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REVIEW

Latent tuberculosis infection – Revisiting and revising concepts

Padmini Salgame [a], Carolina Geadas [b], Lauren Collins [c], Edward Jones-López [b], Jerrold J. Ellner [b, *]

[a] Division of Infectious Diseases, Department of Medicine, New Jersey Medical School, Rutgers University, Newark, NJ, USA
[b] Section of Infectious Diseases, Department of Medicine, Boston Medical Center and Boston University School of Medicine, Boston, MA, USA
[c] Department of Internal Medicine, Duke University Medical Center, Durham, NC, USA

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SUMMARY

Host- and pathogen-specific factors interplay with the environment in a complex fashion to determine the outcome of infection with *Mycobacterium tuberculosis* (Mtb), resulting in one of three possible outcomes: cure, latency or active disease. Although much remains unknown about its pathophysiology, latent tuberculosis infection (LTBI) defined through immunologic evidence of Mtb infection is a continuum between self-cure and asymptomatic, yet active tuberculosis (TB) disease. Strain virulence, intensity of exposure to the index case, size of the bacterial inoculum, and host factors such as age and comorbidities, each contribute to where one settles on the continuum.

Currently, the diagnosis of LTBI is based on reactive tuberculin skin testing (TST) and/or a positive interferon-gamma release assay (IGRA). Neither diagnostic test reflects on the activity of the infectious focus or the risk of progression to active TB. This is a critical shortcoming, as accurate and efficient detection of those with LTBI at higher risk of progression to TB disease would allow for provision of targeted preventive therapy to those most likely to benefit. Host biomarkers may prove of value in stratifying risk of development of TB. New guidelines are required for interpretation of discordance between TST and IGRA, which may be due in part to a lack of stability (that is reproducibility) of IGRA or TST results or to a delay in conversion of IGRA to positivity compared to TST.

In this review, the authors elaborate on the definition, diagnosis, pathophysiology and natural history of LTBI, as well as promising methods for better stratifying risk of progression to TB. The review is centered on the human host and the clinical and epidemiologic features of LTBI that are relevant to the development of new and improved diagnostic tools.

1. Introduction

Exposure to *Mycobacterium tuberculosis* (Mtb) often results in the development of latent tuberculosis infection (LTBI) with a 5–10% lifetime risk of progressing to active tuberculosis (TB), the majority of TB cases occurring within the first two years after infection [1,2]. LTBI comprises a reservoir for new disease and ongoing Mtb transmission within communities and thereby perpetuation of the disease cycle at a population level. Given that one third of the world's population is latently infected with TB, the ability to accurately and efficiently identify those with LTBI at greatest risk of progression and provide targeted preventive therapy, may be essential for achieving TB eradication globally.

2. LTBI requires redefinition or renaming

LTBI is classically defined as measurable immune sensitization to Mtb in the absence of active disease manifestations, such as fever, chills, night sweats, weight loss, cough, hemoptysis, or a new opacity on chest radiograph. Immune reactivity to Mtb is assessed by either tuberculin skin testing (TST) or interferon-gamma release assay (IGRA), with a positive result by either method indicating LTBI. However, this definition does not address the duration and activity of the latent focus, which is not a homogenous entity, but rather varies person to person based on timing and on host- and pathogen-specific factors. The definition also is problematic given that LTBI at the local level is a spectrum from viable organisms actively replicating (or "percolating") to a status where the infection has been cleared (or rendered "quiescent") with substantial memory T cell responses to Mtb antigens.

* Corresponding author. 650 Albany Street, Room 608, Boston MA 02118, USA.
E-mail address: jerrold.ellner@bmc.org (J.J. Ellner).

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There is evidence that bacterial replication occurs in LTBI. For example, this is indicated by the well established efficacy of treatment in markedly diminishing the risk of progression from LTBI to TB. The most commonly used agent for chemoprevention in LTBI is isoniazid (INH), an inhibitor of cell wall synthesis, which is a process that occurs only with active replication of the organism. Further, ESAT-6 and CFP-10 which are used to elicit interferon-gamma (IFN-γ) production for IGRA testing, are secreted during active bacterial replication although there is some induction in hypoxic environments [3]. Lastly, states of acquired immune suppression such as infection with human immunodeficiency virus (HIV) and therapy with tumor necrosis factor alpha (TNF-alpha) inhibitors substantially increased the risk of reactivation TB further indicating that some latent foci contain viable Mtb. The proportion of 'latent' Mtb foci that are active and the contribution of immunosuppression to activity remain unknown. Further confounding the current definition of LTBI, TST and IGRA, may be falsely negative in the case of immunosuppression or active TB disease, may revert to negative after treatment of LTBI or if the initial infection occurred in the distant past.

In evaluating contacts of TB cases for LTBI, patients undergo screening for classic symptoms and signs of disease by thorough history and physical examination, respectively, and then chest radiograph and sputum smear for acid-fast bacilli (AFB) are obtained if the clinical suspicion is high enough to rule out active disease. In areas of high TB prevalence or in high risk populations (e.g. HIV-infected), more sensitive tests may be necessary to rigorously exclude disease such as sputum culture on liquid medium or nucleic acid amplification tests such as the XpertMtb/RIF®. This is relevant in that an individual with no or few clinical symptoms that is initially categorized as LTBI but is found to have a positive sputum culture should be reclassified as asymptomatic, sometimes termed "subclinical" disease. By current definitions and methods of testing, it is not uncommon for misclassification to occur in children, or those with immunocompromise or advanced age: for example, the child is not diagnosed with LTBI when the patient is indeed latently infected, or LTBI may be diagnosed when the patient actually has active disease given the notorious difficulty in establishing the diagnosis of pediatric TB.

It seems obvious that LTBI is not a homogenous entity and that its name is misleading. As a starting point, it would be better to drop "latent" in favor of "Mtb infection" – this term leaves residual uncertainty as the infection may be active or inactive. Alternatively, LTBI could be dropped entirely in favor of "positive TST" or "positive IGRA". Beyond the name, we also have to deal with discordance. TST and IGRA identify Mtb immune sensitization in overlapping but not identical populations. Their discordance is not fully understood and is complicated by a voluminous, rapidly evolving and sometimes contradictory literature (PubMed as of 12/2014 contained 1054 references for the Quantiferon assay). Recent reviews provide a general overview of IGRAs [4] and issues related to their reproducibility [5] and potential for occupational screening [6]. Our review is focused on providing an epidemiologic, immunologic and clinical overview that will serve the scientific community a launching pad for future translational studies that will move us beyond semantics to improve LTBI definitions, diagnostics, and most importantly predictors of progression to active TB disease.

3. The natural history of TB

3.1. Grading infectiousness in pulmonary TB

The natural history of TB begins with the exposure of a susceptible host to an infectious case of pulmonary TB (PTB). Historically, infectiousness has correlated with AFB smear positivity, a rough indicator of bacillary load. As compared to PTB cases that are smear-negative, individuals with positive sputum smears are more likely to have cavitary lung lesions with caseous necrosis that allows for extracellular replication, amplifying the bacterial load. They also have frequent and more severe cough, presumably generating more infectious aerosols, the infectious moiety for transmission of Mtb [7]. However, even smear-positive cases with cavitary PTB show great variability in infectiousness and some may be "super-spreaders". In a model of guinea pigs exposed to humans with PTB of varying infectiousness, 8.5% of human cases accounted for 98% of Mtb infections in the animals [8]. It is possible, but unproven that superspreaders have the highest bacterial loads and/or more forceful and frequent coughs. We have shown that cough strength is predictive of high transmission in households of TB cases [9]. Perhaps time to positive culture on liquid medium would allow a more quantitative assessment of bacterial load.

We have taken a different tack to assess infectiousness; the measurement of culturable Mtb in aerosols using an apparatus invented by Dr. Kevin Fennelly called the cough aerosol sampling system (CASS). Aerosols are collected in the CASS apparatus during two separate five-minute periods of coughing and are then cultured in solid media (Middlebrook). Of the untreated PTB cases, 60–65% have culture-positive Mtb aerosols on initial assessment, which decreases to 25%–45% 1–4 days, on average after initiation of TB treatment [10,11] The presence of high grade aerosols (≥10 colony forming units, CFU) in PTB cases was associated with greater risk of transmission of infection to household contacts (HHC), as evidenced by TST conversion, when compared to grading of sputum AFB smear [10]. Discrepancies in infectiousness between sputum smear-positive/aerosol-positive cases and sputum smear-positive/aerosol-negative cases are not well understood, but could relate to both organism-specific (survival in aerosols) and host-specific (cough strength/frequency, viscosity of sputum) factors.

3.2. Host resistance to infection

TB is primarily a pulmonary disease. Infection occurs with deposition of a single droplet nucleus 2–5 um diameter containing 1–3 tubercle bacilli in the terminal bronchioles or alveoli. Experimental data indicate that 10–50 infectious units must be inhaled to reliably establish infection [12]. In humans, the pathologic finding of primary infection is a single small tubercle suggesting that infection is initiated by a single infective droplet [13]. This observation raises the question of whether primary infection leads to a protective response that prevents implantation of additional infectious foci.

Experimental models of Mtb infection have demonstrated that after initial exposure, there is a three day lag before initiation of bacilli replication which occurs for 19–20 days, and then is controlled by the emerging adaptive immune response [14]. The establishment of protective immunity appears to vary considerably within each host and may be influenced by genetics. There is continuing interest in those individuals frequently Mtb-exposed who do not become infected as they may represent "elite-controllers," a term borrowed from HIV infection. Of course are they truly uninfected, is the infection contained by an effective innate immune response without triggering an adaptive immune response or are they incapable of developing a delayed-type hypersensitivity (DTH) reaction? There is a genetic locus *TST1* associated with persons demonstrating persistently negative TSTs despite close and prolonged contact with a PTB case [15]. Genetic factors on 11p14–15 have also been implicated in possible 'resistance' to establishment of Mtb infection; interestingly, this 11p14–15 region overlaps with *TNF1*, which controls Mtb-stimulated TNF production, a key cytokine in establishing the host immune response to Mtb [16]. It is not clear whether the negative TST in such individuals indicates protection against infection as above or inability to develop TST response once infected with Mtb.

3.3. Mtb transmission dynamics and strain fitness

To remain prevalent at steady state on a population level, each TB case must infect 20 contacts. This derives from a model [17] whereby 10% of Mtb-exposed contacts develop active TB (n = 2) and one half of these individuals (n = 1) become highly infectious, thus a ratio of 1:20 (PTB index case: Mtb-exposed contacts) results in perpetuation of TB prevalence. A recent analysis indicates that each TB index case, in fact, infects 2.6–5.9 contacts [18], suggesting assumptions in the simplified model may fail to account for a greater risk of progression to disease in some hosts (or some organism) and/or an increased likelihood of reinfection.

The predominant strain in a discrete geographic area may vary over time and that more virulent strains may replace those less 'fit' [19]. The development of molecular genotyping has led to significant advancement in the study of transmission dynamics, as evidenced by cluster analyses. The term "cluster" denotes a group of TB cases caused by the same microorganism as evaluated by restriction fragment length polymorphism (RFLP) analysis or spoligotyping. The distribution and size of Mtb clusters reflects the pattern and extent of recent Mtb transmission within a specified community, and has implications for specific strain virulence in terms of propensity to establish infection after Mtb exposure, and to cause progression from infection to disease [20].

3.4. Divergent outcomes of Mtb infection

Once a host is exposed to Mtb and primary infection is established, LTBI is diagnosed. Host factors clearly influence the outcome of LTBI - most significantly, host age, immune status, and interaction dynamics with the index case including infectiousness and nature of exposure.

The sole evidence of Mtb infection may be a TST or IGRA conversion, although local inflammation detected radiographically or pathologically 5–7 weeks after exposure is also possible [21]. In most cases, primary infection is associated with transient and mild symptoms that do not prompt an individual to seek medical attention. The primary lung lesion is usually solitaire and together with the adjoining enlarged bronchopulmonary lymph nodes is referred to as the Ghon complex. Reviewing post-mortem TB cases, the caseous necrosis in adjacent lymph nodes is more advanced than in the lung, however, lymphadenitis often is not apparent clinically. This issue is being revisited with modern imaging such as positron emission tomography-computed tomography (PET-CT) scans. The extent, metabolic activity and nature (lymph node versus parenchymal involvement) of the infectious focus may influence the likelihood of progression from LTBI to active disease. Most cases of primary infection are self-limited. Following primary TB, there may, however, be evidence of dissemination to common sites of delayed reactivation. For example, calcified apical foci known as "Simon foci" are found in 3% of children that heal primary TB [22].

Individuals progressing from new Mtb infection to disease within 1–2 years presumably evolve through a phase of percolating infection with active bacterial replication before developing obvious symptomatology and infectiousness. From a clinical and programmatic standpoint, these individuals are problematic because the bacterial load is sufficiently high that it may contain resistant organisms and in that case, preventive therapy with a single drug may promote the emergence of drug resistance. Further, if they are HIV-infected with subclinical TB, the initiation of antiretroviral therapy (ART) can lead to "unmasking TB," a form of immune reconstitution inflammatory syndrome (IRIS). In this case, recovery of a previously suppressed immune system results in a robust and even excessive inflammatory response to previously acquired organisms, with a high case-fatality rate [23].

3.4.1. Age and TB susceptibility

Age has a profound influence on the outcome of primary infection, including disease progression and severity. As stated by Vynnycky and Fine [24] "the risk of infection is the single most important factor affecting the magnitude of the tuberculous morbidity in the population as it determines both the age pattern of initial infection (and hence the risk of developing disease) and the risk of reinfection." Infants and children have immature immune systems and are unable to adequately control initial Mtb infection. There may be asymptomatic shedding of organisms as well as chest radiograph abnormalities. Of >700 newly Mtb-infected children, 7.7–15% had Mtb in sputum cultures for at least 6 months even in the absence of symptoms and 70% had intra-thoracic adenopathy on chest radiographs [25]. Even when the chest radiograph is normal, sputum, gastric aspirate or urine samples may be culture-positive, and CT scan or MRI often demonstrates lymphadenopathy. Some or most children would resolve primary infection without treatment. Nonetheless, when "TB" is identified radiographically or bacteriologically, multi-drug treatment is started. This is appropriate because of the risk of progression to life-threatening TB.

It is of interest that young children are more likely to manifest a primary TB lesion and develop chest radiographic abnormalities [26]. Further, the risk of severe extra-pulmonary disease is extremely high particularly in the first year of life [27]. In a large controlled trial of BCG vaccination in Puerto Rico, over 82,000 TST reactors were followed for 18–20 years [28]. The highest incidence of TB was in 1–4 year olds, over twice that of older children. A meta-analysis of contact investigations indicated that children <5years of age were at high risk of developing TB [29]. A somewhat different age-related trend is seen in progressive pulmonary TB. The frequency of progressive PTB in TST convertors manifest by upper lobe cavitation is highest in those who are 15–24 years [26]. An observational study of 400,000 persons noted relatively low disease incidence in TST-positive individuals aged 6–10 years, however, there was a rapid rise of TB incidence in the adolescent period (10–19 years old).

The varying susceptibility of the 1–6 year olds to progress to extra-pulmonary disease and adolescents to progress to PTB may be related to immune system immaturity and hormonal modulation, respectively. Across age groups, however, recent infection is the key risk factor for immediate development of TB. In one study [26], 13.2% of infected patients develop cavitary disease in the first year after infection, 7.5% in the second year, and only 1% in the following 2–7 years. Based on such data, the lifetime risk is of development of disease is low in those with "stable" LTBI for more than 5 years [30].

3.4.2. Intense exposure begets disease

Emerging literature suggests that higher intensity of exposure to infectious cases of TB is associated not only with greater risk of Mtb infection but also with increased occurrence of TB. Data derived from HHC studies, high-risk occupational cohorts, and an epidemic in a closed environment all indicate that the infectious inoculum is a key determinant of whether primary TB infection progresses to active disease. A classic study by Grzybowski et al. in Canada found higher rates of secondary TB disease among contacts exposed to sputum smear-positive cases, compared to smear-negative cases and community controls [31]. In children exposed to a HHC with known sputum smear- and culture-positive PTB, 66% tested TST-positive and 35.6% developed primary TB [32]. For those exposed to smear-negative/culture-positive cases, 25.7% tested TST-positive and 5.7% progressed to disease. In a study conducted in a rural community in South India, the adjusted hazard ratio for TB disease was 3.4 for HHCs of smear-positive cases and 1.7 for smear-negative cases with a referent control of non-contacts [33]. A similar frequency of LTBI was seen in contacts of smear-negative and smear-positive TB, however, the frequency of TB disease in HHC of smear-negative cases was only

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0.19 that of smear-positive cases [34]. These findings suggest that the risk of infection progressing to disease is disproportionately high in those exposed to smear-positive cases and cannot be wholly explained by differences in rates of infection.

A particularly revealing TB epidemic occurred on a naval vessel [35]. 308 crew members were exposed to the index case. Of the cohort, 45% developed Mtb infection and 5% developed active disease. For crew members sharing the same berthing compartment, 71% developed Mtb infection and 12.8% disease, compared to those in a different berthing compartment with shared ventilation, which resulted in 53% developing Mtb infection and 2.3% disease. This outbreak demonstrated that high intensity exposure to the source case resulted in a modest increase in infection yet a marked increase in disease, and that this increased risk of disease required direct or close contact. As another example, medical and nursing students are at high occupational risk because they are potentially exposed to multiple infectious TB cases depending on the local disease prevalence. In the pre-chemoprophylaxis era, 14–31% developed TB within 2.5 years of follow-up [36,37]. This reflects an inordinately large risk of developing TB, given that they most likely became infected in the course of training, so 2–6 years previously.

Host factors such as immunocompetence also significantly impact the risk of progression from primary infection to disease. For example, 40% of HIV-infected persons exposed to a PTB case will develop disease and do so within an accelerated time-frame, that is within 120 days [38]. This accelerated natural history leads to many new infectious TB cases perpetuating the epidemic. Similarly, TNF inhibitor therapy increases the risk that primary TB will progress to active disease [39].

3.5. The shifting balance between reactivation TB versus reinfection TB

In low prevalence countries, the dogma has been that delayed reactivation occurs more frequently than progressive primary infection; in part, this may be explained by highly prevalent LTBI in the elderly, as they were infected in the distant past when TB was more common and now comprise a large reservoir for reactivation. Data from the Netherlands indicate, however, that the median interval between primary and secondary cases attributed to reactivation (confirmed by IS6110 RFLP analysis to be the same strain) was 1.26–1.44 years after Mtb infection, with 85% of cases occurring during the first five years and 95% during the first 10 years [40]. This study highlights the relative predominance of progressive primary TB in a country with low and declining disease prevalence. In evaluating immigrant populations that travel from high-to-low prevalence countries, numerous studies have confirmed that the development of TB is uncommon (0.8%) more than 15 years after initial Mtb exposure [41]. Additionally, modeling based on the rapid decrease in TB incidence upon immigration from a high prevalence setting to the United States has provided strong evidence that what classically was considered "reactivation TB" may rather represent re-exposure and reinfection [42]. It is not possible to distinguish reactivation from reinfection disease clinically, therefore, given these data, it is likely that reactivation TB is less common than previously thought, and reinfection disease is likely a dominant mechanism for the development of active TB in high prevalence settings.

3.5.1. How often do latent Mtb organisms persist in LTBI?

In principle, reactivation TB requires that viable organisms persist for years or decades. How often do viable Mtb persist in LTBI, that is, with a chronic stable positive TST or IGRA? In a high prevalence setting, 8.5% of HIV-infected individuals with LTBI upon active screening with sputum culture had prevalent asymptomatic TB defined by positive cultures [43]. Recent data indicate that even at high CD4 counts (≥350/dl) a significant proportion of HIV-infected have lesions on PET-CT scan highly suggestive of active TB. TB in HIV may reactivate at a relatively high CD4 count reflecting that it is a virulent organism and that active immunity is necessary to prevent activation of a latent focus. The risk of TB in HIV-infected individuals is usually stated as 5–10% per year, however, the determinants driving the timing of reactivation are not known. Further, in large trials of preventive therapy the risk of TB was 3.4% over two years of follow-up [44]. In TST-positive persons who decline preventive therapy and are treated with TNF-alpha inhibitors, 5–10% develop TB [45]. Similarly, among the HIV-infected, those who are TST-positive and are not treated with preventive therapy, TB develops in 10–12%, although these data are confounded by administration of ART [46,47]. Estimates for the overall risk of TB in TST-positive HIV-infected in the absence of ART is about 40%, although the proportion of these individuals that reactivate relative to those with progressive primary infection or reinfection is not clear. Taken together, these limited observations suggest that "self-cure" is common and only the minority of those with LTBI are at risk of reactivation TB.

Modeling also provides insights into the risk of reactivation TB [48]. The lifetime risk of reactivation TB is 10–20% in children five years of age or younger (TST reaction ≥10 mm), in recent TST converters, in persons younger than 35 years of age treated with infliximab (TNF inhibitor) therapy (TST ≥ 15 mm), and with HIV infection or old healed TB on chest radiographs (TST ≥ 10 mm).

There are well-described clinical risk factors outside of age that may hasten diminished integrity, or "breakdown", of an established latent Mtb focus. Factors that confer the highest risk are HIV infection, solid organ transplantation, immunosuppressive therapies (TNF-inhibitors, prednisone ≥15 mg/day daily dose for ≥ one month), and presence of fibrotic lesions on chest radiograph consistent with prior TB. Factors conferring slightly less risk are malnutrition (≥15% weight change, defining moderate protein-energy malnutrition), silicosis, diabetes mellitus (particularly insulin-dependent or poorly controlled cases), renal failure, hemodialysis, gastrectomy, jejuno-ileal bypass, carcinoma of the head and neck, lung cancer, lymphoma, and leukemia. The presence of these additional risk factors may be just the force needed to cross the threshold from controlled Mtb infection to the development of primary and/or reactivation TB.

3.5.2. The special case of untreated prior TB

The mechanism of development of LTBI is different in those with fibrous apical scarring on chest radiography. In LTBI following exposure to an infectious TB patient, there is immunologic control of primary infection. In cases with apical scarring, there has been immune control of active disease. The residual bacterial burden may be higher, both in terms of the proportion of individuals with viable bacteria and the burden of Mtb per individual. Consistent with this possibility, 70% of sporadic cases of TB have pre-existing apical scarring [49,50]. Recently, in a group of individuals that had self-healed TB (median of 58 years since recovery from TB), 15 out of 19 had evidence of ongoing bacterial replication (circulating T effector memory cells), exceeding other estimates of the fraction of LTBI patients with viable organisms [51]. Presumably the number of viable organisms and bacterial replication is sufficient to re-prime the immune response but insufficient to cause disease. We previously had shown evidence of a pro-inflammatory factor in the plasma of individuals that had been treated for TB [52].

3.5.3. The role of genetics in TB risk

The genetic predisposition to TB based on defects in IFN-γ and IL-12 pathways is well-established. Recent studies have highlighted the role of other genetic and acquired factors in modifying risk of

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disease. Innate immune mechanisms are affected by toll-like receptor (TLR) polymorphisms that regulate DTH and cytokine response to Mtb, modifying the risk for TB disease [53,54]. Leukotriene A4 hydrolase (LTA4H) regulates inflammation by affecting the balance between leukotriene B4 associated with increased TNF-alpha and lipoxin A, which inhibits TNF production [55]. Both low and high levels of LTA4H are associated with TB disease. Polymorphisms in the promoter modulate the inflammatory response and outcome of TB meningitis as well as the response of TB meningitis to adjunctive anti-inflammatory therapy with dexamethasone [56,57]. Plasma levels of lipoxin and prostaglandin E2 are associated with TB susceptibility by modulating the relationship between IL-1 and Type I interferons [58]. Seasonal fluxes in Vitamin D levels [59] and viral infections that modulate interferon type alpha/beta responses [60,61] also have been cited as predisposing to reactivation TB.

3.5.4. When LTBI is protective against exogenous reinfection and when it is not

Reinfection with Mtb is important to the natural history of the disease, especially in endemic areas. Whereas the possibility of reinfection indicates that prior LTBI or active disease is not uniformly protective against exogenous reinfection, there appears to be considerable immunologic protection garnered by prior Mtb exposure. One meta-analysis indicated that TST-positive individuals demonstrate 79% protection against reinfection upon re-exposure compared to those without LTBI [62]. This observation may be geographically dependent, as a HHC study in Peru demonstrated that household contacts of index TB cases had 35% reduction in the risk of disease [63], whereas national database from England/Wales suggest 16–41% protection [64]. Notably, in approximately one third of secondary TB cases that occur in the household of an index case, the Mtb strain differs from the strain isolated from the source case [65]. This finding raises the possibility that re-exposure to Mtb may reactivate a prior latent focus of infection or in highly endemic areas there may be mixed infection.

Also of interest, PTB patients that have been treated for active TB show increased risk of developing re-infection disease [66]; in one study from South Africa, a high prevalence country, 51% of recurrent TB cases were due to re-infection. Treated TB cases may be at greater risk of progression from re-infection to disease because of underlying host susceptibility that may be acquired as a consequence of prior TB [67]. An alternate possibility is that they return to an environment where re-exposure is likely. Nonetheless, host predisposition must be a factor in progression from infection to disease.

4. Diagnosis of LTBI

There are currently two accepted methods for screening Mtb-exposed individuals for LTBI: TST and IGRA. The former was developed more than a century ago and is cheaper and more widely used; the latter was adopted into clinical practice more than a decade ago and while global distribution and implementation are limited by its higher cost, extensive use in the developed world has revealed many operational limitations that further hampers its utility.

4.1. Shortcomings of IGRA testing

Extensive use of IGRA for LTBI diagnosis has highlighted several issues with the interpretation of results, including significant within-subject variability [68–73] and unusual measurements, such as high IFN-γ in the control unstimulated blood [74]. Serial testing of healthcare workers with IGRA in low risk settings (U.S. and Canada) has revealed unusually high IGRA conversion rates (4–7%) when compared to historical or concurrent TST conversions rates (0.0–0.9%) [75–77]; importantly, 60–75% of individuals with IGRA conversion in these studies reverted to IGRA negative upon repeat testing. Unusually high rates of IGRA conversions and reversions have also been reported in high TB prevalence settings [78–80]. In our recent study in Uganda, 23% of contacts with TST-/IGRA+ discordance at baseline reverted to concordant negative upon further testing (unpublished data). Taken together, these data are reminiscent of the known instability of TST results across populations with variable risk for Mtb infection [81–84]. In their latest comprehensive review, Pai et al. identified several sources of variability (manufacturing, pre-analytical, analytical and immunological) that may impact the reproducibility of IGRA results [4]. Also, recent studies from Uganda, South Africa and Brazil [80,85] indicate that a delay in IGRA conversion, when compared to TST, should be added to the multitude of considerations when interpreting discordant TST/IGRA results in recently exposed contacts.

In a HHC study conducted in Brazil, we found that IGRAs were less sensitive in detecting new or recent infection with Mtb, a particularly high-risk group for progression to disease [85]. IGRA was performed in 357 HHC of smear-positive PTB cases, and were positive in 82% of those with a positive TST, 48% of those that underwent TST conversion and 12% of those with a negative TST. The discordance may, in part, be due to delayed conversion of IGRAs relative to TST as suggested by other recent findings such as a TB outbreak in a military camp in South Korea where PTB contacts were systematically tested with QuantiFERON®-TB Gold in-tube (QFT-GIT) at various time points after the start of the investigation. QFT-GIT conversions were observed between 4 and 22 weeks, suggesting that IGRAs may have a broad and at times lengthy window period for conversion [86]. During a contact investigation in Spain, agreement between TST and QFT-GIT, and correlation with intensity of Mtb exposure, improved after the tuberculin window period which is generally accepted as two months [87]. Collectively, these data suggest that IGRAs have a specific window period for conversion that may be delayed compared to TST. Current CDC guidelines for HHC investigations recommend that IGRA testing (if used for initial assessment) be repeated 8–10 weeks after baseline testing in those HHC who test negative [88], however, testing then may lead to results that are difficult to interpret if the window period is indeed prolonged.

4.2. TST and IGRA discordance-relationship to exposure

Despite the CDC recommendation and acknowledgment that TST and IGRAs identify overlapping but not identical populations, discordance may create uncertainty regarding the diagnosis of LTBI. In cases where TST and IGRA are in disagreement, subjects are usually considered Mtb-infected unless there is strong suspicion of a false positive (as in repeated BCG-vaccination or NTM sensitization likely affecting TST reactivity). Age and degree of recent exposure to TB might influence test agreement since discordant results are more likely to occur in persons of younger age [89] and less exposure [87].

The absence of a gold standard for Mtb infection makes the interpretation of tests to detect LTBI dependent on the pre-test probability of infection. Knowledge of exposure, therefore, is critical in assessing individuals for LTBI. Measuring Mtb viability and burden directly in aerosols allows for comparisons between sputum and aerosols, adding a further dimension to quantifying exposure. In a recent HHC study conducted in Uganda (Jones Lopez et al., submitted), we found that sputum AFB grading progresses linearly as PTB severity advances over time. In contrast, aerosol colony counts (CFU of Mtb), as measured by CASS, are unrelated to markers of TB disease severity or sputum bacilli load and thus could represent a phenotype of the TB case (or the organism) that is

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stable over the natural history of TB disease. With aerosols, the infection hazard follows a standard dose-response curve, with increasing risk of infection paralleling aerosol CFU number.

The availability of aerosols also informed transmission dynamics within the household. The overall prevalence of LTBI at baseline in HHC as defined by positive TST (75%) and IGRA (73%) was similar, and did not significantly differ between exposure groups (sputum AFB vs. aerosol CFU). Discordance between TST and IGRA was 18% and decreased to 8% at 6 weeks. At 6 weeks, there was a significant dose-response association between aerosol CFU and TST conversion ($p = 0.04$) or IGRA ($p = 0.01$) conversion from negative to positive. By comparison, the same analysis using sputum AFB smear grade to classify exposure did not show a clear or consistent risk stratification. Importantly, discordance of TST and IGRA results in HHC was associated with several markers of less intense exposure, such as less crowding and non-cavitary disease in the source case. This is similar to our published data in Brazil where discordance was more likely with less exposure of the HHC to the index case and was more frequent in TST convertors [85]. These data suggest that less intense exposure may lead to delayed TST and IGRA conversion and increased TST-IGRA discordance – the latter due to longer window period for IGRA conversion. BCG vaccination also was associated with discordance in TST converters (TST+/IGRA−). As this was stable across age groups (<10years vs. ≥10years) the data suggest that BCG may have delayed or blocked IGRA conversion. It is also controversial whether increasing TST induration correlates with IGRA positivity and/or level of exposure [90–93], or if discordance between results could be improved by adjusting IGRA cut-off values.

4.3. Assessing risk of progression to TB

A positive TST or IGRA result cannot distinguish contacts with LTBI from those with prevalent or incident active disease [94–96]. In fact, we have shown that PTB is associated with suppression of the IFN-γ response to Mtb antigens, although this result is subject to variation based on the extent of TB and may be location and assay dependent. Both the TST reaction size and the magnitude of IGRA readout correlate with gradient of exposure and Mtb antigen load in the source case [97–99]. Most notably, it is well established that TST size predicts risk of progression to disease [28,100], and there is a growing body of evidence showing quantitative IGRA readouts are associated with increased risk of disease [94,96,101,102]. In a recent review [96], Rangaka et al. showed that individuals who are dually positive (compared to individuals who are either TST-positive or IGRA-positive) are at greater risk of progression to disease. There are, however, inconsistencies in the literature. In an adolescent cohort from a high transmission setting (South Africa), the risk of progressing to TB was 0.60/100pyo for TST positives comparable to 0.64/100pyo for IGRA positives [103]. Contrarily, a HHC study in Hamburg reported that 4.8% of TST positives compared to 12.9% of IGRA positives developed TB [104]. In our studies from Uganda, contacts exposed to patients producing aerosols with high bacterial burden (≥10 CFU/ml) also may be at high risk of progression to disease as indicated by high rates of concomitant TST and IGRA conversion, larger TST diameters and higher IGRA readouts compared to other contacts. These findings are consistent with the key role of inoculum size in determining immunopathology and survival in experimental conditions [105–107].

An immediate question is whether TST convertors are at different risk of progressing to disease compared to other HHC that are TST-positive. In children, the risk is similar [108]. In the case of children in HHC settings, the duration of LTBI in the TST-positives is unknown but may presumably be recent – particularly in low prevalence areas. This may mitigate any additional risk of TST conversion as a proxy for new infection. Arguably the risk may be different in low prevalence settings. TST conversion also is associated with TST and IGRA discordance and therefore less intense exposure and potentially smaller infectious inoculum. Separately and together the results suggest that TST convertors and TST/IGRA discordance may be at lower risk of progression to TB.

In a study conducted in Uganda involving the provision of INH prevention therapy (IPT), 20.5% of TST convertors treated with IPT for nine months reverted to TST-negative three months after therapy completion [109]. In comparison to the stable convertors, conversion in this group that reverted occurred at a later time interval, the TST induration size was smaller and there was no increment in IFN-γ production at the time of conversion. This observation suggests that individuals who experience regression of TST positivity after completion of IPT may have been exposed to a smaller infectious inoculum than those who remained TST positive. In the absence of IPT, TST reactivity is a stable host phenotype. We found that 19 of 22 persons with a positive TST remained TST positive with strong *in vitro* blastogenic responses to PPD after 19 years [110]. There was, in fact, an increase in the mean size of the TST reaction from 19 mm to 32 mm. This finding strongly suggests ongoing or repeated boosting through bacterial replication.

Application of a TST may lead to transient boosting of a subsequent IGRA test [111,112]. The current consensus is that tuberculin-induced boosting of IFN-γ production is rare [113] and first noted three days after the TST [113–115]. Most cases of post-TST IGRA boosting recorded a quantitative increment in response insufficient to cause conversion to positivity, and therefore unlikely to affect decisions regarding provision of preventive therapy to those diagnosed with LTBI. The boosting effect diminishes after the first month, and is considered insignificant after three months [113,114,116].

5. Biomarkers to stratify risk in LTBI

Modeling indicates that achievement of the WHO goal for TB Elimination (<1 case/million) by the year 2050 will require a 2-month treatment regimen and mass treatment of LTBI [117]. One third of the world's population has LTBI and the risk of progressing to TB is heterogeneous. It is not plausible or practical to provide mass treatment for LTBI given that at most 5–10% are at risk of disease. An effective public health intervention, therefore, will require new biomarkers to identify those with LTBI at highest risk of progression to TB for targeting preventive therapy. Predictive biomarkers are most needed for HHC of patients with infectious PTB, as they have the higher than population risk of developing active disease and, therefore, are an important focus for TB prevention programs.

TST and IGRAs are weak predictors of risk of progression. In the adolescent cohort in South Africa, the positive predictive value of a positive TST was 1.4% and a positive IGRA 1.5% [103]. As indicated it is possible that magnitude and concordance of positive TST and IGRA (particularly if coupled with aerosol CFU, a surrogate for inhaled dose) could be a marker of risk of TB but this is unproven.

Several approaches are promising and will be discussed in turn:

5.1. Whole blood transcriptomics

Studies carried out in TB endemic areas have exploited microarray technology to obtain gene signatures that differentiate between the different clinical states of Mtb infection. *Ex-vivo* whole blood gene expression profiling separated treated PTB cases at risk of recurrent TB from cured subjects and from those with LTBI [118]. Using transcriptional profiles, a distinction could also be made between active TB, LTBI and uninfected individuals [119]. A study by Berry et al. performing whole blood gene expression profiling

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reported that a 86-transcript Type I-interferon inducible signature was specific for active TB; the fidelity of the signature was associated with the radiographic extent of PTB and was extinguished following treatment [120]. The finding that about 10% of those with LTBI had signatures that resembled that of active TB was tantalizing. Although the TB biosignature overlaps with other inflammatory conditions notably sarcoidosis, it contains distinctive genes related to higher metabolic activity and anti-microbial response-related genes; other genes such as matrix metallopeptidase 14 were specific to sarcoidosis [121]. The robustness of the association of Type-1 interferon inducible signature with active TB has been corroborated by studies conducted with patients from different genetic backgrounds [122–124]. These studies demonstrate that whole blood transcriptomics has the potential to provide biomarkers to identify those with LTBI at highest risk of TB. In fact, existing biorepositories of serial samples collected from HHC of TB cases may allow definitive evaluation of the approach. Whereas transcriptomic signatures of unstimulated whole blood or PBMC may be of value in patients with "percolating" LTBI in the process of progressing from Mtb infection to disease or with asymptomatic TB, a different approach will be necessary to address risk of (re) activation in LTBI in which replication rate is slow [125].

5.2. Multiparametric flow cytometry to evaluate proportion of T central and T effector memory subsets and multifunctional T cells

Based on differential expression of CD45Ra, CCR7, CD42L, CD27 and IL-7αR, T cells can be separated by flow cytometric analysis into T effector memory (TEM), T central memory (TCM), T effector (TE) and naïve T cells [126]. Typically in response to infection, naïve T cells differentiate into TE cells and subsequently TEM and TCM cells develop. As infection is cleared from the host, the TCM compartment expands and stabilizes over time [127,128]. TEMs are found in the lung and rapidly differentiate to TE on re-exposure to the infectious agent or antigens. TCMs populate lymph nodes and spleen. They show a delayed response on re-exposure. The ratio of TEM to TCM can serve as a biomarker of the presence of infection as suggested by studies that indicate that antigen clearance is associated with higher TCM [129–132]. Studies have analyzed the presence of both TCM and TEM in PBMC of individuals with LTBI [133–136] and some have found a similar TEM association with antigen presence in the host. For example, bi-functional IFN-γ^+TNF-α^+CD4$^+$ T cells and TE phenotype were associated with active TB and not LTBI whereas the response to RD1 stimulation in LTBI and "cured TB" was characterized by a TCM phenotype [137]. In HIV infection, similarly, a poly-functional CD4 response with a high proportion of IFN-γ^+TNF-α^+ cells and effector-memory phenotype was seen in TB cases but not LTBI [138]. Evaluation of T cell responses to ESAT-6 and CFP-10 in 28 individuals with self-cured TB from the pre-antibiotic era revealed two patterns of memory T cell response [51]. 15 of 19 individuals had a profile characteristic of TEM cells whereas 7 of 10 that showed no IFN-γ response had a TCM profile. A phenotypically distinct subset of memory T cells also differentiated latently-infected individuals from clinically cured TB patients [134]. In these studies, the Mtb exposure history or duration of the latent status of LTBI was not known. Nonetheless, the findings indicate that memory T cells have a predominantly TCM profile in individuals with no replicating bacteria whereas TEM cells are likely present when there is replicating bacteria. A recent study identified a panel of markers on CD4$^+$ T cells that could discriminate between active and cured TB [139]. Whether such a panel will be sensitive to differentiate "cured" LTBI from those who are still infected remains to be determined. Another study found that following therapy, there was a decrease in the multifunctional T cells in TB patients and a concomitant increase in single and double cytokine producers [140] suggesting that a multifunctional T cell profile is associated with the presence of live Mtb rather than operating as a marker of protection. Together, these findings indicate that multiparametric flow analysis of T cells in a large cohort of persons with LTBI has the potential to distinguish those with replicating bacteria from those who have naturally cured their infection. This will allow targeting of those with LTBI with replicating bacteria for preventive therapy.

5.3. Nonspecific biomarkers

Host markers of activation of immune and inflammatory pathways may be useful in predicting risk of progression of LTBI. There are a group of serologic biomarkers that are increased in TB compared to those with LTBI and decrease during effective therapy. As a group, these would be amenable to lateral flow modifications and could be rapid point-of-care dipstick assays. CRP is an acute phase reactant synthesized by the liver in response to inflammation. A number of assays are available to measure this because of the use of CRP in cardiovascular disease. In a recent study, rapid CRP >8 mg/dl had a sensitivity of 95% and specificity of 51% for the diagnosis of TB in symptomatic HIV-infected persons with negative sputum smears [141]. Neopterin is produced by activated macrophages and dendritic cells. We and others have shown extremely high levels of neopterin in individuals with HIV-TB coinfection [142]. Persistently high levels after TB treatment suggest risk of relapse or HIV progression [143,144]. IP-10 is a marker of inflammation and potent downstream effector of Th1 and innate immunity and suPAR is a constitutively expressed on immune cells and increased with inflammation. Both IP-10 and suPAR are expressed at high levels in the serum of TB patients and decline rapidly with treatment [144–146]. Pentraxin (PTX)-3 is an acute phase reactant that is increased in TB and declines with therapy. It may be of particular interest in unmasked TB because it was elevated in five contacts of TB cases that progressed to active TB [145]. sICAM-1 is expressed on vascular endothelial cells and is essential for attraction and local expression of immune cells during inflammation. It is increased in TB patients and may be somewhat specific as levels were higher than in lung cancer and pneumonia [147,148]. Osteopontin is produced in early stages of T cell and macrophage activation and correlates with extent of PTB [149].

There are other assays that distinguish active TB from LTBI and have potential application to stratifying risk of progression. Several have been proposed for the identification of recent infection. We have shown [150] that IL-10 and TGF-β in supernatants of Mtb-stimulated PBMC are found at higher levels in TB patients when compared to healthy subjects with LTBI. Other promising approaches include proteomics [151], metabolomics [152], and serodiagnosis [153].

FDG-PET/CT scanning is a novel imaging technique that has the potential to identify persons with both active pulmonary inflammation. The positron-emitting tracer, ^{18}F-fluoro-2-deoxy-glucose (^{18}F-FDG, a labeled glucose analog) is administered and measured by PET/CT technology. Uptake of FDG reflects cell glycolysis and is found in activated neutrophils, macrophages, and lymphocytes that are prominent in TB and other granulomatous inflammatory processes. This surrogate for inflammatory response has been quantified and used to distinguish between active and inactive disease and response to therapy [154–156]. Interestingly, two studies, both in TB endemic countries, reported that a small percentage of "normal" subjects who underwent whole-body FDG-PET scanning demonstrated increased FDG uptake in mediastinal lymph nodes [157,158]. Unfortunately, neither TST nor IGRA was performed on these subjects to determine if they were latently infected with Mtb. A recent FDG-PET/CT study in LTBI, albeit on only 5 subjects, reported increased cellular activity in thoracic lymph nodes in four of the five

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subjects and also found a strong correlation between cellular activity observed in the lymph nodes and IGRA results [159]. Uptake in the mediastinal and hilar lymph nodes regressed during IPT. Although FDG-PET/CT shows inflammation and not bacterial foci *per se*, this imaging approach may well accelerate biomarker discovery. PET-CT may provide an intermediate endpoint to evaluate biomarkers that stratify risk of progression from LTBI to disease.

6. Is LTBI vaccine preventable?

Most effective vaccines prevent infection rather than disease. Two observational studies suggest that BCG vaccination is effective in prevention of Mtb infection: (1) a HHC study in Uganda demonstrated that the presence of a BCG scar was associated with an odds ratio of 0.57 for TST conversion (95% CI: 0.34–0.98) [160] and similarly (2) in Turkey, BCG scarring was associated with an odds ratio of 0.60 (95% CI: 0.43–0.83) for Mtb infection (ELISPOT). The similarity in magnitude of effect is striking, as is the fact that protection is afforded long after perinatal vaccination with BCG.

A pre-exposure vaccine theoretically would prevent establishment of LTBI and could serve as a short-track for characterization of immunologic correlates of protection crucial for interfering with the evolution of infection and possible development of disease. Alternatively, a post-exposure vaccine would theoretically prevent progression from LTBI to disease. A number of vaccine candidates are in the developmental pipeline although screening in animal models does not shed light on their activity to prevent Mtb infection.

One such therapeutic vaccine currently undergoing phase II trials is RUTI, made from a semi-purified preparation of virulent Mtb, it is grown in stressful conditions, fragmented, detoxified, heat inactivated and introduced as a liposomal preparation. The conditions in which the bacteria are grown induce expression of relevant stress or dormancy antigens important for targeting the immunopathogenecity of LTBI. RUTI induces a strong humoral and cellular immune response against antigens of both active and latent bacilli, but also against structural antigens. RUTI is administered after one month of chemotherapy for LTBI, at which point it is theorized to facilitate immune clearance of persisting bacilli; further, it may potentially prevent the establishment of LTBI. It is of interest that heat-killed *Mycobacterium vaccae* prevented the development of TB in BCG-immunized HIV-infected persons [161].

A leading vaccine candidate to prevent LTBI is Hybrid 56/Aeras-456 (H56), a multi-stage subunit vaccine originally reported to contain not only antigens Ag85B and ESAT-6 but also Rv2660c, an antigen selectively expressed during periods of bacillary starvation and stress. A recent study has shown the transcriptional signal ascribed to Rv2660c to be associated with an sRNA encoded on the opposite strand of the DNA rather than the mRNA [162]. The existence of Rv2660c transcripts or protein derivatives in Mtb isolates remains controversial, and the true mechanism of action of the H56 vaccine is therefore not yet fully understood. However, when administered to cynomolgus macaques with adjuvant IC31 as a booster to BCG, this vaccine reduced clinical disease after challenge with Mtb and prevented reactivation of latent foci [163]. The more recent ID93 includes a fusion protein with four different antigens. Similar to H56, ID93 also expresses stress-induced antigens and is intended for post-exposure vaccination although it could prevent infection [164].

LTBI itself serves as a model for vaccine development. Mimicking and modifying the mechanisms that successfully control and maintain Mtb in its dormant stage could allow for a vaccine capable of containing infection and avoiding progression to disease. As previously described, this mostly consists of adaptive pro-inflammatory Th1 immune response mechanisms. The adaptive immune response does not, however, necessarily achieve a cure as sterile eradication of bacteria is not achieved. For a vaccine to achieve cure after infection, it would induce not the mechanisms seen in those individuals with LTBI that succeed in orchestrating "self-cure".

7. Conclusions

Table 1
Conclusions.

	What we think we know	What remains unknown
Infectiousness of the index case	The infectious inoculum is a key determinant of the subsequent course of Mtb infection. The contact exposed to intense exposure is more likely to progress to TB.	Can infectiousness be resolved into density of Mtb in sputum/aerosols, cough strength and frequency?
	Cough aerosols may be a better index of infectiousness of the index case than sputum smear for AFB.	Does Mtb strain or sputum quality play a role in infectiousness?
Development of latent tuberculosis infection	80% of heavily exposed HHC develop LTBI.	How does re-exposure to Mtb affect the original latent focus?
	LTBI is a continuum between asymptomatic TB and self-cure.	What determines whether LTBI confers protection against re-infection or susceptibility to re-infection disease?
	LTBI therefore is a misnomer - more appropriate would be Mtb Infection or "TST-positive" or "IGRA- positive".	Is immunity specific and strain dependent?
	Most individuals with LTBI contain or eradicate the latent focus and do not develop TB even when immunosuppressed.	Can multiple Mtb strains be latent in the same host?
	Progression of primary infection accounts for most TB cases.	
	Reinfection may be more common and reactivation less common than previously appreciated.	
Limitations of the currently available diagnostic methods	Discordance of TST and IGRA may reflect a smaller infectious inoculum, delayed TST conversion, or longer window period for IGRA conversion.	Is discordance a marker for lower risk of TB?
	Neither positive TST nor positive IGRA are sensitive indicators of risk of progression to TB.	Does strongly positive TST and IGRA indicate greater intensity of exposure and higher risk of progression ?
	TST still is preferred to IGRAs for annual screening because of problems in reproducibility and stability of IGRAs particularly when values are close to the cutoff point.	
Biomarkers for risk stratification	Biomarkers that stratify risk of progression are urgently needed for public health interventions.	What are the biomarkers that most accurately predict risk of progression from latent infection to active tuberculosis disease?
	Biomarkers that define Mtb infection as new or recent would be enormously useful for targeting interventions because of the great risk of disease in the first year.	Can biomarkers determine the timing of Mtb infection?

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