumption, and cigarette smoking. Cigarette smoking was ascertained by first asking, "Have you smoked cigarettes in the past 6 months?" Participants who responded "Yes" were asked, "In the past 6 months, how many cigarettes did you usually smoke per day?" Based on preliminary analysis, we anticipated a high prevalence of cigarette smoking in this population and, consequently, insufficient number of nonsmokers for categorization. Therefore, we used the average number of cigarettes smoked daily during the 18-month study period as the exposure of interest. This exposure was stratified into quartiles (0–5, 6–10, 11–15, and ≥16 cigarettes) for the primary analysis.

IGRA conversion at 18 months was ascertained using QuantiFERON-TB Gold In-Tube ((QFT) Cellestis, VIC, Australia). For this test, whole blood samples were collected in three separate tubes: a Nil Control tube, TB Antigen tube, and a Mitogen Control tube. The tubes were incubated at 37°C for 16 to 24 hours and centrifuged. The interferon-gamma (IFN-γ) released in the Nil Control tube was then measured using enzyme-linked immunosorbent assay (ELISA) and subtracted from that found in the TB Antigen Tube. QFT was administered at baseline for all participants. However, because of an unexpected delay in procuring the supplies necessary for specimen collection and testing, only a subset of the participants who were QFT negative using the manufacturer recommended cutoff of <0.35 IU/mL at baseline were retested at 18 months. For the primary analysis, we used a previously published conservative definition of QFT conversion (i.e., baseline IFN-γ < 0.35 IU/mL and IFN-γ ≥ 0.70 IU/mL at followup), which reduces false positive conversions that could potentially arise due to within-subject variability observed in serial QFT tests [22]. In a secondary sensitivity analysis, we used the cutoff of 0.35 IU/mL at 18 months to define conversion.

2.3. Statistical Analysis. The Pearson's χ^2 test was used for comparisons involving categorical variables, and the Wilcoxon rank-sum and the Kruskal-Wallis tests were used for continuous variables. We considered statistical tests to be significant at α of 0.05. We constructed Poisson regression models with robust variance estimation, via generalized estimating equation (GEE), to determine risk ratios (RRs) for QFT conversion for participants in each smoking exposure quartile compared to those in the first quartile [23, 24]. The models were weighted by inverse probability weights derived using the RDS Analytical Tool [25]. The GEE algorithm also accounted for clustering by recruiter assuming an exchangeable correlation structure.

The base model included covariates representing established risk factors for *M. tuberculosis* infection, including age, gender, education, and alcohol use, regardless of their association with QFT conversion in our study population. We also evaluated the effect of drug use behavior using the "change-in-estimate" approach; drug use variables were added to the base model only if their inclusion changed the RRs between smoking and QFT by >10% [26]. Drug use variables evaluated included frequency and duration of heroin, methamphetamine, cocaine and marijuana use, including smoking of these substances. To account for the

Table 1: Demographic and behavioral characteristics of participants with negative QFT results (IFN-γ < 0.35 IU/mL) at baseline, included versus not included in the analysis; Tijuana, Mexico, 2006–2008.

Characteristic	Included $N = 129$ n (%)	Not included $N = 212$ n (%)	P value*
Gender			0.806
Male	107 (82.9)	178 (84.0)	
Female	22 (17.1)	34 (16.0)	
Age, median (IQR)	38 (32–43)	37 (30–42)	0.247
Education			0.389
Up to primary	39 (30.2)	76 (35.8)	
Primary to middle	60 (46.5)	83 (39.2)	
High school and higher	30 (23.3)	53 (25.0)	
Unstable housing			0.357
No	113 (87.6)	178 (84.0)	
Yes	16 (12.4)	34 (16.0)	
History of incarceration			0.719
No	78 (60.5)	124 (58.5)	
Yes	51 (39.5)	88 (41.5)	
HIV infection			0.366
No	123 (95.3)	197 (92.9)	
Yes	6 (4.7)	15 (7.1)	
Alcohol			0.154
None	76 (58.9)	134 (63.2)	
Less than twice per week	31 (24.0)	57 (26.9)	
Twice per week or more	22 (17.1)	21 (9.9)	
Smoked cigarette during study periods (18 months)			0.919
No	4 (3.1)	7 (3.3)	
Yes	125 (96.9)	205 (96.7)	
Number of cigarettes smoked daily, median (IQR)	10.5 (6–15)	12.5 (7–19)	0.023
Number of cigarettes smoked daily (quartiles)			0.058
0–5	30 (23.3)	41 (19.3)	
6–10	36 (27.9)	50 (23.6)	
11–15	37 (28.7)	49 (23.1)	
16+	26 (20.2)	72 (34.0)	
QFT conversion at 18 mos (IFN-$\gamma \geq 0.70$ IU/mL)			
No	77 (59.7)	—	
Yes	52 (40.3)	—	

* P values for the difference between included versus excluded participants were generated using the Pearson's χ^2 test for categorical variables and the Wilcoxon rank sum for continuous variables.
IFN-γ: interferon-gamma; QFT: QuantiFERON-TB Gold In-Tube; IQR: interquartile range.

possible loss of statistical power due to overfitting the final model with covariates, we also constructed a reduced model that included the stratified smoking exposure variable and only the covariates that were statistically significant predictors of QFT conversion. For the final model, we calculated tolerance and condition index statistics to assess multicollinearity, and Pearson residuals, Cook's distance, and leverage statistics to identify outlier observations [27]. SAS 9.3 (Cary, North Carolina) was used for all analyses.

3. Results

Of the 1056 participants enrolled during April 2006–April 2007, 341 had negative QFT (IFN-γ < 0.35 IU/mL) at baseline. Of these, 129 (37.8%) who had QFT results available at 18 months were included in the analysis. Among included participants, the median age was 38 (interquartile range (IQR) = 32–43), 107 (82.9%) were male, and 99 (76.7%) had obtained middle school education or less (Table 1). Nearly

Table 2: QFT conversion (IFN-$\gamma \geq 0.70$ IU/mL) at 18 months by quartiles of number of cigarettes smoked among persons who inject drugs in Tijuana, Mexico, 2006–2008.

Number of cigarettes smoked daily (quartiles)	QFT conversion	P value
0–5	13/30 (43.3)	0.716
6–10	16/36 (44.4)	
11–15	15/37 (40.5)	
16+	8/26 (30.8)	

* P value for the difference in QFT conversion at 18 months across quartiles was generated using the Pearson's χ^2 test.
IFN-γ: interferon-gamma; QFT: QuantiFERON-TB Gold In-Tube.

all of the included participants (96.9%) reported smoking at least one cigarette during the followup period. On average, participants included in the analysis smoked fewer cigarettes per day compared to the 212 participants who were excluded due to missing QFT results at 18 months (median of 10.5 [IQR = 6–15] versus 12.5 [IQR = 7–19] cigarettes per day, resp., $P = 0.023$). None of the other characteristics differed between included and excluded participants (Table 1). At 18 months, 52 (40.3%) participants met the primary QFT conversion definition.

Across quartiles of self-reported daily cigarettes smoked, the median IFN-γ concentrations were 0.61, 0.56, 0.19, and 0.315 IU/mL, respectively (Figure 1), and the proportion of participants with QFT conversion was 43.4%, 44.4%, 40.5%, and 30.8%, respectively (Table 2). There was no association between IFN-γ distribution or QFT conversion across quartile levels of daily cigarettes smoked ($P = 0.523$ and $P = 0.716$, resp.). In the bivariate model adjusted for RDS weights, which included only the smoking quartiles as the independent variable, the RRs for QFT conversion for each quartile of daily number of cigarettes smoked compared to the lowest exposure quartile were 0.75 (95% confidence interval [CI] 0.37–1.55), 0.53 (95% CI 0.23–1.20), and 0.59 (95% CI 0.24–1.43), respectively (Table 3).

In multivariable analysis adjusted for RDS, inclusion of drug use variables to the base model did not change the association between cigarette smoking quartiles and QFT conversion. Therefore, the final model consisted of daily cigarette smoking quartiles, age, gender, education, and alcohol use as independent variables (Table 3). The adjusted RRs for QFT conversion for each quartile of daily number of cigarettes smoked compared to the lowest exposure quartile were 0.86 (95% CI 0.46–1.63), 0.54 (95% CI 0.25–1.17), and 0.74 (95% CI 0.33–1.64), respectively (Table 3; Figure 2). There was no statistically significant difference in the risk of QFT conversion at any of the quartiles of daily cigarettes smoked compared with that of the lowest exposure quartile. Furthermore, age, gender, education, and alcohol use were not statistically significant predictors of QFT conversion. In the reduced model that included the smoking variables and educational attainment only, having attained less than high school education compared with higher education was found to increase the risk of QFT conversion (RR = 2.83; 95% CI 1.08–7.42). As with the full model, higher levels of daily

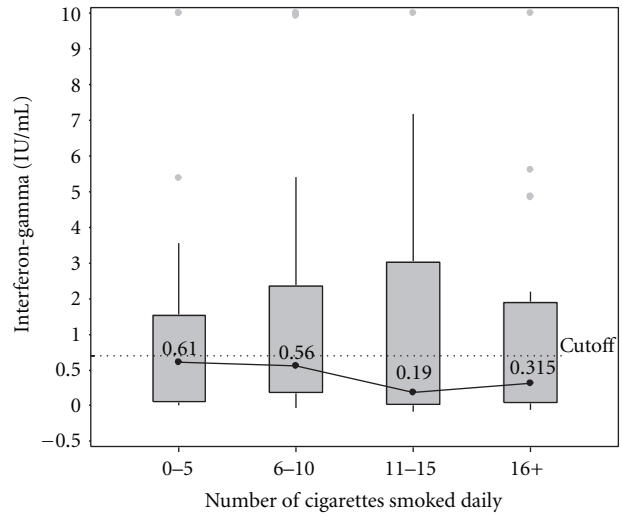


Figure 1: Median IFN-γ and interquartile range at 18 months by quartiles of number of cigarettes smoked. IFN-$\gamma > 10$ IU/mL were set to 10 IU/mL due to imprecision at high concentration levels. The dotted line represents the 0.70 IU/mL cutoff which was used to define QFT conversion. IFN-γ: interferon-gamma. QFT: QuantiFERON-TB Gold In-Tube.

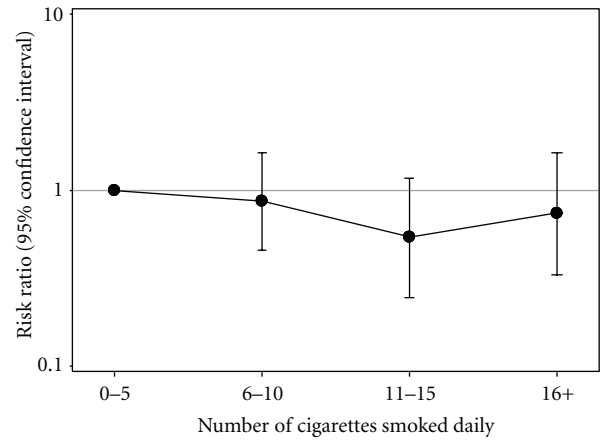


Figure 2: Adjusted risk ratios for QFT conversion (IFN-$\gamma \geq 0.70$ IU/mL) at 18 months based on the final multivariable Poisson regression model with robust variance which included the following covariates: quartiles of the number of cigarettes smoked daily, education, age, gender, and alcohol use. IFN-γ: interferon-gamma. QFT: QuantiFERON-TB Gold In-Tube.

cigarette smoking exposure quartiles were not associated with QFT conversion risk in this model (Table 3).

All tolerance estimates were greater than 0.10, and the highest condition index was 11.8 in the final model, indicating that multicollinearity did not affect our findings. We found five potential outliers based on residual and influence statistics, but removing these had no effect on our findings. Additionally, using a cutoff of 0.35 IU/mL instead of 0.70 IU/mL at 18 months to define conversion and fitting the final model with daily number of cigarettes smoked as a continuous variable did not alter our findings. In post

Table 3: Adjusted risk ratios for QFT conversion (IFN-$\gamma \geq 0.70$ IU/mL) at 18 months based on multivariable Poisson regression models with robust variance; Tijuana, Mexico, 2006–2008.

Variable	Risk Ratio (95% Confidence Interval)		
	Bivariate Model	Reduced Model	Final Model
Number of cigarettes smoked daily (quartiles)			
0–5	1.00	1.00	1.00
6–10	0.75 (0.37–1.55)	1.04 (0.53–2.04)	0.86 (0.46–1.63)
11–15	0.53 (0.23–1.20)	0.73 (0.36–1.51)	0.54 (0.25–1.17)
≥16	0.59 (0.24–1.43)	0.79 (0.34–1.83)	0.74 (0.33–1.64)
Education			
High school or higher		1.00	1.00
Less than high school		2.83 (1.08–7.42)	2.60 (0.96–7.03)
Age			
+10 years			1.25 (0.80–1.94)
Gender			
Male			1.00
Female			0.81 (0.34–1.93)
Alcohol use			
<2x per week			1.00
≥2x per week			1.04 (0.52–2.08)

IFN-γ: interferon-gamma; QFT: QuantiFERON-TB Gold In-Tube.

hoc power analysis, assuming 43.3% QFT conversion risk that we found among participants in the lowest cigarette-smoking quartile, our sample size of 129 provided 28.1%, 55.6%, and 82.6% power to detect a RR of 1.4, 1.6, and 1.8, respectively, for QFT conversion among participants in the highest exposure quartile.

4. Discussion

In our analysis of longitudinal cohort data from PWID in Tijuana, we were not able to detect a dose-response relationship between the number of cigarettes smoked per day and QFT conversion. Previous studies evaluating dose-response relationships between cigarette-smoking and *M. tuberculosis* infection have shown mixed results for this putative association. A study of population survey data in South Africa found no evidence of a dose-response relationship between pack-years and TST positivity [9]. Likewise, a study of people with silicosis in Hong Kong found no relationship between the number of cigarettes smoked per day or cigarette pack-years and TST positivity [10]. In contrast, a study of prisoners in Pakistan found that TST prevalence increased with number of cigarettes smoked per day [11]. However, these previous studies employed cross-sectional or case-control study designs, which limit their ability to evaluate temporality between cigarette-smoking exposures and *M. tuberculosis* infection.

Cigarette smoking has been hypothesized to increase the risk of *M. tuberculosis* infection by adversely affecting the innate immune system of the host and/or causing structural damage to the respiratory tract [28]. First, exposure to cigarette smoke might impair the ability of alveolar macrophages to clear the *M. tuberculosis* bacilli before T cells are primed for adaptive immunity. Under this model, increased exposure to cigarette smoke in the lungs would result in increased acute susceptibility to *M. tuberculosis* infection. We were unable to generate evidence to support this model in our study. Smoking also impairs the mucociliary clearance of pathogens and causes other changes to the respiratory tract that could increase the risk for *M. tuberculosis* infection over time [28–30]. Because we did not collect information regarding lifetime history of smoking, we were not able to evaluate the long-term effect of cigarette smoking on *M. tuberculosis* infection.

Our findings should be interpreted with consideration of the following limitations. First, we were not able to compare QFT conversion risk between smokers and nonsmokers because nearly all of our study participants reported smoking during the study followup period. If even low levels of cigarette smoking increase IGRA conversion substantially, there might have been minimal increased risk for higher frequency smokers, and our study might not have had sufficient power to detect a dose-response relationship. While we had adequate sample size to detect a RR of 1.8 or greater for QFT conversion between participants in the lowest and highest smoking exposure quartiles, the study was underpowered to conclude that there is no association between smoking and QFT conversion. We were also unable to control for history of exposure to persons with TB disease, which is a necessary causal factor for incident *M. tuberculosis* infection. The inclusion of participants who were not exposed to persons with TB disease in our analysis could have biased

our results towards the null. However, controlling for a proxy variable "Have you ever known someone who had TB?" did not alter our findings (data not shown). Future longitudinal studies should investigate the risk of cigarette smoking on *M. tuberculosis* infection among study participants recruited from persons with known history of exposure to someone with TB disease.

It is also possible that our study participants were already at high risk for *M. tuberculosis* infection due to other risk factors, which might have overshadowed an incremental increase in risk due to cigarette smoking. In addition, as with TSTs, QFT assays have significant within-subject variability such that conversions and reversions often occur around the 0.35 IU/mL cutoff during serial testing even among persons who are at low risk for *M. tuberculosis* infection [31, 32]. While we used a conservative definition of QFT conversion to minimize misclassification in our analysis, the conversions observed in our study might not represent incident *M. tuberculosis* infection. Furthermore, since the recommended QFT cutoff of 0.35 IU/mL was derived to maximize specificity for *M. tuberculosis* infection, the use of this cutoff as an inclusion criterion could have resulted in the inclusion of some participants who were already infected with *M. tuberculosis* at baseline. However, restricting the analysis to only those participants with baseline QFT of <0.20 did not alter our findings (data not shown). Participants included in our study smoked fewer cigarettes than the participants who were excluded due to unavailable QFT results at 18 months. Therefore, our findings might not be generalizable to all PWID at risk for *M. tuberculosis* infection. Smoking levels were ascertained by self-report, which might have insufficient precision to evaluate a dose-response relationship. Lastly, we did not collect information regarding secondhand smoke exposure, which has been shown to be associated with *M. tuberculosis* infection among children [33].

5. Conclusions

The present study is the first longitudinal cohort study to explore the relationship between cigarette-smoking intensity and *M. tuberculosis* infection, and the first to use IGRA conversion as the outcome. Given our findings and the limitations of previous research on this topic, additional research is needed to determine whether there is a causal relationship between smoking and *M. tuberculosis* infection. For example, a recent mathematical modeling study concluded that intensified tobacco control efforts could prevent 27 million TB-related deaths by 2050 [34]. However, the authors of that study assumed a RR of 2.0 for the effect of smoking on *M. tuberculosis* infection in their model to arrive at this conclusion. Stronger evidence from larger longitudinal studies is needed to justify such assumptions. Ideally, such a study would be conducted among persons at high risk for *M. tuberculosis* infection, such as those with household exposure to persons with TB disease, and consists of sufficient numbers of smokers and nonsmokers. While the evidence of a causal relationship between smoking and *M. tuberculosis* infection is weak, substantial evidence exists that implicates smoking as an independent risk factor for the development of TB disease [1–8]. Therefore, integration of tobacco and TB control interventions remains a high priority for global health [1].

Acknowledgments

The authors wish to thank the study participants and the staff at Pro-COMUSIDA who assisted with data collection. The parent study was supported by U.S. National Institute on Drug Abuse (NIDA; R37DA019829). S. S. Shin received support from NIDA Dissertation Grant 1R36DA033152. Rodwell received support from a NIAID Career Development Award K01AI083784. Garfein received support from a NIDA grant (R01DA031074).

References

[1] WHO & The Union, *Monograph on TB and Tobacco Control: Joining Efforts to Control Two Related Global Epidemics*, World Health Organization & International Union Against Tuberculosis and Lung Disease, Geneva, Switzerland, 2007.

[2] H. H. Lin, M. Ezzati, and M. Murray, "Tobacco smoke, indoor air pollution and tuberculosis: a systematic review and meta-analysis," *PLoS Medicine*, vol. 4, article e20, 2007.

[3] K. Slama, C. Y. Chiang, D. A. Enarson et al., "Tobacco and tuberculosis: a qualitative systematic review and meta-analysis," *International Journal of Tuberculosis and Lung Disease*, vol. 11, no. 10, pp. 1049–1061, 2007.

[4] M. N. Bates, A. Khalakdina, M. Pai, L. Chang, F. Lessa, and K. R. Smith, "Risk of tuberculosis from exposure to tobacco smoke: a systematic review and meta-analysis," *Archives of Internal Medicine*, vol. 167, no. 4, pp. 335–342, 2007.

[5] R. N. Van Zyl Smit, M. Pai, W. W. Yew et al., "Global lung health: the colliding epidemics of tuberculosis, tobacco smoking, HIV and COPD," *European Respiratory Journal*, vol. 35, no. 1, pp. 27–33, 2010.

[6] N. K. Schneider and T. E. Novotny, "Addressing smoking cessation in tuberculosis control," *Bulletin of the World Health Organization*, vol. 85, no. 10, pp. 820–821, 2007.

[7] S. H. Jee, J. E. Golub, J. Jo, I. S. Park, H. Ohrr, and J. M. Samet, "Smoking and risk of tuberculosis incidence, mortality, and recurrence in South Korean men and women," *American Journal of Epidemiology*, vol. 170, no. 12, pp. 1478–1485, 2009.

[8] H. H. Lin, M. Ezzati, H. Y. Chang, and M. Murray, "Association between tobacco smoking and active tuberculosis in Taiwan: prospective cohort study," *American Journal of Respiratory and Critical Care Medicine*, vol. 180, no. 5, pp. 475–480, 2009.

[9] S. Den Boon, S. W. P. Van Lill, M. W. Borgdorff et al., "Association between smoking and tuberculosis infection: a population survey in a high tuberculosis incidence area," *Thorax*, vol. 60, no. 7, pp. 555–557, 2005.

[10] C. C. Leung, W. W. Yew, W. S. Law et al., "Smoking and tuberculosis among silicotic patients," *European Respiratory Journal*, vol. 29, no. 4, pp. 745–750, 2007.

[11] H. Hussain, S. Akhtar, and D. Nanan, "Prevalence of and risk factors associated with Mycobacterium tuberculosis infection in prisoners, North West Frontier Province, Pakistan," *International Journal of Epidemiology*, vol. 32, no. 5, pp. 794–799, 2003.

[12] A. J. Plant, R. E. Watkins, B. Gushulak et al., "Predictors of tuberculin reactivity among prospective Vietnamese migrants: the effect of smoking," *Epidemiology and Infection*, vol. 128, no. 1, pp. 37–45, 2002.

[13] S. A. McCurdy, D. S. Arretz, and R. O. Bates, "Tuberculin reactivity among California Hispanic migrant farm workers," *American Journal of Industrial Medicine*, vol. 32, pp. 600–605, 1997.

[14] M. Nisar, C. S. D. Williams, D. Ashby, and P. D. O. Davies, "Tuberculin testing in residential homes for the elderly," *Thorax*, vol. 48, no. 12, pp. 1257–1260, 1993.

[15] M. Pai, "Spectrum of latent tuberculosis existing tests cannot resolve the underlying phenotypes," *Nature Reviews Microbiology*, vol. 8, article 242, 2010.

[16] P. A. Lobue and K. G. Castro, "Is it time to replace the tuberculin skin test with a blood test?" *The Journal of The American Medical Association*, vol. 308, pp. 241–242, 2012.

[17] R. S. Garfein, R. Lozada, L. Liu et al., "High prevalence of latent tuberculosis infection among injection drug users in Tijuana, Mexico," *International Journal of Tuberculosis and Lung Disease*, vol. 13, no. 5, pp. 626–632, 2009.

[18] R. S. Garfein, "Serial testing using QuantiFERON TB Gold In-tube assay in a high-risk population," in *Proceedings of the 3rd Annual Symposium on Interferon Gamma Release Assays*, Waikoloa, Hawaii, USA, 2012.

[19] S. A. Strathdee, R. Lozada, R. A. Pollini et al., "Individual, social, and environmental influences associated with HIV infection among injection drug users in Tijuana, Mexico," *Journal of Acquired Immune Deficiency Syndromes*, vol. 47, no. 3, pp. 369–376, 2008.

[20] D. D. Heckathorn, "Respondent-driven sampling II: deriving valid population estimates from chain-referral samples of hidden populations," *Social Problems*, vol. 49, no. 1, pp. 11–34, 2002.

[21] M. J. Salganik, "Respondent-driven sampling in the real world," *Epidemiology*, vol. 23, pp. 148–150, 2012.

[22] M. Pai, R. Joshi, S. Dogra et al., "Serial testing of health care workers for tuberculosis using interferon-γ assay," *American Journal of Respiratory and Critical Care Medicine*, vol. 174, no. 3, pp. 349–355, 2006.

[23] L. A. McNutt, C. Wu, X. Xue, and J. P. Hafner, "Estimating the relative risk in cohort studies and clinical trials of common outcomes," *American Journal of Epidemiology*, vol. 157, no. 10, pp. 940–943, 2003.

[24] D. Spiegelman and E. Hertzmark, "Easy SAS calculations for risk or prevalence ratios and differences," *American Journal of Epidemiology*, vol. 162, no. 3, pp. 199–200, 2005.

[25] D. Abramovitz, E. M. Volz, S. A. Strathdee, T. L. Patterson, A. Vera, and S. D. W. Frost, "Using respondent-driven sampling in a hidden population at risk of HIV infection: who do HIV-positive recruiters recruit?" *Sexually Transmitted Diseases*, vol. 36, no. 12, pp. 750–756, 2009.

[26] S. Greenland, "Modeling and variable selection in epidemiologic analysis," *American Journal of Public Health*, vol. 79, no. 3, pp. 340–349, 1989.

[27] D. G. Kleinbaum, L. L. Kupper, K. E. Muller, and N. Azhar, *Applied Regression Analysis and Other Multivariable Methods*, Duxbury Press, North Scituate, Mass, USA, 4th edition, 2008.

[28] L. Arcavi and N. L. Benowitz, "Cigarette smoking and infection," *Archives of Internal Medicine*, vol. 164, no. 20, pp. 2206–2216, 2004.

[29] J. Garmendia, P. Morey, and B. J. Antonio, "Impact of cigarette smoke exposure on host-bacterial pathogen interactions," *European Respiratory Journal*, vol. 39, no. 2, pp. 467–477, 2012.

[30] R. H. Anderson, F. S. Sy, S. Thompson, and C. Addy, "Cigarette smoking and tuberculin skin test conversion among incarcerated adults," *American Journal of Preventive Medicine*, vol. 13, no. 3, pp. 175–181, 1997.

[31] R. N. van Zyl-Smit, A. Zwerling, K. Dheda, and M. Pai, "Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review," *PLoS ONE*, vol. 4, no. 12, Article ID e8517, 2009.

[32] A. Zwerling, S. van den Hof, J. Scholten, F. Cobelens, D. Menzies, and M. Pai, "Interferon-gamma release assays for tuberculosis screening of healthcare workers: a systematic review," *Thorax*, vol. 67, pp. 62–70, 2012.

[33] S. Den Boon, S. Verver, B. J. Marais et al., "Association between passive smoking and infection with Mycobacterium tuberculosis in children," *Pediatrics*, vol. 119, no. 4, pp. 734–739, 2007.

[34] S. Basu, D. Stuckler, A. Bitton, and S. A. Glantz, "Projected effects of tobacco smoking on worldwide tuberculosis control: mathematical modelling analysis," *British Medical Journal*, vol. 343, article d5506, 2011.

Hindawi Publishing Corporation
Pulmonary Medicine
Volume 2012, Article ID 469128, 6 pages
doi:10.1155/2012/469128

Research Article

Mannose-Binding Lectin Promoter Polymorphisms and Gene Variants in Pulmonary Tuberculosis Patients from Cantabria (Northern Spain)

J.-Gonzalo Ocejo-Vinyals,[1] Lucía Lavín-Alconero,[1] Pablo Sánchez-Velasco,[1] M.-Ángeles Guerrero-Alonso,[1] Fernando Ausín,[1] M.-Carmen Fariñas,[2] and Francisco Leyva-Cobián[1]

[1] *Servicio de Inmunología, Hospital Universitario Marqués de Valdecilla, Avenida de Valdecilla s/n, 39008 Santander, Spain*
[2] *Unidad de Enfermedades Infecciosas, Departamento de Medicina Interna, Hospital Universitario Marqués de Valdecilla, Universidad de Cantabria, 39011 Santander, Spain*

Correspondence should be addressed to J.-Gonzalo Ocejo-Vinyals, jgocejo@humv.es

Received 25 September 2012; Revised 16 November 2012; Accepted 18 November 2012

Academic Editor: José R. Lapa e Silva

Mannose-binding lectin is a central molecule of the innate immune system. Mannose-binding lectin 2 promoter polymorphisms and structural variants have been associated with susceptibility to tuberculosis. However, contradictory results among different populations have been reported, resulting in no convincing evidence of association between mannose-binding lectin 2 and susceptibility to tuberculosis. For this reason, we conducted a study in a well genetically conserved Spanish population in order to shed light on this controversial association. We analysed the six promoter and structural mannose-binding lectin 2 gene variants in 107 patients with pulmonary tuberculosis and 441 healthy controls. Only D variant and HYPD haplotype were significantly more frequents in controls which would indicate that this allele could confer protection against pulmonary tuberculosis, but this difference disappeared after statistical correction. Neither the rest of alleles nor the haplotypes were significantly associated with the disease. These results would indicate that mannose-binding lectin promoter polymorphisms and gene variants would not be associated with an increased risk to pulmonary tuberculosis. Despite the slight trend of the D allele and HYPD haplotype in conferring protection against pulmonary tuberculosis, susceptibility to this disease would probably be due to other genetic factors, at least in our population.

1. Introduction

Tuberculosis (TB) is one of the world's most important infectious causes of death worldwide. More than 90 million TB patients were reported to WHO between 1980 and 2005, most of them in Asia and sub-Saharan Africa [1]. Spain is one of the European countries with the highest rates of incidence and prevalence of TB [2].

Approximately 90%–95% of individuals infected with *Mycobacterium tuberculosis* (MTB) are able to mount an immune response that halts the progression from latent TB infection to active TB disease. This is one of the main reasons that would indicate the need to identify and treat all those with risk factors for TB disease [3, 4].

Susceptibility to TB seems to be multifactorial, and the development of active disease would probably be the result of a complex interaction between the host and pathogen influenced by environmental and genetic factors. Numerous host genes are likely to be involved in this process [5–7].

Mannose-binding lectin (MBL) is an acute phase protein primarily produced by the liver. One of its main roles is to activate the complement system suggesting that it is one of the most important constituents of the innate immune system [8–12]. The gene encoding MBL has been associated with susceptibility to TB and other infectious diseases [8, 13]. The first mutation in *MBL2*, the gene encoding MBL was found in 1991 [14]. Three structural mutations, affecting

codons 52, 54, and 57, in the first exon of the *MBL2* gene (MBL1 is a pseudogene) have been found, and the corresponding alleles were designated D, B, and C, respectively (A is the wild-type allele for all three positions). Moreover, three polymorphisms have also been identified in the *MBL2* promoter and 5′-untranslated regions: H/L at position −550, X/Y at position −221, and P/Q at position +4 [15].

The effect of the three structural mutations in the *MBL2* first exon involves the impairment of MBL multimerization. This caused decreasing ligand binding and, consequently, a lack of complement activation [16]. In general, all these genetic variants result in a phenotype of low serum MBL levels, which influences the susceptibility to TB and the course of different diseases [13, 17, 18].

To date, controversial results have been reported regarding the relationship between structural genetic variants or polymorphisms of the *MBL2* gene and an increased risk of TB in different populations [19–30]. Several studies have found a significant association between the frequency of structural alleles or promoter polymorphisms and serum MBL levels with susceptibility to TB [20, 23, 24, 26–30] while others did not find any significant association [19, 21, 25]. Recently a meta-analysis of 17 human trials considering the effect of *MBL2* genotype and/or MBL levels and TB infection did not found significant association between *MBL2* genotype and pulmonary TB infection [22]. The majority of studies analysed did not report neither the *MBL2* haplotype nor the promoter polymorphisms. The aim of our study was to analyse if gene variants, promoter polymorphisms, haplotypes, or diplotypes could contribute to increase the risk of active pulmonary TB (PTB) in a human immunodeficiency virus negative genetically homogeneous population (Cantabria, Northern Spain), containing newly diagnosed patients with active disease.

2. Material and Methods

2.1. Study Population. To investigate the possible association between *MBL2* polymorphisms and PTB infection in our population, we recruited a total of 107 active PTB patients and 441 randomly selected healthy blood donors from Cantabria (northern Spain). All of them were HIV negative.

The study was conducted at a 1,200-bed community and teaching hospital. Both, blood donors (mean age, 48 years; range, 18–65 years; male/female ratio, 1.3) and PTB patients (mean age, 56 years; range, 23–76 years; male/female ratio, 1.5) were of Caucasian background. The PTB patients group was selected from patients admitted to the Infectious Unit and the Department of Respiratory Medicine (Hospital Universitario Marqués de Valdecilla) from 2008 to 2011 and who fulfilled clinical, radiological, and bacteriological criteria of active PTB according the standards for the diagnosis and classification of TB developed by the American Thoracic Society and the Centers for Disease Control and Prevention (http://www.cdc.gov/mmwr/). Diagnosis of PTB was made clinically and by X-rays and confirmed by bacteriological (microscopy and culture) procedures. We excluded patients with extrapulmonary TB due to dissemination and subsequent involvement of single or multiple nonpulmonary sites. In the same way, we excluded patients with autoimmune or neoplastic diseases, chronic renal failure, transplant individuals, and patients suffering from alcoholism or drug abuse. Controls had neither previous history of TB nor contact with infected patients. Furthermore, we ruled out the presence of active or latent TB in the control group by performing an interferon-gamma release assay (Quantiferon TB Gold, Cellestis Ltd., Carnegie, Victoria, Australia). All the procedures used in the study conformed to the principles outlined in the Declaration of Helsinki. Informed consent was obtained and data anonymously recorded. The study protocol was accepted and approved by the Research Ethics Board of the Hospital.

2.2. DNA Extraction and Amplification of Genomic DNA for MBL2 Genotyping. Blood was collected in EDTA-stabilized tubes in compliance with approved protocols from our institution. Genomic DNA from patients and controls was extracted from peripheral blood by using the Maxwell 16 Genomic DNA Purification system. For *MBL2* gene amplification and genotyping, we used the INNO-LiPA *MBL2* (Innogenetics Diagnóstica Iberia S.L.U, Barcelona, Spain), following the manufacturer's instructions. The INNO-LIPA *MBL2* is a line probe assay, designed for genotyping the 6 variations in the human *MBL2* gene (−550G > C, −221G > C, +4C > T, R52C, G54D, and G57E) which leads to analyse the seven common haplotypes and the 28 possible resulting diplotypes.

3. Statistical Analysis

Frequencies of alleles and diplotypes of patients and healthy controls were estimated by direct counting. Alleles and genotypic (dyplotypes) frequencies were compared by χ^2 test or the Fisher's exact test when necessary. P values with Yates correction and odds ratio (OR) with 95% confidence intervals (CI) were calculated using SPSS version 12.0 (SPSS Inc, Chicago, IL, USA). $P < 0.05$ was considered statistically significant. Hardy-Weinberg equilibrium (HWE) was tested in patients and controls for all the analysed parameters. Bonferroni correction for multiple comparisons was applied in order to avoid false positive results.

For haplotype analysis, frequencies and linkage disequilibrium were calculated trough the expectation maximization algorithm using the SNPStats web-based tool (http://bioinfo.iconcologia.net/SNPstats/). To determine the linkage disequilibrium between pairs of alleles, we calculated the D′ statistic. Comparisons between patients and controls were performed by χ^2 test or the Fisher's exact test considering each haplotype like an allele.

Statistical power was calculated by using the PS power and sample size calculation software version 3.0, (http://biostat.mc.vanderbilt.edu/PowerSampleSize).

4. Results and Discussion

4.1. MBL2 Structural Variants, Promoter Polymorphisms, and Genotypes. Frequencies of *MBL2* alleles are shown in Table 1. All the structural genetic variants and the promoter

Table 1: Allelic frequencies of *MBL2* structural variants and promoter polymorphisms in healthy controls and patients with pulmonary tuberculosis.

	Structural variants				Promoter polymorphisms					
	A	B	C	D	L	H	Y	X	P	Q
Controls										
($n = 441$)	0.782	0.143	0.008	**0.067**	0.655	0.345	0.760	0.240	0.815	0.195
PTB Patients										
($n = 107$)	0.836	0.131	0.009	**0.023**	0.678	0.322	0.776	0.224	0.757	0.243
P value[a,b]				**0.014**						
OR				**0.33**						
(95% CI)				**(0.13–0.84)**						

[a]Only D variant showed significant differences between the two groups. The rest of structural and promoter alleles did not show significant differences.
[b]The study had 74% power for detecting an odds ratio (OR) ≥ 2.

Table 2: Frequency of *MBL2* haplotypes in controls and PTB patients from Cantabria compared with other previously reported populations.

Haplotype	Population										
	Controls ($n = 441$)	PTB ($n = 107$)	CAN ($n = 344$)	ESK ($n = 72$)	DAN ($n = 250$)	JAP ($n = 218$)	KEN ($n = 61$)	MOZ ($n = 154$)	CHIR ($n = 43$)	MAP ($n = 25$)	WAR ($n = 190$)
HYPA	0.28	0.30	0.24	0.81	0.31	0.44	0.08	0.06	0.54	0.38	0.75
LYQA	0.23	0.23	0.22	0	0.19	0.16	0.25	0.27	0.01	0	0.01
LYPA	0.07	0.08	0.08	0.04	0.04	0.07	0.13	0.30	0.02	0.08	0.23
LXPA	0.21	0.23	0.19	0.03	0.26	0.11	0.24	0.13	0.01	0.04	0.01
LYPB	0.14	0.13	0.17	0.12	0.11	0.22	0.02	0	0.42	0.46	0
LYQC	0.01	0.01	0.03	0	0.03	0	0.24	0.24	0	0.04	0
HYPD	0.07	**0.02**[a]	0.07	0	0.06	0	0.04	0	0	0	0.003
H[b]	0.81	0.82	0.82	0.33	0.79	0.72	0.81	0.76	0.54	0.66	0.39

Controls, healthy population from Cantabria, PTB, patients with pulmonary tuberculosis from Cantabria, CAN, and Gran Canaria p; ESK, Eskimo; DAN, Danish; JAP, Japanese; KEN, Kenya; MOZ, Mozambique; CHIR, Chiriguano (South America); MAP, Mapuche (South America); WAR, Warlpiri (Australia) populations.
[a]*P* value 0.014 (OR 0.33 and 95% CI 0.13–0.84): frequency of HYPD haplotype in PTB patients versus control subjects from Cantabria.
[b]Average heterozygosity of the seven alleles when they were considered together.

polymorphisms were within the range of HWE. The average heterozygosity in the control group was 0.88 when the seven alleles of the *MBL2* gene were analysed, being this frequency higher than others previously reported in different populations [30].

There was no significant difference in the frequencies of the different promoter polymorphisms. Regarding the structural variants, only D allele was significantly more frequent in controls ($P = 0.014$, OR 0.33, and CI 95% 0.13–0.84), but this significance disappeared after Bonferroni correction.

Table 2 shows the frequency of the *MBL2* haplotypes in controls and PTB patients from Cantabria compared with other previously reported populations. As with D structural variant, HYPD showed the same significant difference between PTB patients and controls which disappeared after Bonferroni correction. All the promoter polymorphisms and structural variants conforming the different haplotypes were in linkage disequilibrium ($P < 2e - 16$).

4.2. MBL2 Complete Genotypes (Diplotypes) in PTB Patients and Healthy Controls. The frequencies of all the possible combinations of the seven haplotypes that appeared in both groups are shown in Table 3. No significant differences were found in any of the complete diplotypes between PTB patients and healthy controls.

All seven haplotypes were present in both groups following the same order of frequency in them, giving rise to 22 different *MBL2* diplotypes in our subjects. Eighteen of these diplotypes were present in both groups, 5 in either the PTB patients or the control group, and six diplotypes were not observed in any of the groups.

HYPA was the most frequent haplotype, followed by LYQA, LXPA, LYPB, LYPA, HYPD, and LYQC, respectively. When we regrouped the 22 observed diplotypes in all the possible genotypic combinations (structural-structural, structural-polymorphism, and polymorphism-polymorphism), we did not find any significant difference (data not shown).

5. Discussion

Innate immunity is one of the most important barriers against invading pathogens. The complement system gets activated when these microorganisms are detected, resulting in biochemical pathways that lead to the destruction of the infectious agent. One of these pathways that make up

Table 3: Frequencies of complete diplotypes in Spanish PTB patients and controls.

MBL2 diplotypes*	PTB patients *n* (%)	Controls *n* (%)	*P* value	OR	(95% CI)
LYQA/HYPA	17 (15.9)	54 (12.24)	0.40	1.35	(0.75–2.45)
LYQA/LYPB	10 (9.3)	29 (6.58)	0.43	1.46	(0.69–3.11)
LYQA/LYPA	6 (5.6)	19 (4.31)	0.60	1.32	(0.51–3.39)
LYQA/LYQA	3 (2.8)	19 (4.31)	0.59	0.64	(0.19–2.20)
LYQA/HYPD	1 (0.9)	10 (2.27)	0.70	0.41	(0.05–3.21)
LYQA/LYQC	0 (0)	2 (0.45)	1.0	NA	NA
LYQC/HYPD	0 (0)	1 (0.23)	1.0	NA	NA
LXPA/HYPA	15 (14.0)	45 (10.20)	0.34	1.43	(0.77–2.69)
LXPA/LYQA	10 (9.3)	53 (12.02)	0.54	0.75	(0.37–1.54)
LXPA/LXPA	7 (6.5)	14 (3.17)	0.15	2.14	(0.84–5.43)
LXPA/LYPB	4 (3.7)	31 (7.03)	0.30	0.51	(0.18–1.49)
LXPA/HYPD	3 (2.8)	13 (2.95)	1.0	0.95	(0.27–3.39)
LXPA/LYPA	1 (0.9)	10 (2.27)	0.70	0.41	(0.05–3.21)
LXPA/LYQC	1 (0.9)	2 (0.45)	0.48	2.07	(0.19–23.05)
HYPA/HYPA	10 (9.3)	38 (8.62)	0.96	1.09	(0.53–2.27)
LYPB/HYPA	8 (7.5)	38 (8.62)	0.88	0.86	(0.39–1.89)
LYPB/LYPB	1 (0.9)	7 (1.59)	1.0	0.58	(0.07–4.81)
LYPB/LYQC	1 (0.9)	1 (0.23)	0.35	4.15	(0.26–66.90)
LYPB/HYPD	0 (0)	9 (2.04)	0.22	NA	NA
LYPA/HYPA	4 (3.7)	18 (4.08)	1.0	0.91	(1.30–2.75)
LYPA/LYPB	3 (2.8)	4 (0.91)	0.14	3.15	(0.69–14.30)
LYPA/LYPA	1 (0.9)	2 (0.45)	0.48	2.07	(0.19–23.05)
LYPA/LYQC	0 (0)	1 (0.23)	1.0	NA	NA
LYPA/HYPD	1 (0.9)	2 (0.45)	0.48	2.07	(0.19–23.05)
HYPD/HYPA	0 (0)	14 (3.17)	0.08	NA	NA
HYPD/HYPD	0 (0)	5 (1.13)	0.59	NA	NA

*Frequencies of the rest of combined diplotypes (LYQC/HYPA, LYQC/LYQC) were 0 in both groups.

the complement system is the lectin pathway in which MBL plays the main role. MBL protein is therefore important, especially in first-line defense against invading pathogens. It has been reported that low levels of circulating MBL may predispose against infectious diseases [8, 31, 32].

Structural variants are found at the coding regions of the *MBL2* gene that lead to low or near absent serum MBL levels in heterozygosis and homozygosis, respectively. Low-serum levels of MBL are associated with defects in opsonization, resulting in recurrent infections mainly during infancy [31]. Due to a strong linkage disequilibrium between the polymorphisms present in the promotor and the structural variants in exon 1 of the human *MBL2* gene, only seven common haplotypes have been described (HYPA, LYPA, LXPA, LYQA, HYPD, LYPB, and LYQC) which give rise to 28 possible haplotype combinations. The frequencies of the seven haplotypes vary considerably between populations [17, 18]. Among haplotypes carrying the wild-type A allele, HYPA results in the production of higher amounts of MBL, whereas LXPA is associated with lower serum MBL levels.

Several groups have studied *MBL2* genetic variants and PTB, suggesting a partial protective effect of heterozygosity for *MBL2* variant alleles against PTB [33–35], whereas others have pointed toward a susceptibility to PTB for homozygous carriers of *MBL2* variant alleles [36].

Previous studies have found controversial results, at least at a genetic level. Some authors have reported a lower frequency of allele B in Afro-Americans, but not in Caucasian or in the so-called "Hispanic" TB patients [28]. However, other reports have not found any significant differences in the frequency of structural *MBL2* alleles between PTB Caucasian patients and control subjects. Nevertheless, when they included the promoter polymorphisms according to high serum MBL levels (*YA/YA*, *YA/XA*, *XA/XA*, and *YA/O*), low MBL levels (*XA/O*), and deficient MBL individuals (*O/O*),a significant difference in diplotype frequency was revealed [25, 27, 29]. Finally, another study has found a significantly increased frequency of *O/O* diplotype of structural polymorphism and of *Y/Y* diplotype of promoter polymorphisms in HIV-TB+ patients compared with controls [24].

In the present study, we have investigated whether structural variations or promoter polymorphisms in the *MBL2* gene considering them individually of regrouped might be associated with PTB in Northern Spain. Our results after statistical correction show that there is no differences neither in the frequencies of polymorphisms in the promoter and 5′-untranslated region nor in the structural variants of the exon 1 of the *MBL2* gene between PTB patients and control subjects when we considered them individually.

Lack of concordance of our results and those from other studies, specifically another Spanish report that studied *MBL2* gene variants in the population from Canary Islands, [27] could be explained, at least, by the genetic characteristics of both pathogens and hosts. There are, consequently, two possibilities to understand these differences among insular and peninsular Spaniards.

First of all, it should be considered the different ethnic background of geographically apart populations [37–40]. Genetic studies have demonstrated that an aboriginal African background still persists in inhabitants from Canary Islands. Estimates of genetic contribution to the Canary Islanders from their putative parental populations based on mtDNA and other genetic markers are 43% Berbers, 35% Peninsular Spaniards, and 21% Guineans (being the Spanish nuclear contribution due to males and practically all the Berber and Guinean due to females). On the other hand, Cantabrians, at the North of the Iberian Peninsula, appear as a semi-isolated result of an ancient indigenous substrate more or less mixed with more recent immigrants. This population seems to be a genetically well-differentiated community, as deduced from uniparental and autosomal markers, perhaps to a higher level than their neighbours, the Basques, the most reputed European isolate on linguistic grounds [41–43].

Secondly, another explanation could be due to the genetic background within *M. tuberculosis* because variability among different strains of *M. tuberculosis* in their surface, oligosaccharides, might have led to differences in the MBL levels associated with resistance or susceptibility against PTB [27, 44, 45]. Consequently, when geographic variation in

pathogen polymorphism is superimposed on host genetic heterogeneity, considerable variation may occur in detectable allelic association [5]. These factors could explain our findings in the analysed Northern Spanish population.

6. Conclusion

The results obtained in our study show a significant higher prevalence of *MBL2* D allele and HYPD haplotype in controls than in PTB patients (6.7% versus 2.3%, $P = 0.014$, OR 0.33, and CI 95% 0.13–0.84).

Although after statistical correction, the significance disappeared; this trend of the P values to significance could indicate a role of D allele and HYPD haplotype in conferring protection against PTB. For this reason, we cannot argue that *MBL2* D allele or HYPD haplotype would act as a factor of resistance to PTB, and susceptibility to this disease would probably be determined by other environmental and genetic factors, at least in our population [20, 46–50].

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

This work was partly supported by Grants PI05-0503 and G03-104 to F. Leyva-Cobián. and Grant PI04-1086 to M.-Carmen Fariñas. from the Fondo de Investigaciones Sanitarias (Ministry of Health, Spain). All authors read and approved the final version of the paper for publication.

References

[1] World Health Organization, "Global tuberculosis control: surveillance, planning, financing," WHO Report 2007, World Health Organization, Geneva, Switzerland, (WHO/HTM/TB/2007. 376).

[2] M. Díez, C. Huerta, T. Moreno et al., "Tuberculosis in Spain: epidemiological pattern and clinical practice," *International Journal of Tuberculosis and Lung Disease*, vol. 6, no. 4, pp. 295–300, 2002.

[3] M. Pai, S. Kalantri, and K. Dheda, "New tools and emerging technologies for the diagnosis of tuberculosis: part I. Latent tuberculosis," *Expert Review of Molecular Diagnostics*, vol. 6, no. 3, pp. 413–422, 2006.

[4] M. Pai, S. Kalantri, and K. Dheda, "New tools and emerging technologies for the diagnosis of tuberculosis: part II. Active tuberculosis and drug resistance," *Expert Review of Molecular Diagnostics*, vol. 6, no. 3, pp. 423–432, 2006.

[5] P. Selvaraj, "Host genetics and tuberculosis susceptibility," *Current Science*, vol. 86, no. 1, pp. 115–121, 2004.

[6] E. Schurr, "Is susceptibility to tuberculosis acquired or inherited?" *Journal of Internal Medicine*, vol. 261, no. 2, pp. 106–111, 2007.

[7] R. Bellamy, "Genome-wide approaches to identifying genetic factors in host susceptibility to tuberculosis," *Microbes and Infection*, vol. 8, no. 4, pp. 1119–1123, 2006.

[8] D. L. Worthley, P. G. Bardy, and C. G. Mullighan, "Mannose-binding lectin: biology and clinical implications," *Internal Medicine Journal*, vol. 35, no. 9, pp. 548–555, 2005.

[9] W. I. Weis, K. Drickamer, and W. A. Hendrickson, "Structure of a C-type mannose-binding protein complexed with an oligosaccharide," *Nature*, vol. 360, no. 6400, pp. 127–134, 1992.

[10] I. P. Fraser, H. Koziel, and R. A. B. Ezekowitz, "The serum mannose-binding protein and the macrophage mannose receptor are pattern recognition molecules that link innate and adaptive immunity," *Seminars in Immunology*, vol. 10, no. 5, pp. 363–372, 1998.

[11] S. Thiel, T. Vorup-Jensen, C. M. Stover et al., "A second serine protease associated with mannan-binding lectin that activates complement," *Nature*, vol. 386, no. 6624, pp. 506–510, 1997.

[12] O. Neth, D. L. Jack, A. W. Dodds, H. Holzel, N. J. Klein, and M. W. Turner, "Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition," *Infection and Immunity*, vol. 68, no. 2, pp. 688–693, 2000.

[13] D. P. Eisen, "Mannose-binding lectin deficiency and respiratory tract infection," *Journal of Innate Immunity*, vol. 2, no. 2, pp. 114–122, 2010.

[14] M. Sumiya, M. Super, P. Tabona et al., "Molecular basis of opsonic defect in immunodeficient children," *The Lancet*, vol. 337, no. 8757, pp. 1569–1570, 1991.

[15] H. O. Madsen, P. Garred, S. Thiel et al., "Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein," *Journal of Immunology*, vol. 155, no. 6, pp. 3013–3020, 1995.

[16] F. Larsen, H. O. Madsen, R. B. Sim, C. Koch, and P. Garred, "Disease-associated mutations in human mannose-binding lectin compromise oligomerization and activity of the final protein," *The Journal of Biological Chemistry*, vol. 279, no. 20, pp. 21302–21311, 2004.

[17] P. Garred, F. Larsen, J. Seyfarth, R. Fujita, and H. O. Madsen, "Mannose-binding lectin and its genetic variants," *Genes and Immunity*, vol. 7, no. 2, pp. 85–94, 2006.

[18] J. L. Casanova and L. Abel, "Human mannose-binding lectin in immunity: friend, foe, or both?" *Journal of Experimental Medicine*, vol. 199, no. 10, pp. 1295–1299, 2004.

[19] N. Singla, D. Gupta, A. Joshi, N. Batra, J. Singh, and N. Birbian, "Association of mannose-binding lectin gene polymorphism with tuberculosis susceptibility and sputum conversion time," *International Journal of Immunogenetics*, vol. 39, no. 1, pp. 10–14, 2012.

[20] Z. B. Liu, R. J. Zheng, H. P. Xiao et al., "The correlation between polymorphisms of genes with susceptibility to tuberculosis and the clinical characteristics of tuberculosis in 459 Han patients," *Zhonghua Jie He He Hu Xi Za Zhi*, vol. 34, no. 12, pp. 923–928, 2011.

[21] H. A. Solğun, D. Taştemir, N. Aksaray, I. Inan, and O. Demirhan, "Polymorphisms in NRAMP1 and MBL2 genes and their relations with tuberculosis in Turkish children," *Tuberkuloz ve Toraks*, vol. 59, no. 1, pp. 48–53, 2011.

[22] J. T. Denholm, E. S. McBryde, and D. P. Eisen, "Mannose-binding lectin and susceptibility to tuberculosis: a meta-analysis," *Clinical and Experimental Immunology*, vol. 162, no. 1, pp. 84–90, 2010.

[23] O. P. Dossou-Yovo, C. Lapoumeroulie, M. Hauchecorne et al., "Variants of the mannose-binding lectin gene in the benin population: heterozygosity for the p.G57E allele may confer a selective advantage," *Human Biology*, vol. 81, no. 5-6, pp. 899–909, 2009.

[24] K. Alagarasu, P. Selvaraj, S. Swaminathan, S. Raghavan, G. Narendran, and P. R. Narayanan, "Mannose binding lectin gene variants and susceptibility to tuberculosis in HIV-1 infected patients of South India," *Tuberculosis*, vol. 87, no. 6, pp. 535–543, 2007.

[25] W. Liu, F. Zhang, Z. T. Xin et al., "Sequence variations in the MBL gene and their relationship to pulmonary tuberculosis in the Chinese Han population," *International Journal of Tuberculosis and Lung Disease*, vol. 10, no. 10, pp. 1098–1103, 2006.

[26] P. Selvaraj, M. S. Jawahar, D. N. Rajeswari, K. Alagarasu, M. Vidyarani, and P. R. Narayanan, "Role of mannose binding lectin gene variants on its protein levels and macrophage phagocytosis with live *Mycobacterium tuberculosis* in pulmonary tuberculosis," *FEMS Immunology and Medical Microbiology*, vol. 46, no. 3, pp. 433–437, 2006.

[27] M. I. García-Laorden, M. J. Pena, J. A. Caminero et al., "Influence of mannose-binding lectin on HIV infection and tuberculosis in a Western-European population," *Molecular Immunology*, vol. 43, no. 14, pp. 2143–2150, 2006.

[28] H. M. El Sahly, R. A. Reich, S. J. Dou, J. M. Musser, and E. A. Graviss, "The effect of mannose binding lectin gene polymorphisms on susceptibility to tuberculosis in different ethnic groups," *Scandinavian Journal of Infectious Diseases*, vol. 36, no. 2, pp. 106–108, 2004.

[29] C. Søborg, H. O. Madsen, A. B. Andersen, T. Lillebaek, A. Kok-Jensen, and P. Garred, "Mannose-binding lectin polymorphisms in clinical tuberculosis," *Journal of Infectious Diseases*, vol. 188, no. 5, pp. 777–782, 2003.

[30] M. I. García-Laorden, A. Manzanedo, A. Figuerola, F. Sánchez-García, and C. Rodríguez-Gallego, "Mannose-binding lectin polymorphisms in a Canary Islands (Spain) population," *Genes and Immunity*, vol. 2, no. 5, pp. 292–294, 2001.

[31] F. Özbaş-Gerçeker, I. Tezcan, A. I. Berkel et al., "The effect of mannose-binding protein gene polymorphisms in recurrent respiratory system infections in children and lung tuberculosis," *Turkish Journal of Pediatrics*, vol. 45, no. 2, pp. 95–98, 2003.

[32] L. E. Mombo, C. Y. Lu, S. Ossari et al., "Mannose-binding lectin alleles in sub-Saharan Africans and relation with susceptibility to infections," *Genes and Immunity*, vol. 4, no. 5, pp. 362–367, 2003.

[33] A. V. S. Hill, "The immunogenetics of human infectious diseases," *Annual Review of Immunology*, vol. 16, pp. 593–617, 1998.

[34] P. Garred, C. Richter, A. B. Andersen et al., "Mannan-binding lectin in the sub-saharan HIV and tuberculosis epidemics," *Scandinavian Journal of Immunology*, vol. 46, no. 2, pp. 204–208, 1997.

[35] E. G. Hoal-van Helden, J. Epstein, T. C. Victor et al., "Mannose-binding protein B allele confers protection against tuberculous meningitis," *Pediatric Research*, vol. 45, no. 4 I, pp. 459–464, 1999.

[36] P. Selvaraj, P. R. Narayanan, and A. M. Reetha, "Association of functional mutant homozygotes of the mannose binding protein gene with susceptibility to pulmonary tuberculosis in India," *Tubercle and Lung Disease*, vol. 79, no. 4, pp. 221–227, 1999.

[37] J. C. Delgado, A. Baena, S. Thim, and A. E. Goldfeld, "Ethnic-specific genetic associations with pulmonary tuberculosis," *Journal of Infectious Diseases*, vol. 186, no. 10, pp. 1463–1468, 2002.

[38] C. Flores, N. Maca-Meyer, J. A. Pérez, A. M. González, J. M. Larruga, and V. M. Cabrera, "A predominant European ancestry of paternal lineages from Canary Islanders," *Annals of Human Genetics*, vol. 67, no. 2, pp. 138–152, 2003.

[39] F. Pinto, A. M. González, M. Hernández, J. M. Larruga, and V. M. Cabrera, "Genetic relationship between the Canary Islanders and their African and Spanish ancestors inferred from mitochondrial DNA sequences," *Annals of Human Genetics*, vol. 60, no. 4, pp. 321–330, 1996.

[40] F. M. Pinto, A. M. González, M. Hernández, J. M. Larruga, and V. M. Cabrera, "Sub-Saharan influence on the Canary Islands population deduced from G6PD gene sequence analysis," *Human Biology*, vol. 68, no. 4, pp. 517–522, 1996.

[41] E. Esteban, J. M. Dugoujon, E. Guitard et al., "Genetic diversity in Northern Spain (Basque Country and Cantabria): GM and KM variation related to demographic histories," *European Journal of Human Genetics*, vol. 6, no. 4, pp. 315–324, 1998.

[42] P. Sánchez-Velasco, J. Escribano de Diego, J. E. Paz-Miguel, G. Ocejo-Vinyals, and F. Leyva-Cobián, "HLA-DR, DQ nucleotide sequence polymorphisms in the Pasiegos (Pas valleys, Northern Spain) and comparison of the allelic and haplotypic frequencies with those of other European populations," *Tissue Antigens*, vol. 53, no. 1, pp. 65–73, 1999.

[43] P. Sánchez-Velasco, E. Gómez-Casado, J. Martínez-Laso et al., "HLA alleles in isolated populations from north Spain: origin of the basques and the ancient Iberians," *Tissue Antigens*, vol. 61, no. 5, pp. 384–392, 2003.

[44] M. Kato-Maeda, P. J. Bifani, B. N. Kreiswirth, and P. M. Small, "The nature and consequence of genetic variability within *Mycobacterium tuberculosis*," *Journal of Clinical Investigation*, vol. 107, no. 5, pp. 533–537, 2001.

[45] B. López, D. Aguilar, H. Orozco et al., "A marked difference in pathogenesis and immune response induced by different *Mycobacterium tuberculosis* genotypes," *Clinical and Experimental Immunology*, vol. 133, no. 1, pp. 30–37, 2003.

[46] N. Keicho, M. Hijikata, and S. Sakurada, "Human genetic susceptibility to tuberculosis," *Nihon Rinsho*, vol. 69, no. 8, pp. 1363–1367, 2011.

[47] R. J. Wilkinson, "Human genetic susceptibility to tuberculosis: time for a bottom-up approach?" *Journal of Infectious Diseases*, vol. 205, no. 4, pp. 525–527, 2012.

[48] J. L. Rowell, N. F. Dowling, W. Yu, A. Yesupriva, L. Zhang, and M. Gwinn, "Trends in population-based studies of human genetics in infectious diseases," *PloS ONE*, vol. 7, no. 2, Article ID e25431, 2012.

[49] T. Qidwai, F. Jamal, and M. Y. Khan, "DNA sequence variation and regulation of genes involved in pathogenesis of pulmonary tuberculosis," *Scandinavian Journal of Immunology*, vol. 75, no. 6, pp. 568–587, 2012.

[50] A. K. Azad, W. Sadee, and L. S. Schlesinger, "Innate immune gene polymorphisms in tuberculosis," *Infection and Immunity*, vol. 80, no. 10, pp. 3343–3359, 2012.

Hindawi Publishing Corporation
Pulmonary Medicine
Volume 2012, Article ID 745483, 10 pages
doi:10.1155/2012/745483

Review Article

Role of TNF-Alpha, IFN-Gamma, and IL-10 in the Development of Pulmonary Tuberculosis

Yone Vila Nova Cavalcanti,[1,2] Maria Carolina Accioly Brelaz,[2] Juliana Kelle de Andrade Lemoine Neves,[2] José Candido Ferraz,[3] and Valéria Rêgo Alves Pereira[2]

[1] *Departamento de Biologia, UFRPE, Dom Manoel de Medeiros, S/N, Dois Irmãos, 52171-900 Recife, PE, Brazil*
[2] *Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães (CPqAM/FIOCRUZ), Avenida Professor Moraes Rego S/N, Cidade Universitária, Campus da UFPE, 50670-420 Recife, PE, Brazil*
[3] *Núcleo de Educação Física e Ciências do Esporte, Centro Acadêmico de Vitória, Universidade Federal de Pernanbuco (UFPE), Vitória de Santo Antão, PE, Brazil*

Correspondence should be addressed to Valéria Rêgo Alves Pereira, valeriaph@gmail.com

Received 30 August 2012; Revised 31 October 2012; Accepted 5 November 2012

Academic Editor: José R. Lapa e Silva

Host immune response against *Mycobacterium tuberculosis* is mediated by cellular immunity, in which cytokines and Th1 cells play a critical role. In the process of control of the infection by mycobacteria, TNF-alpha seems to have a primordial function. This cytokine acts in synergy with IFN-gamma, stimulating the production of reactive nitrogen intermediates (RNIs), thus mediating the tuberculostatic function of macrophages, and also stimulating the migration of immune cells to the infection site, contributing to granuloma formation, which controls the disease progression. IFN-gamma is the main cytokine involved in the immune response against mycobacteria, and its major function is the activation of macrophages, allowing them to exert its microbicidal role functions. Different from TNF-alpha and IFN-gamma, IL-10 is considered primarily an inhibitory cytokine, important to an adequate balance between inflammatory and immunopathologic responses. The increase in IL-10 levels seems to support the survival of mycobacteria in the host. Although there is not yet conclusive studies concerning a clear dichotomy between Th1 and Th2 responses, involving protective immunity and susceptibility to the disease, respectively, we can suggest that the knowledge about this responses based on the prevailing cytokine profile can help to elucidate the immune response related to the protection against *M. tuberculosis*.

1. Introduction

The genus *Mycobacterium* displays more than 100 known species, with a broad geographic distribution, habitat diversity, and diverse relations with other organisms, including more than 20 species presenting different degrees of pathogenicity to humans [1]. *Mycobacterium tuberculosis* (*M. tuberculosis*) (MTB), an intracellular facultative bacillus, is the most frequent species isolated in human tuberculosis (TB) cases.

Pulmonary tuberculosis is a global public health problem, presenting high incidence in Brazil. It is still the world's leading cause of death from a single infectious agent. Most infections are asymptomatic and latent however around 5% to 10% of infected people progress to the disease development at each year, pulmonary tuberculosis being found in most cases. Each second a person is infected with *M. tuberculosis* in the world. From the lungs, the organism is efficiently transmissible through aerosol. It is estimated that, on average, a person with active TB can infect between 10 and 15 individuals per year [2]. The World Health Organization (WHO) works intensively to significantly reduce the TB cases and halve this disease death number until 2015 [3]; however, the number of multidrug-resistant TB cases is rising, and this increasingly compromises the disease control [4, 5].

Latent TB is defined as an infection with *M. tuberculosis* that remains within macrophages without replicate, but that retains the ability to exit latency and cause active disease when there is an interruption of the protective immune response. The reactivation of a latent infection requires the activation of the quiescent bacilli. Several factors can trigger the development of active disease from reactivation of latent infection, which usually involves the decline of the immune response. HIV infection is the most important risk factor for progression to active disease due to depletion of $CD4^+$ T cells [6]. Advanced age, malnutrition, and medical conditions that compromise the immune system are also risk factors for the reactivation [7, 8].

Tuberculosis progression is associated with the immune *status*. It is known that host protective immune response against this pathogen is mediated by cellular immunity, in which certain cytokines and Th1 cells have a critical role [9]. Understanding the mechanisms involved in this response, and in particular the function of the cytokine network involved in this disease, is of significant relevance to reach advances in the development of effective control and prevention [10].

2. Cytokines

Cytokines are molecules that mediate mainly the intercellular communication in the immune system, being produced by different cell types. Cytokines have pleiotropic and regulatory effects and participate in the host defense and in inflammatory and tissue reparation processes [11].

In tuberculosis, an effective and coordinated participation of different cytokines was already identified, such as interleukin-12 (IL-12), IL-23, IL-27, IL-18, IL-1, IL-7, and IL-15 [5]. An important aspect associated with the production of cytokines in MTB infections is the activation of macrophages in response to IFN-γ and TNF-α signaling [12]. Nonactivated macrophages are the usual habitat of MTB, which resists in the intracellular environment, blocking the phagosome fusion with the lysosome, thus avoiding its exposure to low pH and to reactive nitrogen intermediates (RNIs), important to its destruction [12]. IFN-γ activated macrophages transpose this blockage and form phagolysosomes expressing RNIs able to eliminate MTB in the infection sites [13]. The cord factor, the 19KDa lipoprotein, and other MTB components induce the production of IL-12 by macrophages, thereby mobilizing the Th1 cytokine pathway. Figure 1 shows the initial immune protection to *M. tuberculosis*.

Several cytokines, including interleukin IL-12, IL-17, and IL-23, contribute to the host response to mycobacteria, improving the development of Th1 cells [14]. Among Th1 cytokines, IFN-γ and TNF-α were identified as the most important agents of the antimycobacterial cytokine cascade. This is due to the formation as well as the maintenance of the granuloma, which is mediated by TNF-α acting synergistically with IFN-γ in the activation of macrophages to produce effector molecules [15].

Recently, a new population of cells was identified and named Th17. These cells produce IL-17, IL-21, and IL-22 as signature cytokines [16, 17]. The IL-17 receptor is expressed in different organs including the liver, lung, and spleen, and different cell types are able to respond to IL-17, such as dendritic cells, macrophages, lymphocytes, epithelial cells, and fibroblasts. The responses induced by the IL-17 gene include expression of proinflammatory genes, chemokines, IL-6, IL-8, and antimicrobial proteins. Recent data suggest a superior and more complex role for these cells and their cytokines in different intracellular infections, including bacteria, fungi, and viruses in different mucosal surfaces [18]. Therefore, the balance between protection and Th17 cell-mediated pathology is the key in the definition of consequences in mucosal infections [19].

Th17 cells also participate in the inflammatory response at an early mycobacterial infection; however, the production of IL-17 in the lungs is mainly immunosuppressive of IFN-γ. The protective potential role of Th17 cells during the early phase of infection with *M. tuberculosis* is unknown [20]. There are evidences on the role of IL-17 during mycobacterial infections. Pulmonary infection with BCG or *M. tuberculosis* stimulated the early secretion of IL-17 from the day 1 to 14 and sequentially the development of T cells secreting IFN-γ. Pulmonary infected IL-17 deficient mice with BCG showed a reduction in the delayed hypersensitivity responses, with a deficiency in granuloma formation in the lungs, suggesting that IL-17 is required for an efficient development of Th1 responses [20].

The secretion of IL-23 is essential for the secretion of IL-17, and people with deficiency in the IL-12Rβ1 gene have low capacity to produce IL-23, and they have a lower production of IFN-γ. IL-12 is a cytokine that reduces the expression of IL-17, and this appears to show a self-regulation on inflammation. The balance between the secretions of IL-23/IL-17 and IL-12/IFN-γ appears to be essential for the regulation of inflammation in response to *M. tuberculosis* and other mycobacteria [21].

Among the cytokines that are being studied in response to *M. tuberculosis*, we can also emphasize interleukin-1. Interleukin-1 is necessary for the control of infection with *M. tuberculosis*, but the role of its two ligands, IL-1α and IL-1β, and its regulation *in vivo* are poorly understood. An important feature of IL-1 is its control on transcription, arranging the levels of transcription and signal transduction, as evidenced by the variety of immunopathologies and autoinflammatory diseases that occur in the absence of regulation of IL-1 [22, 23]. Little is known about the expression and processing of IL-1 in the context of infection with *M. tuberculosis in vivo*. The populations of cells that produce IL-1 during infection have not yet been characterized [24].

Guler et al. [25] investigated the role of IL-1α and IL-1β during chronic infection with *M. tuberculosis* and spontaneous reactivation of it in mice. Blockade of IL-1α, but not IL-1β, resulted in increased susceptibility to chronic infection with *M. tuberculosis*. When they neutralized IL-1α or IL-1β alone, they did not observe an increase in the reactivation of latent tuberculosis. The generation of antibodies neutralizing IL-1α and IL-1β simultaneously did not influence weight gain during reactivation, and they observed a slight increase in the lung bacilli count when compared to the immunized

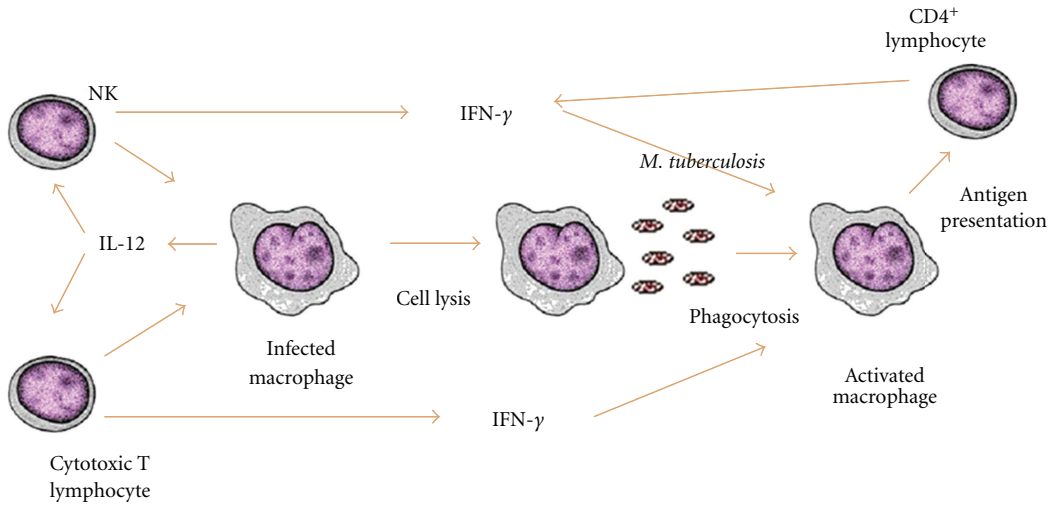


Figure 1: Initial protective response to *M. tuberculosis*-Th1 profile.

control group. Thus, their results suggested that IL-1α is the prime mediator of the IL-1RI-dependent and protective innate immune responses to *M. tuberculosis* in mice.

In a recent study the role of IL-1 in host resistance was demonstrated by inducing antibodies against this cytokine, which resulted in an increased mortality during chronic infection [25].

The granuloma is a typical structure of this disease, where we can find CD4 and CD8 T lymphocytes, B lymphocytes, macrophages, neutrophils, fibroblasts, and giant multinucleated cells. IFN-γ-producing CD4 T lymphocytes contribute to the generation of granulomas, besides being important costimulators to the adequate activation of CD8 T lymphocytes. The importance of CD4 T lymphocytes function is seen in patients with HIV, where the risk of TB increases with the decrease of the cells counting [26].

Many events mediated by cytokines are important to the establishment of immunity against MTB and the expression of host resistance [11].

Response against M. tuberculosis. $CD4^+$ T cells exert regulatory activity on macrophage function, as well as cytolytic $CD8^+$ lymphocytes. The effector function for the bacterial elimination is mediated by macrophages that are activated by cytokines derived from T lymphocytes, particularly IFN-γ and TNF-α.

3. Tumor Necrosis Factor (TNF-α)

The tumor necrosis factor (TNF, TNF-α) was originally characterized as a necrosis inductor in sarcomas *in vivo* [27]. TNF-α is a proinflammatory cytokine which exerts multiple biological effects. TNF-α expression is strictly controlled, since its superproduction can mediate damaging effects found in the septic shock such as arterial hypotension, disseminated vascular coagulation, and lethal hypoglycemia.

In the process of mycobacterial infection control, TNF-α seems to have a primordial role, acting upon a wide variety of cells. The main producing cells are activated macrophages, T lymphocytes, and dendritic cells [27–29]. This cytokine acts in synergy with IFN-γ, stimulating the production of reactive nitrogen intermediates (RNIs), thus mediating the tuberculostatic function of macrophages [30, 31]. TNF-α also stimulates the migration of immune cells to the infection site, contributing to the granuloma formation, capable of controlling the disease progression [32].

TNF-α blocking has dramatic effects on the progression of tuberculosis in experimental models. Neutralization of TNF-α in murine models results in tuberculosis aggravation or reactivation [32]. The excision of the TNF-α gene or its receptor results in deviant granulomas or fulminant acute tuberculosis [33, 34]. Studies have also revealed that TNF-α is expressed in MTB-infected tissues during the whole latent phase of infection [35], suggesting a contribution, with other cytokines like IFN-γ, in the control of the bacillus multiplication.

Increased levels of TNF-α are commonly detected in culture supernatants of peripheral blood mononucleated cells (PBMCs) from patients with pulmonary tuberculosis stimulated with mycobacterial antigens [10, 36, 37]. Moura [38] evaluating the immune response of patients prior to and after treatment noticed that patients with active pulmonary tuberculosis produced increased levels of TNF-α; however they did not observe significant difference in these cytokine levels after treatment, concluding that these results reinforce this cytokine's role at both the physiopathology and in the protective immunity of the disease.

A recent study investigated the role played by the nucleotide-binding oligomerization domain-containing protein 2 (NOD2) in the human alveolar macrophage innate responses and revealed that significant levels of IL-1β, IL-6, and TNF-α were produced after the recognition of the ligand with the muramyl dipeptide (MDP). Alveolar macrophage treatment with MDP has improved the control of intracellular growth of *M. tuberculosis*, activity associated with a significant production of TNF-α and IL-6 [39].

One of the most overwhelming lines of evidence of the protective effects of TNF-α is, perhaps, provided by the observation that patients with rheumatoid arthritis under treatment with TNF-α antagonists (monoclonal antibodies against TNF-α or TNF-α soluble receptors) have a significant increased risk of reactivating latent TB [40–42].

On the other hand, there is also evidence showing that TNF-α may be associated with immunopathological responses in tuberculosis, aforementioned also as the head mediator of the destruction of the pulmonary tissue [43]. Elevated levels of TNF-α are related to an excessive inflammation with necrosis and cachexy [44, 45].

Tumor necrosis factor (TNF-α) relative roles in MTb have been a subject of controversy. It was described that mycobacteria decreases the production of TNF in human PBMCs, skill which probably contributes to its ability to establish chronic infections [46]. Produced by macrophages, lymphocytes, neutrophils, and some endothelial cells, TNF-α coordinates the inflammatory response via induction of other cytokines (IL-1 and IL-6), and the recruitment of immune and inflammatory cells through the induction of chemokine and supraregulation of adhesion molecules. Experimental models have shown that TNF-α plays an important role not only in host response against *M. tuberculosis* but also in the immunopathology of tuberculosis [47].

TNF-α increases the capacity of macrophages to phagocytose and kill mycobacteria and stimulates apoptosis of macrophages, depriving bacilli of host cells and leading to death and presentation by dendritic cells of mycobacterial antigens [48]. *In vivo* TNF-α is required for the formation and maintenance of granulomas. Neutralization of TNF-α produced by mice chronically infected with *M. tuberculosis* specific monoclonal antibodies disrupts the integrity of granulomas, exacerbates infection, and increases mortality [49].

M. tuberculosis evolved and has developed mechanisms which interact and modulate the host immune response. Mycobacterium expresses surface antigens that can induce the production of IL-10 and IL-4, which typically have anti-inflammatory effects [50, 51]. The high expression of IL-4 has been implicated as a virulence factor, both for the anti-inflammatory ability and also for its apparent capacity to promote tissue damage in association with TNF-α [52]. These studies suggest that IL-4 (alone or jointly with TNF-α) may play a role in tissue destruction and/or cell death during infection by *M. tuberculosis*. TNF-α is one of the most powerful controlling factors for the recruitment of monocytes and is a potent inducer of cell death by apoptosis [53]. Necrosis, on the other hand, is associated with the lysis of the infected cell, the release of feasible *M. tuberculosis*, and damage to the surrounding tissues [54]. TNF-α is also a key cytokine involved in this event.

4. Interferon Gamma (IFN-γ)

Interferons (IFNs) are substances originally identified at cellular culture supernatants infected by virus and that appeared to interfere directly in the viral replication, hence its denomination [55]. Divided into two major types, type I IFNs are induced and act effectively in responses against viruses: IFN-α is secreted mainly by leucocytes, and IFN-β is produced by fibroblasts. Type II interferon, now referred to as IFN-γ, is synthesized mostly by T lymphocytes and NK cells after this cells activation with immune and inflammatory stimuli, rather than viral infection [56]. IFN-γ is the chief cytokine involved in the protective immune response against mycobacterial infection. It is produced primarily by CD4 and CD8 T lymphocytes and NK cells. It is also known that natural killer T cells (NKT) and $\gamma\delta$ T lymphocytes, cells with a narrow repertoire of antigen recognition, can also produce IFN-γ in response to mycobacterial stimulation, displaying protection against *M. tuberculosis* infection both *in vitro* and *in vivo* [12].

The main function of IFN-γ is macrophage activation, rendering them able to exert its microbicidal functions. It operates also enhancing the antigen presentation through the induction of the expression of molecules from the major histocompatibility complex (MHC) class I and II and promoting the differentiation of CD4 T lymphocytes to the Th1 subpopulation [57–59]. IFN-γ induces the transcription of more than 200 genes in macrophages, including those for the production of antimicrobial molecules such as oxygen free radicals and nitric oxide, which represent one of the best effector mechanisms for elimination of *M. tuberculosis* [60]. However, some mycobacterial antigens, such as the 19 kDa lipoprotein, have the potential to mitigate the response of macrophages by blocking the transcription of subsets of genes responsive for IFN-γ [61, 62].

A series of clinical and experimental studies have demonstrated the importance of IFN-γ production in the control of tuberculosis [63–65]. Experiments in mice revealed that IFN-γ is an essential cytokine for macrophage activation and mycobacteria death in the intracellular environment. Cooper et al. [66] and Flynn et al. [67] have demonstrated that mice deprived from the IFN-γ genes have experienced fulminant infection by *M. tuberculosis*.

Individuals with a deficiency in the IFN-γ receptor gene have shown to be extremely susceptible to mycobacterial infections [68]. The complete deficiency of IFN-γ receptor in humans is associated with increased severity in the course of infection, poor formation of granulomas, multibacillary lesions, and progressive infection [69]. Studies with individuals that presented genetic mutations in the IFN-γ receptor have also proven that they presented high susceptibility to atypical mycobacterial infections [70].

The interleukin-12 (IL-12)/interferon-γ (IFN-γ) axis is determinant to the generation of Th1 lymphocytes, activation of macrophages by T cells, and further elimination of bacteria. A series of mutations associated to these axis components were identified in humans: these include mutation in the IL-12Rβ1, IL-12p40, IFN-γR2 genes, and the signal transducer and activator of transcription-1 (STAT-1). Most infections associated with these Mendelian disorders arise from the use of BCG or environmental mycobacteria. Nevertheless, some of the disorders are also associated with an increased susceptibility to *M. tuberculosis* (IFN-γR2 and IL-12p40) [71, 72].

IL-12 enhances IFN-γ production by NK cells and expands antigen specific Th1 cells. Other cytokines such as IL-23, IL-18, and IL-27 are also important inducers of IFN-γ. About 20 days are enough to produce IFN-γ by Th1 lymphocytes, which results in its accumulation in the lungs and bacterial growth arrest [73]. IL-18, a cytokine produced by monocytes, macrophages, and dendritic cells, cooperates with IL-12 to induce IFN-γ production [61]. Studies clearly indicate that IL-18 contributes to protect against infection by mycobacteria [74, 75]. Moreover, IL-18 deficient mice when infected with *M. tuberculosis* present reduced levels of IFN-γ compared with normal mice, despite the standard levels of IL-12 [76].

Morosini and colleagues [77] emphasize through data that they found in their study the view that in humans, at least at certain stages of pulmonary tuberculosis, there is a differential compartmentalization of IFN-γ and of the regulatory cytokine IL-12 and IL-10, where the protection factor associated with the secretion of IL-12 is present in the lungs and the component associated with immunosuppressive IL-10 secretion is predominant in peripheral blood. Furthermore, their results indicate a more critical role for IL-18 in the host response to *M. tuberculosis* in humans, suggesting that IL-18 may act as a factor for induction of IFN-γ in the lungs, whereas one can have immunoregulatory activity on peripheral circulation [77].

Studies report that patients with less severe forms of pulmonary tuberculosis have a predominance of Th1 cytokines such as IFN-γ, whereas the increase in IL-4 levels, a Th2 type cytokine, is related to the disease severity [78, 79]. Torres et al. [80] studied the immune response of patients' PBMCs with active tuberculosis and their healthy household contacts in response to the 30-KDa antigen from *M. tuberculosis*. Their results demonstrated a defect in the IFN-γ production by patients in response to the investigated antigen and a strong response to this antigen by the healthy communicants' cells, suggesting a protective role of IFN-γ in those individuals.

After inhalation and subsequent infection with *M. tuberculosis* in the lungs, dendritic cells infected with the bacilli migrate to the regional lymph node, which occurs, on average, around 14 days after infection, initiating the activation of T cells [81]. A model study used dendritic cells infected with *M. tuberculosis* inoculated intratracheally in the lung and as a result found that dendritic cells exposed to *M. tuberculosis* prior to inoculation are better in migrating to the lymph node and in T cell activation [82, 83]. These findings, in association with information about the secretion of cytokines and activation of the populations of $CD4^+$ T cells, indicate that different subtypes of $CD4^+$ T cells involved in protection in tuberculosis are activated in the initial phases of infection and produce cytokines classically considered immune protectors such as IL-2, IFN-γ and TNF-α [84, 85].

There is evidence that $CD4^+$ T cells may contribute both to the control of *M. tuberculosis* as well as of immunopathology, contributing to morbidity and mortality in tuberculosis disease. Reference [86] quantified the variation of IFN-γ/IL-17 in response to specific antigens of *M. tuberculosis* in patients with a positive PPD test and healthy individuals and observed a large variation in the amounts of IL-17 and IFN-γ secreted in response to the various antigens used.

5. Interleukin-10 (IL-10)

Due to its ability to inhibit the T lymphocyte production of cytokines, IL-10 was originally described as a cytokine synthesis inhibitory factor (CSIF) [86]. Subsequent studies have demonstrated that IL-10 could also inhibit Th1 and Th2 subpopulations *in vitro* [87, 88]. IL-10 acts inhibiting the production of pro-inflammatory cytokines (IFN-γ, TNF-α and IL-12) and the action of antigen presenting cells, blocking the activation of T lymphocytes through the inhibition of expression of MHC class II molecules [89, 90]. Therefore, it has an immunoregulatory function [91].

IL-10 is produced by macrophages and T lymphocytes during *M. tuberculosis* infection. Unlike TNF-α and IFN-γ, IL-10 is considered primarily an inhibitory cytokine, important to the adequate balance between inflammatory and immunopathological responses. However, the increase in IL-10 levels appears to support the mycobacterial survival in the host. Mice with defective IL-10 exhibit an increase in the antimycobacterial immunity [92]. IL-10 reduces the protective response to MTB in the CBA mice strain, in which IL-10 is produced by phagocytes in the interior of the pulmonary lesion and where a reduction in the TNF and IL-12p40 expression can be observed [93]. IL-10 is also able to induce the reactivation of tuberculosis in animals [94].

IL-10 is increased in samples obtained from patients with TB, and a higher capacity of IL-10 production is associated with an increase in the disease incidence. In human tuberculosis, IL-10 production is higher in anergic patients, suggesting that *M. tuberculosis* induces IL-10 production, suppressing an effective immune response [95]. Macrophages from patients suffering from tuberculosis are suppressed *in vitro*, and the inhibition of IL-10 reverts partially this suppression [90]. In another study, IL-10 was capable of directly inhibiting the responses of CD4 T lymphocyte from donors with latent TB and also reduced the expression of MHC class I and II, CD40, B7-1, and B7-2 of monocytes infected with MTB [96].

Lowering the protective cellular immune response is the *M. tuberculosis* aim to survive in the host. IL-10 and other inhibitory mediators of the inflammatory response (TGF-βRII, IL-1Rn e IDO) are detected in the sputum samples of patients with TB, whereas 30 days after treatment their level decreases considerably, while an increase in the Th1 response is observed [97].

In some human populations, an increase in IL-10 expression was identified, being possible to correlate it with an inefficiency in the BCG (Bacillus Calmette-Guérin) vaccination [98]. The analysis of the IL-10 gene polymorphisms involved in the development of infectious diseases suggests that this polymorphism has a critical role in the immunity and progression of inflammation. The increase in IL-10 production can, in particular, suppress the immune response and promote progression of the disease [99].

Regarding the immunopathogeny of TB, it is unquestionable the immunosuppressor role presented by IL-10

[100, 101]. Nonetheless, some studies have not detected increased levels of this cytokine in PBMCs from patients with active TB in response to mycobacterial antigens [102].

6. Cytokines in Household Tuberculosis Contact Cases

The study of household contacts of TB cases is of essential importance to the programs for combat and control of tuberculosis, in other words, the epidemiologic surveillance of household contacts as a means for early diagnosis of TB cases and the decrease of the spread of the disease [103].

Some studies assessed the cytokine profile in groups of healthy contacts individuals. From these works, it is important to mention Demissie [104], who conducted a study comparing the immune response of infected individuals in the latent stage with TB patients. The results demonstrated that TB patients presented a low production of IFN-γ and IL-2 cytokines when compared to individuals with latent infections. This suggests that the control of TB in the latent stage is not only associated with increased expression of Th1 cytokines, but also with the suppression of IL-4 activity [104]. Later, the same group [105] compared the expression of IL-4, IL-4δ2, and IFN-γ in the peripheral blood of household contacts of TB patients presenting positive sputum. The results demonstrated that the expression of IL-4 was slightly higher in household contacts when compared to the controls from the community. However, when the household contacts were divided into groups with or without immunological signs of infection with *M. tuberculosis,* the expression of IL-4 was clearly elevated in the positive ESAT-6 (signal transducer and activator of transcription 6, an MTB antigen) group and the expression of Th1 cytokines such as IFN-γ was low. Thus, they suggest that a strong response to the antigen ESAT-6 in individuals exposed to *M. tuberculosis* correlates with a low expression of IFN-γ and higher expression of IL-4 and that possibly this profile is associated with a poor prognosis [105]. The results founded by our group [106] demonstrated that individuals with or without a previous history of tuberculosis and exposed to *M. tuberculosis* showed a Th1 (TNF-α and IFN-γ) and Th2 (IL-10 and TGF-β) profile of cytokines, similar to that found by Demissie [105], with an IFN-γ production relatively low when compared to IL-10. These cytokines would be involved in shifting the state of latency to the stage of clinical tuberculosis [106].

The presence of high levels of IL-10 in the plasma of household contacts was unexpected for the group of [107], since they have also found high levels of IL-10 in the studied patients. However, there are few reports in the literature on the production of IL-10 by household contacts of patients with tuberculosis [107]. According to [107], these levels are due to stimulation of mycobacterial antigens that induce this cytokine production by mononuclear cells. Moreover, we can suggest that IL-10 was involved in the natural defense that goes against excessive proinflammatory responses generated by TNF-α. Therefore, the simultaneous presence of IL-10 and TNF-α in household communicating TB patients might be beneficial to these individuals [105]. IL-10 may be required to modulate proinflammatory effects in patients and in healthy household tuberculosis individuals.

Table 1: Studies of cytokines associated to *M. tuberculosis* infection.

Cytokine	Tuberculosis study	References
TNF-α	Studies in murine models	[32–35, 44]
	Studies in patients with pulmonary tuberculosis	[36–39]
IFN-γ	Studies in murine models	[45, 66, 67, 76]
	Clinical studies	[63–65, 77–80, 85]
	Genetic studies	[68–72]
IL-10	Studies in murine models	[92–94]
	Studies in patients with tuberculosis	[95–102]
Other cytokines	Studies in tuberculosis	[14, 16–21, 24, 25]

Table 1 summarizes the main cytokines, associated studies, and references described in this paper.

7. Final Thoughts

The host resistance against infection with *M. tuberculosis* starts with the innate immunity, involving the interaction of the bacillus with macrophages and dendritic cells. Little is known about the transition between the initial control of infection and the establishment of latent infection, which is largely due, in part, to the lack of appropriate animal models [108].

Although there are still no conclusive studies about a clear dichotomy between Th1 versus Th2 response, involving protective immunity and disease susceptibility, respectively, we can conclude that the knowledge of Th1 and Th2 responses helps to elucidate the immune protection profile of the host against *M. tuberculosis*.

References

[1] D. N. Sato, "Mycobacterium," in *Bacteriologia—um texto ilustrado*, C. H. P. M. e Silva, Ed., pp. 285–315, Eventos, Rio de Janeiro, Brazil, 1999.

[2] WHO, *Global Tuberculosis Control*, 2011.

[3] WHO, *The Global Plan Tuberculosis to Stop TB, 2011–2015*, 2012.

[4] M. Baliza, A. H. Bach, G. L. de Queiroz et al., "High frequency of resistance to the drugs isoniazid and rifampicin among tuberculosis cases in the City of Cabo de Santo Agostinho, an urban area in Northeastern Brazil," *Revista da Sociedade Brasileira de Medicina Tropical*, vol. 41, no. 1, pp. 11–16, 2008.

[5] H. Tomioka, Y. Tatano, C. Sano, and T. Shimizu, "Development of new antituberculous drugs based on bacterial virulence factors interfering with host cytokine networks," *Journal of Infection and Chemotherapy*, vol. 17, no. 3, pp. 302–317, 2011.

[6] E. L. Corbett, C. J. Watt, N. Walker et al., "The growing burden of tuberculosis: global trends and interactions with

the HIV epidemic," *Archives of Internal Medicine*, vol. 163, no. 9, pp. 1009–1021, 2003.

[7] T. R. Frieden, T. R. Sterling, S. S. Munsiff, C. J. Watt, and C. Dye, "Tuberculosis," *The Lancet*, vol. 362, no. 9387, pp. 887–899, 2003.

[8] K. E. Dooley and R. E. Chaisson, "Tuberculosis and diabetes mellitus: convergence of two epidemics," *The Lancet Infectious Diseases*, vol. 9, no. 12, pp. 737–746, 2009.

[9] Y. Kinjo, K. Kawakami, K. Uezu et al., "Contribution of IL-18 to Th1 response and host defense against infection by *Mycobacterium tuberculosis*: a comparative study with IL-12p40," *Journal of Immunology*, vol. 169, no. 1, pp. 323–329, 2002.

[10] C. H. Wang, H. C. Lin, C. Y. Liu et al., "Upregulation of inducible nitric oxide synthase and cytokine secretion in peripheral blood monocytes from pulmonary tuberculosis patients," *International Journal of Tuberculosis and Lung Disease*, vol. 5, no. 3, pp. 283–291, 2001.

[11] A. Gupta, A. Kaul, A. G. Tsolaki, U. Kishore, and S. Bhakta, "*Mycobacterium tuberculosis*: immune evasion, latency and reactivation," *Immunobiology*, vol. 217, no. 3, pp. 363–374, 2012.

[12] A. M. Cooper and S. A. Khader, "The role of cytokines in the initiation, expansion, and control of cellular immunity to tuberculosis," *Immunological Reviews*, vol. 226, no. 1, pp. 191–204, 2008.

[13] S. D. Lawn, L. Myer, D. Edwards, L. G. Bekker, and R. Wood, "Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa," *AIDS*, vol. 23, no. 13, pp. 1717–1725, 2009.

[14] S. A. Khader, G. K. Bell, J. E. Pearl et al., "IL-23 and IL-17 in the establishment of protective pulmonary $CD4^+$ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge," *Nature Immunology*, vol. 8, no. 4, pp. 369–377, 2007.

[15] P. G. Lin and J. L. Flynn, "Understanding latent tuberculosis: a moving target," *Journal of Immunology*, vol. 185, no. 1, pp. 15–22, 2010.

[16] L. E. Harrington, R. D. Hatton, P. R. Mangan et al., "Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages," *Nature Immunology*, vol. 6, no. 11, pp. 1123–1132, 2005.

[17] H. Park, Z. Li, X. O. Yang et al., "A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17," *Nature Immunology*, vol. 6, no. 11, pp. 1133–1141, 2005.

[18] M. Kader, X. Wang, M. Piatak et al., "$\alpha 4^+\beta 7^{hi}CD4^+$ memory T cells harbor most Th-17 cells and are preferentially infected during acute SIV infection," *Mucosal Immunology*, vol. 2, no. 5, pp. 439–449, 2009.

[19] E. Torrado and A. M. Cooper, "IL-17 and Th17 cells in tuberculosis," *Cytokine and Growth Factor Reviews*, vol. 21, no. 6, pp. 455–462, 2010.

[20] T. M. Wozniak, B. M. Saunders, A. A. Ryan, and W. J. Britton, "*Mycobacterium bovis* BCG-specific Th17 cells confer partial protection against *Mycobacterium tuberculosis* infection in the absence of γ interferon," *Infection and Immunity*, vol. 78, no. 10, pp. 4187–4194, 2010.

[21] I. L. D. Moutinho, "Tuberculose: aspectos imunológicos na infecção e na doença," *Revista Médica de Minas Gerais*, vol. 21, no. 1, pp. 42–48, 2011.

[22] C. A. Dinarello, "Immunological and inflammatory functions of the interleukin-1 family," *Annual Review of Immunology*, vol. 27, pp. 519–550, 2009.

[23] C. Garlanda, D. Di Liberto, A. Vecchi et al., "Damping excessive inflammation and tissue damage in *Mycobacterium tuberculosis* infection by toll IL-1 receptor 8/single Ig IL-1-related receptor, a negative regulator of IL-1/TLR signaling," *Journal of Immunology*, vol. 179, no. 5, pp. 3119–3125, 2007.

[24] D. M.-B. Katrin, B. B. Andrade, D. L. Barber et al., "Innate and adaptive interferons suppress IL-1a and IL-1b production by distinct pulmonary myeloid subsets during *Mycobacterium tuberculosis* infection," *Immunity*, vol. 35, no. 6, pp. 1023–1034, 2011.

[25] R. Guler, S. P. Parihar, G. Spohn, P. Johansen, F. Brombacher, and M. F. Bachmann, "Blocking IL-1α but not IL-1β increases susceptibility to chronic *Mycobacterium tuberculosis* infection in mice," *Vaccine*, vol. 29, no. 6, pp. 1339–1346, 2011.

[26] E. A. Carswell, L. J. Old, Kassel, R. L. Green, N. Fiore, and B. Williamson, "An endotoxin-induced serum factor that causes necrosis of tumors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 72, no. 9, pp. 3666–3670, 1975.

[27] P. F. Barnes, S. Lu, J. S. Abrams, E. Wang, M. Yamamura, and R. L. Modlin, "Cytokine production at the site of disease in human tuberculosis," *Infection and Immunity*, vol. 61, no. 8, pp. 3482–3489, 1993.

[28] C. H. Ladel, G. Szalay, D. Riedel, and S. H. E. Kaufmann, "Interleukin-12 secretion by *Mycobacterium tuberculosis*-infected macrophages," *Infection and Immunity*, vol. 65, no. 5, pp. 1936–1938, 1997.

[29] N. V. Serbina and J. L. Flynn, "Early emergence of CD8+ T cells primed for production of type 1 cytokines in the lungs of *Mycobacterium tuberculosis*-infected mice," *Infection and Immunity*, vol. 67, no. 8, pp. 3980–3988, 1999.

[30] K. Yu, C. Mitchell, Y. Xing, R. S. Magliozzo, B. R. Bloom, and J. Chan, "Toxicity of nitrogen oxides and related oxidants on mycobacteria: *M. tuberculosis* is resistant to peroxynitrite anion," *Tubercle and Lung Disease*, vol. 79, no. 4, pp. 191–198, 1999.

[31] C. A. Scanga, V. P. Mohan, K. Yu et al., "Depletion of $CD4^+$ T cells causes reactivation of murine persistent tuberculosis despite continued expression of interferon γ and nitric oxide synthase 2," *Journal of Experimental Medicine*, vol. 192, no. 3, pp. 347–358, 2000.

[32] V. P. Mohan, C. A. Scanga, K. Yu et al., "Effects of tumor necrosis factor α on host immune response in chronic persistent tuberculosis: possible role for limiting pathology," *Infection and Immunity*, vol. 69, no. 3, pp. 1847–1855, 2001.

[33] J. L. Flynn, M. M. Goldstein, J. Chan et al., "Tumor necrosis factor-α is required in the protective immune response against *Mycobacterium tuberculosis* in mice," *Immunity*, vol. 2, no. 6, pp. 561–572, 1995.

[34] A. G. D. Bean, D. R. Roach, H. Briscoe et al., "Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol *Mycobacterium tuberculosis* infection, which is not compensated for by lymphotoxin," *Journal of Immunology*, vol. 162, no. 6, pp. 3504–3511, 1999.

[35] J. L. Flynn, C. A. Scanga, K. E. Tanaka, and J. Chan, "Effects of aminoguanidine on latent murine tuberculosis," *Journal of Immunology*, vol. 160, no. 4, pp. 1796–1803, 1998.

[36] D. Dlugovitzky, M. L. Bay, L. Rateni et al., "Influence of disease severity on nitrite and cytokine production by peripheral blood mononuclear cells (PBMC) from patients with pulmonary tuberculosis (TB)," *Clinical and Experimental Immunology*, vol. 122, no. 3, pp. 343–349, 2000.

[37] R. Al-Attiyah, A. El-Shazly, and A. S. Mustafa, "Comparative analysis of spontaneous and mycobacterial antigen-induced secretion of Th1, Th2 and pro-inflammatory cytokines by peripheral blood mononuclear cells of tuberculosis patients," *Scandinavian Journal of Immunology*, vol. 75, no. 6, pp. 623–632, 2012.

[38] E. P. Moura, "Avaliação da resposta immune cellular e humoral antes e depois do tratamento da tuberculose pulmonar," Tese, p. 90, 2002.

[39] E. Juárez, C. Carranza, F. Hernández-Sánchez et al., "NOD2 enhances the innate response of alveolar macrophages to *Mycobacterium tuberculosis* in humans," *European Journal of Immunology*, vol. 42, no. 4, pp. 880–889, 2012.

[40] R. N. Maini, P. C. Taylor, E. Paleolog et al., "Anti-tumour necrosis factor specific antibody (infliximab) treatment provides insights into the pathophysiology of rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 58, no. 1, pp. I56–I60, 1999.

[41] I. Solovic, M. Sester, J. J. Gomez-Reino et al., "The risk of tuberculosis related to tumour necrosis factor antagonist therapies: a TBNET consensus statement," *European Respiratory Journal*, vol. 36, no. 5, pp. 1185–1206, 2010.

[42] A. Stallmach, S. Hagel, and T. Bruns, "Adverse effects of biologics used for treating IBD," *Best Practice and Research: Clinical Gastroenterology*, vol. 24, no. 2, pp. 167–182, 2010.

[43] J. L. Flynn, J. Chan, and P. L. Lin, "Macrophages and control of granulomatous inflammation in tuberculosis," *Mucosal Immunology*, vol. 4, no. 3, pp. 271–278, 2011.

[44] L. G. Bekker, A. L. Moreira, A. Bergtold, S. Freeman, B. Ryffel, and G. Kaplan, "Immunopathologic effects of tumor necrosis factor alpha in murine mycobacterial infection are dose dependent," *Infection and Immunity*, vol. 68, no. 12, pp. 6954–6961, 2000.

[45] S. Ehlers, J. Benini, H. D. Held, C. Roeck, G. Alber, and S. Uhlig, "$\alpha\beta$ T cell receptor-positive cells and interferon-γ, but not inducible nitric oxide synthase, are critical for granuloma necrosis in a mouse model of mycobacteria-induced pulmonary immunopathology," *Journal of Experimental Medicine*, vol. 194, no. 12, pp. 1847–1859, 2001.

[46] B. Jönsson, M. Ridell, and A. E. Wold, "Phagocytosis and cytokine response to rough and smooth colony variants of mycobacterium abscessus byhuman peripheral blood mononuclear cells," *Acta Pathologica, Microbiologica et Immunologica Scandinavica*. In press.

[47] I. Dambuza, N. Allie, L. Fick et al., "Efficacy of membrane TNF mediated host resistance is dependent on mycobacterial virulence," *Tuberculosis*, vol. 88, no. 3, pp. 221–234, 2008.

[48] J. Keane, H. G. Remold, and H. Kornfeld, "Virulent *Mycobacterium tuberculosis* strains evade apoptosis of infected alveolar macrophages," *Journal of Immunology*, vol. 164, no. 4, pp. 2016–2020, 2000.

[49] C. M. Robinson, J. Y. Jung, and G. J. Nau, "Interferon-γ, tumor necrosis factor, and interleukin-18 cooperate to control growth of *Mycobacterium tuberculosis* in human macrophages," *Cytokine*, vol. 60, no. 1, pp. 233–241, 2012.

[50] C. Manca, M. B. Reed, S. Freeman et al., "Differential monocyte activation underlies strain-specific *Mycobacterium tuberculosis* pathogenesis," *Infection and Immunity*, vol. 72, no. 9, pp. 5511–5514, 2004.

[51] Y. van Kooyk and T. B. H. Geijtenbeek, "DC-SIGN: escape mechanism for pathogens," *Nature Reviews Immunology*, vol. 3, no. 9, pp. 697–709, 2003.

[52] G. T. Seah and G. A. W. Rook, "IL-4 influences apoptosis of mycobacterium-reactive lymphocytes in the presence of TNF-α," *Journal of Immunology*, vol. 167, no. 3, pp. 1230–1237, 2001.

[53] S. Stenger, "Immunological control of tuberculosis: role of tumour necrosis factor and more," *Annals of the Rheumatic Diseases*, vol. 64, supplement 4, pp. iv24–iv28, 2005.

[54] M. Bocchino, D. Galati, A. Sanduzzi, V. Colizzi, E. Brunetti, and G. Mancino, "Role of mycobacteria-induced monocyte/macrophage apoptosis in the pathogenesis of human tuberculosis," *International Journal of Tuberculosis and Lung Disease*, vol. 9, no. 4, pp. 375–383, 2005.

[55] A. Isaacs, J. Lindenmann, and R. C. Valentine, "Virus interference. II. Some properties of interferon," *Proceedings of the Royal Society of London, Series B*, vol. 1471, no. 927, pp. 268–273, 1957.

[56] G. H. Schreiber and R. D. Schereiber, "Interferon-γ," in *The Cytokine Handbook*, A. W. Thomson and M. T. Lotze, Eds., vol. 1, chapter 24, pp. 567–601, Elsevier, New York, NY, USA, 4th edition.

[57] I. E. A. Flesch, J. H. Hess, S. Huang et al., "Early interleukin 12 production by macrophages in response to mycobacterial infection depends on interferon γ and tumor necrosis factor α," *Journal of Experimental Medicine*, vol. 181, no. 5, pp. 1615–1621, 1995.

[58] M. J. Fenton, M. W. Vermeulen, S. Kim, M. Burdick, R. M. Strieter, and H. Kornfeld, "Induction of γ interferon production in human alveolar macrophages by *Mycobacterium tuberculosis*," *Infection and Immunity*, vol. 65, no. 12, pp. 5149–5156, 1997.

[59] A. Oberholzer, C. Oberholzer, and L. L. Moldawer, "Cytokine signaling—regulation of the immune response in normal and critically ill states," *Critical Care Medicine*, vol. 28, no. 4, supplement, pp. N3–N12, 2000.

[60] A. M. Cooper, "Cell-mediated immune responses in tuberculosis," *Annual Review of Immunology*, vol. 27, pp. 393–422, 2009.

[61] A. J. Gehring, R. E. Rojas, D. H. Canaday, D. L. Lakey, C. V. Harding, and W. H. Boom, "The *Mycobacterium tuberculosis* 19-kilodalton lipoprotein inhibits γ interferon-regulated HLA-DR and FcγR1 on human macrophages through toll-like receptor 2," *Infection and Immunity*, vol. 71, no. 8, pp. 4487–4497, 2003.

[62] C. V. Harding, R. K. Pai, M. Convery, T. A. Hamilton, and W. Henry Boom, "Inhibition of IFN-γ-induced class II transactivator expression by a 19-kDa lipoprotein from *Mycobacterium tuberculosis*: a potential mechanism for immune evasion," *Journal of Immunology*, vol. 171, no. 1, pp. 175–184, 2003.

[63] R. C. O. Tavares, J. Salgado, V. B. Moreira et al., "Cell proliferation and interferon-γ response to recombinant MBP-3, NarL, MT-10.3, and 16kDa *Mycobacterium tuberculosis* antigens in Brazilian tuberculosis patients," *Memorias do Instituto Oswaldo Cruz*, vol. 101, no. 8, pp. 857–861, 2006.

[64] A. Nienhaus, A. Schablon, and R. Diel, "Interferon-γ release assay for the diagnosis of latent TB infection—analysis of discordant results, when compared to the tuberculin skin test," *PLoS ONE*, vol. 3, no. 7, Article ID e2665, 2008.

[65] J. C. T. Costa, R. Silva, R. Sá, M. J. Cardoso, C. Ribeiro, and A. Nienhaus, "Comparison of interferon-γ release assay and tuberculin test for screening in healthcare workers," *Revista Portuguesa de Pneumologia*, vol. 16, no. 2, pp. 211–221, 2010.

[66] A. M. Cooper, D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell, and I. M. Orme, "Disseminated tuberculosis in interferon γ gene-disrupted mice," *Journal of Experimental Medicine*, vol. 178, no. 6, pp. 2243–2247, 1993.

[67] J. L. Flynn, J. Chan, K. J. Triebold, D. K. Dalton, T. A. Stewart, and B. R. Bloom, "An essential role for interferon γ in resistance to *Mycobacterium tuberculosis* infection," *Journal of Experimental Medicine*, vol. 178, no. 6, pp. 2249–2254, 1993.

[68] M. J. Newport, C. M. Huxley, S. Huston et al., "A mutation in the interferon-γ-receptor gene and susceptibility to mycobacterial infection," *The New England Journal of Medicine*, vol. 335, no. 26, pp. 1941–1949, 1996.

[69] T. H. M. Ottenhoff, F. A. W. Verreck, M. A. Hoeve, and E. van de Vosse, "Control of human host immunity to mycobacteria," *Tuberculosis*, vol. 85, no. 1-2, pp. 53–64, 2005.

[70] E. Jouanguy, F. Altare, S. Lamhamedi et al., "Interferon-γ-receptor deficiency in an infant with fatal bacille Calmette-Guérin infection," *The New England Journal of Medicine*, vol. 335, no. 26, pp. 1956–1961, 1996.

[71] J. L. Casanova and L. Abel, "Genetic dissection of immunity to mycobacteria: the human model," *Annual Review of Immunology*, vol. 20, pp. 581–620, 2002.

[72] G. S. Cooke and A. V. S. Hill, "Genetics of susceptibility to human infectious disease," *Nature Reviews Genetics*, vol. 2, no. 12, pp. 967–977, 2001.

[73] H. M. S. Algood, J. Chan, and J. L. Flynn, "Chemokines and tuberculosis," *Cytokine and Growth Factor Reviews*, vol. 14, no. 6, pp. 467–477, 2003.

[74] V. E. García, K. Uyemura, P. A. Sieling et al., "IL-18 promotes type 1 cytokine production from NK cells and T cells in human intracellular infection," *Journal of Immunology*, vol. 162, no. 10, pp. 6114–6121, 1999.

[75] R. Vankayalapati, B. Wizel, S. E. Weis, B. Samten, W. M. Girard, and P. F. Barnes, "Production of interleukin-18 in human tuberculosis," *Journal of Infectious Diseases*, vol. 182, no. 1, pp. 234–239, 2000.

[76] I. Sugawara, H. Yamada, H. Kaneko, S. Mizuno, K. Takeda, and S. Akira, "Role of interleukin-18 (IL-18) in mycobacterial infection in IL-18- gene-disrupted mice," *Infection and Immunity*, vol. 67, no. 5, pp. 2585–2589, 1999.

[77] M. Morosini, F. Meloni, A. M. Bianco et al., "The assessment of IFN-γ and its regulatory cytokines in the plasma and bronchoalveolar lavage fluid of patients with active pulmonary tuberculosis," *International Journal of Tuberculosis and Lung Disease*, vol. 7, no. 10, pp. 994–1000, 2003.

[78] D. Dlugovitzky, A. Torres-Morales, L. Rateni et al., "Circulating profile of Th1 and Th2 cytokines in tuberculosis patients with different degrees of pulmonary involvement," *FEMS Immunology and Medical Microbiology*, vol. 18, no. 3, pp. 203–207, 1997.

[79] D. Dlugovitzky, M. L. Bay, L. Rateni et al., "In vitro synthesis of interferon-γ, interleukin-4, transforming growth factor-β and interleukin-1β by peripheral blood mononuclear cells from tuberculosis patients: Relationship with the severity of pulmonary involvement," *Scandinavian Journal of Immunology*, vol. 49, no. 2, pp. 210–217, 1999.

[80] M. Torres, T. Herrera, H. Villareal, E. A. Rich, and E. Sada, "Cytokine profiles for peripheral blood lymphocytes from patients with active pulmonary tuberculosis and healthy household contacts in response to the 30-kilodalton antigen of *Mycobacterium tuberculosis*," *Infection and Immunity*, vol. 66, no. 1, pp. 176–180, 1998.

[81] A. J. Wolf, B. Linas, G. J. Trevejo-Nuñez et al., "*Mycobacterium tuberculosis* infects dendritic cells with high frequency and impairs their function in vivo," *Journal of Immunology*, vol. 179, no. 4, pp. 2509–2519, 2007.

[82] S. A. Khader, S. Partida-Sanchez, G. Bell et al., "Interleukin 12p40 is required for dendritic cell migration and T cell priming after *Mycobacterium tuberculosis* infection," *Journal of Experimental Medicine*, vol. 203, no. 7, pp. 1805–1815, 2006.

[83] C. Demangel, P. Bertolino, and W. J. Britton, "Autocrine IL-10 impairs dendritic cell (DC)-derived immune responses to mycobacterial infection by suppressing DC trafficking to draining lymph nodes and local IL-12 production," *European Journal of Immunology*, vol. 32, no. 4, pp. 994–1002, 2002.

[84] Y. J. Jung, L. Ryan, R. LaCourse, and R. J. North, "Properties and protective value of the secondary versus primary T helper type 1 response to airborne *Mycobacterium tuberculosis* infection in mice," *Journal of Experimental Medicine*, vol. 201, no. 12, pp. 1915–1924, 2005.

[85] L. Fan, H. Xiao, Z. Hu, and J. D. Ernst, "Variation of *Mycobacterium tuberculosis* antigen-specific IFN-c and IL-17 responses in healthy tuberculin skin test (TST)-positive human subjects," *PLoS ONE*, no. 8, Article ID e42716, 2012.

[86] D. F. Fiorentino, M. W. Bond, and T. R. Mosmann, "Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones," *Journal of Experimental Medicine*, vol. 170, no. 6, pp. 2081–2095, 1989.

[87] R. de Waal Malefyt, H. Yssel, and J. E. de Vries, "Direct effects of IL-10 on subsets of human $CD4^+$ T cell clones and resting T cells: specific inhibition of IL-2 production and proliferation," *Journal of Immunology*, vol. 150, no. 11, pp. 4754–4765, 1993.

[88] L. Schandene, C. Alonso-Vega, F. Willems et al., "B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10," *Journal of Immunology*, vol. 152, no. 9, pp. 4368–4374, 1994.

[89] S. A. Fulton, J. V. Cross, Z. T. Toossi, and W. H. Boom, "Regulation of interleukin-12 by interleukin-10, transforming growth factor-β, tumor necrosis factor-α, and interferon-γ in human monocytes infected with *Mycobacterium tuberculosis* H37Ra," *Journal of Infectious Diseases*, vol. 178, no. 4, pp. 1105–1114, 1998.

[90] J. L. Flynn and J. Chan, "Immunology of tuberculsis," *Annual Review of Immunology*, vol. 19, pp. 93–129, 2001.

[91] A. D'Andrea, M. Aste-Amezaga, N. M. Valiante, X. Ma, M. Kubin, and G. Trinchieri, "Interleukin 10 (IL-10) inhibits human lymphocyte interferon γ-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells," *Journal of Experimental Medicine*, vol. 178, no. 3, pp. 1041–1048, 1993.

[92] P. J. Murray and R. A. Young, "Increased antimycobacterial immunity in interleukin-10-deficient mice," *Infection and Immunity*, vol. 67, no. 6, pp. 3087–3095, 1999.

[93] G. L. Beamer, D. K. Flaherty, B. D. Assogba et al., "Interleukin-10 promotes *Mycobacterium tuberculosis* disease progression in CBA/J mice," *Journal of Immunology*, vol. 181, no. 8, pp. 5545–5550, 2008.

[94] M. I. Henao, C. Montes, S. C. París, and L. F. García, "Cytokine gene polymorphisms in Colombian patients with different clinical presentations of tuberculosis," *Tuberculosis*, vol. 86, no. 1, pp. 11–19, 2006.

[95] V. A. Boussiotis, E. Y. Tsai, E. J. Yunis et al., "IL-10-producing T cells suppress immune responses in anergic tuberculosis patients," *Journal of Clinical Investigation*, vol. 105, no. 9, pp. 1317–1325, 2000.

[96] R. E. Rojas, K. N. Balaji, A. Subramanian, and W. H. Boom, "Regulation of human $CD4^+\alpha\beta$ T-cell-receptor-positive

(TCR+) and $\gamma\delta$ TCR+ T-cell responses to *Mycobacterium tuberculosis* by interleukin-10 and transforming growth factor β," *Infection and Immunity*, vol. 67, no. 12, pp. 6461–6472, 1999.

[97] A. S. Almeida, P. M. Lago, N. Boechat et al., "Tuberculosis is associated with a down-modulatory lung immune response that impairs Th1-type immunity," *Journal of Immunology*, vol. 183, no. 1, pp. 718–731, 2009.

[98] R. E. Weir, G. F. Black, H. M. Dockrell et al., "Mycobacterial purified protein derivatives stimulate innate immunity: Malawians show enhanced tumor necrosis factor α, interleukin-1β (IL-1β), and IL-10 responses compared to those of adolescents in the United Kingdom," *Infection and Immunity*, vol. 72, no. 3, pp. 1807–1811, 2004.

[99] H. D. Shin, B. L. Park, L. H. Kim, H. S. Cheong, I. H. Lee, and S. K. Park, "Common interleukin 10 polymorphism associated with decreased risk of tuberculosis," *Experimental and Molecular Medicine*, vol. 37, no. 2, pp. 128–132, 2005.

[100] C. B. Pereira, M. Palaci, O. H. M. Leite, A. J. S. Duarte, and G. Benard, "Monocyte cytokine secretion in patients with pulmonary tuberculosis differs from that of healthy infected subjects and correlates with clinical manifestations," *Microbes and Infection*, vol. 6, no. 1, pp. 25–33, 2004.

[101] A. A. Awomoyi, A. Marchant, J. M. M. Howson, K. P. W. J. McAdam, J. M. Blackwell, and M. J. Newport, "Interleukin-10, polymorphism in SLC11A1 (formerly NRAMP1), and susceptibility to tuberculosis," *Journal of Infectious Diseases*, vol. 186, no. 12, pp. 1808–1814, 2002.

[102] J. F. McDyer, M. N. Hackley, T. E. Walsh, J. L. Cook, and R. A. Seder, "Patients with multidrug-resistant tuberculosis with low $CD4^+$ T cell counts have impaired Th1 responses," *Journal of Immunology*, vol. 158, no. 1, pp. 492–500, 1997.

[103] C. E. Gazeta, M. L. S. Santos G, S. H. F. Vendramini, J. M. Pinto Neto, and T. C. S. Villa, "Controle de comunicantes de tuberculose no Brasil: Revisão de literatura (1984–2004)," *Revista Latino-Americana de Enfermagem*, vol. 16, no. 2, pp. 306–313, 2008.

[104] A. Demissie, M. Abebe, A. Aseffa et al., "Healthy individuals that control a latent infection with *Mycobacterium tuberculosis* express high levels of Th1 cytokines and the IL-4 antagonist IL-4δ2," *Journal of Immunology*, vol. 172, no. 11, pp. 6938–6943, 2004.

[105] A. Demissie, L. Wassie, M. Abebe et al., "The 6-kilodalton early secreted antigenic target-responsive, asymptomatic contacts of tuberculosis patients express elevated levels of interleukin-4 and reduced levels of γ interferon," *Infection and Immunity*, vol. 74, no. 5, pp. 2817–2822, 2006.

[106] Y. V. N. Cavalcanti, V. R. A. Pereira, L. C. Reis et al., "Evaluation of memory immune response to mycobacterium extract among household contact of tuberculosis cases," *Journal of Clinical Laboratory Analysis*, vol. 23, no. 1, pp. 57–62, 2009.

[107] J. O. Olobo, M. Geletu, A. Demissie et al., "Circulating TNF-α, TGF-β, and IL-10 in tuberculosis patients and healthy contacts," *Scandinavian Journal of Immunology*, vol. 53, no. 1, pp. 85–91, 2001.

[108] S. A. Sharpe, E. Eschelbach, R. J. Basaraba et al., "Determination of lesion volume by MRI and stereology in a macaque model of tuberculosis," *Tuberculosis*, vol. 89, no. 6, pp. 405–416, 2009.