Latent tuberculosis diagnostics: current scenario and review

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Abstract

This review presents a comprehensive examination of the contemporary landscape pertaining to latent tuberculosis infection (LTBI) diagnostics, with a particular emphasis on the global ramifications and the intricacies surrounding LTBI diagnosis and treatment. It accentuates the imperative of bolstering diagnostic, preventive, and treatment modalities for tuberculosis (TB) to fulfill the ambitious targets set forth by the World Health Organization aimed at reducing TB-related mortalities and the incidence of new TB cases. The document underscores the significance of addressing LTBI as a means of averting the progression to active TB, particularly in regions burdened with high TB prevalence, such as India. An in-depth analysis of the spectrum delineating latent and active TB disease is provided, elucidating the risk factors predisposing individuals with LTBI to progress towards active TB, including compromised immune functionality, concurrent HIV infection, and other immunosuppressive states. Furthermore, the challenges associated with LTBI diagnosis are elucidated, encompassing the absence of a definitive diagnostic assay, and the merits and demerits of tuberculin skin testing (TST) and interferon-γ release assays (IGRAs) are expounded upon. The document underscores the necessity of confronting these challenges and furnishes a meticulous examination of the advantages and limitations of TST and IGRAs, along with the intricacies involved in interpreting their outcomes across diverse demographics and settings. Additionally, attention is drawn towards the heritability of the interferon-γ response to mycobacterial antigens and the potential utility of antibodies in LTBI diagnosis.

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Introduction

Tuberculosis (TB) is a major public health problem worldwide. As per the Global TB Report 2023, globally, 10.6 million people developed TB disease, and about a quarter of the world's TB cases were reported from India in 2021 [1]. The World Health Organization (WHO) "End TB Strategy" aims to reduce TB deaths by 95% and lower the incidence of new TB cases by 90% between 2015 and 2035 [2]. To attain the worldwide goals for reducing the TB disease burden, it is imperative to enhance the diagnostic, preventive, and treatment services for TB [3,4].

Latent TB infection (LTBI) is defined as a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens with no evidence of clinically manifest active TB. The exact global burden is not known due to a lack of definitive diagnostic tests. One in four people in the world is estimated to have LTBI. The global prevalence of LTBI is estimated to be nearly 23% which amounts to 1.7 billion people as per the latest estimates [4]. Among the six high-burden countries within the South-East Asia region, India contributes significantly, accounting for 28 percent of the worldwide TB burden. Notably, India bears the highest global burden of TB infection (TBI). As per findings from the National TB Prevalence Survey conducted in 2021, the crude prevalence of TBI among individuals aged over 15 years was reported at 31.3% [5].

Although efforts to curb the TB burden have resulted in a decline in the disease burden both globally and in India, to achieve the WHO targets, especially in the high TB-burden countries like

India, it is not only crucial to improve the diagnosis and treatment of active TB, but also to prevent the development of active TB. To achieve this, active contact-tracing, integrating TB and HIV control programs, and addressing the key gaps in LTBI diagnosis and treatment may become useful approaches [6-8].

Spectrum of latent tuberculosis and active tuberculosis

Latent and active TB disease are two dynamic parts of the immunological spectrum. People with LTBI are considered to be non-infectious and asymptomatic, but bacilli may reactivate and later cause active TB disease. After initial infection, 5-10% of those infected will develop active TB disease in their lifetime, usually within the first 5 years after initial infection [6]. This risk is much higher in those with HIV and young children, with a ~10% annual risk of reactivation. Under 1 year of age, 40% of LTBI children, 24% in children of 1-10 years, and 16% in those between 11 and 15 years may develop active TB if latent TB is left untreated [9].

Identification and treatment of these LTBI people can reduce the burden of active TB diseases, which is one of the main goals of TB control programs globally. It has been estimated that if we were to treat just 14% of individuals with LTBI per year, this would reduce the TB incidence from 1280 cases per million recorded in 2010 to 20 cases per million by 2050, without any additional intervention [10]. Achieving 90% LTBI treatment coverage by 2025 is therefore one of the key milestones set by the WHO [2].

While the treatment of TBI plays a crucial role in preventing TB disease, it is often underappreciated. Nevertheless, it remains a significant component of India's National Strategic Plan 2017-25 to eliminate TB by 2025, 5 years ahead of the Sustainable Development Goals. The Lancet Commission on TB underscores that efforts to diagnose and treat TB effectively would be ineffective without the inclusion of TB preventive treatment (TPT) in a comprehensive strategy. It is imperative to enhance the implementation of established interventions, such as the adoption of effective new regimens for TPT, and ensure their swift and efficient scaling up.

Risk factors for active tuberculosis

Several factors elevate the risk of individuals with LTBI progressing to active TB. Many of these factors are linked to compromised immune responses, including concurrent HIV infection, cancer, immunosuppressive therapy, renal transplantation, and diabetes. The significance of diabetes is particularly noteworthy, as its prevalence has been on the rise in regions with high TB prevalence, and diabetic individuals are approximately three times more susceptible to developing TB compared to non-diabetic individuals [11,12]. Moreover, certain factors are associated with specific aspects of the host's response, such as macrophage activation, maintenance of granuloma structure, CD4 T-cells, CD8 T-cells, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) production, all of which are pivotal in controlling the pathogen during LTBI [13]. In recent times, studies utilizing whole-blood transcriptomic profiling have been conducted to identify distinct signatures capable of distinguishing between LTBI and active TB, as well as predicting varying treatment outcomes [14-16].

Diagnosis of latent tuberculosis

Lack of a gold standard test for the diagnosis of LTBI remains a major challenge in TB control. As per current the WHO guidelines, the test for LTBI is to be done when the risk of development of active disease is increased in specific high risk population like close contact of a person with TB or immunosuppressed individuals like in the case of young children in contact with those with active TB, people living with HIV infection, or people on medications or with conditions such as uncontrolled diabetes and cancer [6]. Since the positive predictive value of LTBI testing is low, screening for LTBI in people who are healthy and have a low risk of progressing to active disease is not recommended [17]. Secondly, the balance of risk and benefit is also different in high-burden settings, where the risk of reinfection may be high and screening for LTBI will have a low negative predictive value, but the same is not true for children, where the risk-to-benefit ratio is more favorable than for adults [6,17].

Testing for latent tuberculosis infection

Regarding acceptable methods of LTBI diagnosis, the latest WHO guidelines 2018 recommend the tuberculin skin test (TST) and IFN-γ release assay (IGRA) as the two types of tests available for identification of LTBI [6].

Tuberculin skin testing

It was developed by Koch in 1890, but the intradermal technique currently in use was described in 1912 by Charles Mantoux, a French physician [18]. The tuberculin most widely used is purified protein derivative (PPD), prepared according to the method described by Siebert (PPD-S) from *M. tuberculosis*, which is derived from cultures of *M. tuberculosis*. PPD-research tuberculin 23 (PPD-RT23) with Tween 80 of strengths 1 tuberculin unit (TU) and 2 TU are standardized tuberculins available in India, supplied by the Bacillus Calmette-Guérin (BCG) vaccine Laboratory, Guindy, Chennai [18]. The TST is performed using the Mantoux technique [19], which consists of the intradermal injection of 5 TU of PPD-S or 2 TU PPD-RT23 (both are equivalent). A delayed-type hypersensitivity reaction will occur within 48 to 72 hours in a person who has cell-mediated immunity to tuberculin antigens. There will be localized induration of the skin at the injection site, which may be determined by inspection (from a side view against the light as well as by direct light) and by palpation [20]. For standardization, the diameter of induration should be measured transversely to the long axis of the forearm and recorded in millimeters by a trained health person [21]. Reading should be performed in a good light, with the forearm slightly flexed at the elbow. Erythema (redness) should not be measured.

Various manufacturers produce PPD that conforms to the international standard, and there are also commercial brands available under the US Food and Drug Administration (FDA) standard PPD-S2, including Aplisol (manufactured by JHP Pharmaceuticals, Inc. in Rochester, MI, USA) and Tubersol (manufactured by Sanofi Pasteur Limited in Swiftwater, PA, USA).

The immune response observed in the TST has been the focus of numerous studies. These studies have revealed that biological variations among individuals, such as the following factors, can partly account for why some individuals exhibit strong TST responses while others show weak or no response at all (Table 1) [22-24].

Adverse effects

Severe reactions to the test in the form of ulceration, necrosis, vesicles, swelling, and redness of the arm can occur on very rare occasions, particularly in people who have had TB or been infected previously and in those who have previously had the BCG vaccine [25]. Local reactions such as regional lymphangitis and adenitis may also occur on rare occasions. Allergic reactions are also rare complications [25]. There are no chances of developing TB from the test, as live bacteria are not used for the test.

Interpretation of tuberculin reaction

The interpretation is done on the basis of risk-stratified cutoffs for the size of induration (5 mm, 10 mm, or 15 mm) [18,21,26] (Table 2).

Limitations

False-positive and false-negative results can occur with TST, which is the main limitation of this test. Similar antigens from environmental mycobacteria like *M. avium, M. fortuitum, M. kanasasii,* and M. *bovis* can give a positive reaction [17,18]. Due to their ubiquitous nature, a large number of populations in many areas of the world have been exposed and sensitized to antigens of environmental mycobacteria, and due to this exposure, non-tuberculosis mycobacteria (NTMs) may not be clinically important reason for false-positive TST results, except in populations where sensitization with NTM is high like post TB sequalae, immunocompromised and cancer patients [27-30]. The impact of BCG on TST specificity depends on certain factors, like when and how many doses of BCG are given. Impact on TST specificity is minimal if BCG is administered at birth or early infancy and can be ignored while interpreting the results. In contrast, if BCG is given after infancy and/or given multiple times (*i.e.*, booster shots), then TST specificity is affected [29]. False-negative TST results may occur because of cutaneous anergy (anergy is the inability to react to skin tests because of a weakened immune system) in certain patient population (*e.g.*, immunosuppressed individuals due to medical conditions such as HIV infection or malnutrition or those taking immunosuppressive medications like cancer), recent TBI (within 8-10 weeks of exposure), very old TBI (many years), very young age (less than 6 months old), recent live-virus vaccination (*e.g.*, measles and smallpox), and disseminated TB disease [18,31].

This may also occur due to preanalytical or analytical sources of test variability (*e.g.*, improper tuberculin handling or placement or incorrect interpretation of test results) [31]. The inter- and

Table 1. Summary of various immune markers and their influence on the tuberculin skin test.

Factor	Details
CD14 (-159C/T) polymorphism [22]	Associated with a higher likelihood of TST negativity Observed even in individuals vaccinated with BCG Variant found in the CD14 molecule within monocytes and macrophages.
Th1, Th2, or Th17 immune responses [23]	TST reactivity influenced by Th1, Th2, or Th17 immune responses. TST-positive individuals exhibit impaired production of IL-17 and IL-23. Lack of Th17 upregulation is a significant characteristic of TST positivity. Role of Th2 cytokines in TST reactivity may be less pronounced.
Delayed-type hypersensitivity and TST2 locus [24]	Intensity of delayed type hypersensitivity response to tuberculin governed by TST2, a genetic locus. TST2 located on chromosome region 5p15.
TST1 locus on chromosomal region 11p14 [24]	TST1 controls TST response. Signifies resistance to M. tuberculosis independently of T-cell activity. Located on chromosomal region 11p14.

CD14, cluster of differentiation 14; TST, Tuberculin skin test; BCG, Bacillus Calmette Guerin; Th, T helper cells; IL, interleukin.

Table 2. Interpretation of tuberculin reaction.

Induration size	Positive result criteria	Sensitivity [26] (%)	Specificity [26] (%)
≥ 5 mm	- HIV-positive individuals. - Recent contacts of active TB cases. - Chest X-ray abnormalities consistent with old healed TB. - Organ transplant recipients and other immunosuppressed patients. - Patients on long-term corticosteroid therapy (>6 weeks) with prednisone dose ≥15 mg/day or equivalent. - End-stage renal disease patients.	80	95
≥ 10 mm	- Recent arrivals (≤5 years) from high-prevalence countries. - Injectable drug users. - Residents and employees of high-risk congregate settings. - Mycobacteriology lab personnel. - Children <4 years, or those exposed to high-risk adults. - Infants, children, and adolescents exposed to high-risk adults.	81	98
≥ 15 mm	- Individuals with no known TB risk factors; unlikely due to BCG vaccination or environmental mycobacteria exposure.	60	99

intrareader variability in measurements of induration is also seen with TST, which affects the reproducibility of the test [32].

A repeat visit is required to read the test results after 48 to 72 hours. Prolonged follow-up is required to measure the long-term ability of a positive TST to predict the development of active TB. As per previous literature, the association between tuberculin reactivity and the risk of active TB is poor [33]. The various phenomena like immunologic recall of preexisting hypersensitivity to TB (*i.e.*, boosting), conversions (*i.e.*, new infection), and reversions (of positive results to negative) may lead to non-specific variability and make the interpretation of repeat testing's to be complicated [17,32]. Also, only standardized PPD is required, which must be stored at optimum temperature [17].

Deniz and colleagues conducted a study involving 371 patients with chronic kidney disease, a population more vulnerable to TBI and disease. Their findings revealed that elevated levels of parathormone (PTH) and the use of vitamin D treatment were associated with negative TST results, suggesting that these factors might induce a degree of immunosuppression [34].

In children, two noteworthy reports have proposed that helminth infestations could influence the outcomes of immunological tests used to assess M. tuberculosis infection [35]. Furthermore, the ratio of IFN-γ to IL-10 may positively correlate with TST results, indicating the potential significance of the interplay between these two cytokines in TST reactivity [36]. Additionally, this latter report demonstrated that TST outcomes are impacted by BCG vaccination but not by exposure to non-TB mycobacteria (NTMs) [36].

In summary, TST results are influenced by a complex interplay of factors, including age, nutritional and immunological status, the duration between antigen exposure and test administration, BCG vaccination, immunosuppression, genetic background, and the potential for cross-reactivity with environmental NTMs, and possibly other pathogens.

Advantages

It has been used to diagnose latent TB for more than 100 years, and the test has a very low cost. It does not require any withdrawal of blood and can be used in an outpatient clinic without the requirement of any sophisticated lab.

Next-generation skin tests for the detection of TB and Diaskintest are novel skin tests designed to detect LTBI by utilizing specific *M. tuberculosis* (MT) antigens, early secretory antigenic target 6 (ESAT-6) and 10-kDa culture filtrate protein (CFP-10), instead of the traditional tuberculin solution employed in the TST. These tests boast higher specificity compared to TST and are unaffected by prior BCG vaccination or exposure to environmental mycobacteria [37].

Interferon-γ release assays

The IGRA is a recent whole blood test developed to detect IFN-γ production by sensitized T-cells upon *in vitro* stimulation with mycobacterial antigens. Specifically, the test utilizes mycobacterial antigens, including the ESAT-6 and the CFP-10. These antigens are encoded within the region of differentiation 1 (RD1) found in the genomes of *M. tuberculosis* and *M. bovis* and are notably absent in BCG and the majority of environmental mycobacteria [38,39]. Consequently, IGRA results remain unaffected by both BCG vaccination and exposure to environmental mycobacteria.

T-cell IGRA serves as an alternative immunodiagnostic approach to the TST for detecting *M. tuberculosis* infection. IGRAs are *in vitro* whole-blood tests measuring the cell-mediated immune response. This specificity makes them more suitable for *M. tuberculosis* detection than the widely used PPD for TST. However, some evidence of cross-reactivity between ESAT-6 and CFP-10 of *M. tuberculosis* and *M. leprae* exists [34,35].

Until 2015, only two commercially available types of assays were present: QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube (QFT-GIT). These tests, which have replaced QuantiFERON-TB and QuantiFERON-Gold, are the latest generation of IGRA. The US FDA approved QuantiFERON-TB Gold Plus in 2015 and QFT-GIT in 2007 as an aid for detecting latent *M. tuberculosis* infection. The tests quantify IFN-γ released from sensitized lymphocytes in whole blood incubated overnight with PPD from *M. tuberculosis* and control antigens.

Both assays use peptides from the RD1 antigens ESAT-6 and CFP-10, as well as peptides from one additional antigen (TB7.7 [Rv2654c]), which is not an RD1 antigen, in an in-tube format. The results are reported as quantification of IFN-γ in international units per milliliter. If the IFN-γ response to TB antigens is above the test cutoff, an individual is considered positive for *M. tuberculosis* infection. QuantiFERON-TB Gold Plus has a higher sensitivity (98.9%) compared to QFT-GIT (97.9%), while both tests exhibit similar specificity. However, in resource-limited, high TB-burden settings, where cost and logistics are limiting factors, TST remains the preferred method for LTBI diagnosis. TST is still considered the most preferred method for LTBI diagnosis in resource-limited, high TB-burden settings, due to cost and logistical constraints associated with IGRA-based tests.

T-SPOT.TB assay (Oxford Immunotec, Abingdon, United Kingdom)

The T-SPOT.TB assay is also an enzyme-linked immunosorbent spot assay. T-SPOT.TB counts the number of antimycobacterial effector T-cells, white blood cells that produce IFN-γ, in a sample of blood. This gives an overall measurement of the host immune response against mycobacteria, which can reveal the presence of infection with *M. tuberculosis*. Because this does not rely on the production of a reliable antibody response or recoverable pathogen, the technique can be used to detect latent TB [40]. The test received FDA approval in 2008.

It is performed on separated and counted peripheral blood mononuclear cells that are incubated with ESAT-6 and CFP-10 peptides. The result is reported as the number of IFN-producing T-cells (spot-forming cells). If the spot counts in the TB antigen wells exceed a specific threshold relative to the negative-control wells, the individual is considered positive for *M. tuberculosis* infection. In a systematic review and meta-analysis by Diel *et al.* [41], the sensitivity of T-SPOT.TB was 98% and the negative predictive value was 94% showing the effectiveness of this test in ruling out *M. tuberculosis* infection.

Furthermore, various studies have documented the heritability of the IFN-γ response to mycobacterial antigens, including ESAT-6. The percentage of heritability varied among the populations examined, with the highest heritability reported in South African subjects, particularly when studying sibling pairs. In this context, the estimated heritability of the IFN-γ response to ESAT-6 was found to be 58% [42,43].

Test characteristics: sensitivity and specificity, reproducibility for latent tuberculosis infection

IGRAs have a specificity for LTBI diagnosis of 95% in settings with a low TB incidence, and specificity is not affected by BCG vaccination [17,44,45]. The sensitivity for the T-SPOT.TB assay appears to be higher than that for the QuantiFERON-TB assay or TST (approximately 90%, 80%, and 80%, respectively). Sensitivity of IGRAs is decreased in HIV infection and children [46]. NTMs infections do not affect IGRAs [22]. However, infection with *M. marinum* or *M. kansasii*, which express ESAT-6 or CFP-10, may cause positive results in IGRAs, as with the TST [47].

Functional T-cell assays are highly susceptible to variability by numerous factors at multiple levels, including assay manufacturing, preanalytical processing, analytical testing, and immunomodulation. A systematic review on IGRA reproducibility in 2009 showed that variability was substantial, with magnitudes of within-subject IFN-responses varying by up to 80% [48].

Advantages

They require fewer visits than TST for test completion and do not have cross-reactivity with BCG results. The test results are available within 24 to 48 hours, as previously mentioned, they have less cross-reactivity than TST with NTMs [17].

Limitations

The test requires a withdrawal of blood, which may be challenging in children. A well-equipped laboratory, with electricity and trained staff, is needed. The cold chain needs to be maintained for the transport of kits and reagents and for their storage. There is a high likelihood of false-positive conversions during serial testing, and reproducibility is affected by several preanalytical and analytical factors, as well as manufacturing defects. Interpretation of serial IGRAs is complicated by frequent conversions and reversions and a lack of consensus on optimal thresholds [17].

Similar to the challenges observed with the TST, the performance of IGRA tests can be influenced by various factors, primarily associated with compromised immune responses and technical considerations. For instance, the inclusion of interleukin-7 (IL-7) has been shown to enhance test positivity [49].

The clinical accuracy of IGRAs appears to be adversely affected in patients with immune-mediated inflammatory diseases, such as Crohn's disease, where immune cell function is suppressed [50]. Additionally, patients receiving immunomodulatory drugs like teriflunomide, which inhibits T-cell activation, may experience a change in QuantiFERON results from positive to negative, often accompanied by a marked reduction in IFN-γ [51]. Moreover, the administration of high doses of corticosteroids has been linked to a high proportion of indeterminate (QFT-GIT) results in individuals with rheumatoid arthritis and inflammatory bowel disease. Consequently, patients with these conditions should be tested with QFT-GIT before commencing steroid treatment [52].

Interestingly, in TB patients, the sensitivity of IGRA is not compromised by the presence of diabetes. In fact, the sensitivity of QuantiFERON-TB Gold was significantly higher in TB patients with diabetes compared to those without diabetes [53].

Furthermore, technical variations that can impact IGRA results encompass issues related to blood sampling (including time and volume), tube shaking, incubation or processing delays (which may affect cell viability in blood), incubation duration, analytical errors, and manufacturing defects [54].

Application of interferon-γ release assays

Recent studies have assessed interferon assays for various applications, such as:

i) individuals with suspected TB disease – a negative result with IGRAs in HIV-infected people cannot reliably rule out active TB because of suboptimal sensitivity for active TB. Also, IGRAs cannot distinguish between LTBI and active TB, and therefore, the specificity of TB diagnosis will always be poor in countries with high TB burdens [6,55]. In children with suspected active TB, the ability of IGRA alone is poor to rule in or rule out active TB; hence, IGRAs should be used with other clinical data (chest X-ray findings, and history of contact) to support a diagnosis of active TB [17,56];
ii) prognostic value for progression to active TB – the currently available data show that the predictive value of IGRAs for progression to TB disease is low and slightly but not significantly higher than that of the TST. The data suggest that a majority (95%) of those with positive IGRA or TST results do not progress to TB disease during follow-up [17,41];
iii) monitoring of antituberculosis therapy – studies have shown no role of IGRAs in monitoring treatment responses in both active and latent TB [57,58].

Comparing tuberculin skin testing and interferon-γ release assay for the diagnosis of latent tuberculosis infection

While both the TST and the IGRA are employed in clinical practice for diagnosing LTBI, it is important to note that they assess distinct aspects of the immune response that are particularly relevant in immunocompetent individuals (Table 3) [41,59-60]. Latency antigens hold the potential to serve as differentiators between LTBI and active TB.

Numerous research endeavors have been dedicated to identifying mycobacterial antigens that are naturally expressed during LTBI. It is important to distinguish between the terms "latency", which pertains to the state of the host, and "dormancy", which refers to the bacterial state during latency. Dormancy characterizes a reversible metabolic quiescence, representing a condition of reduced bacterial metabolic activity as the bacilli transition from a replicating to a non-replicating state. In this non-replicating state, mycobacterial cells can endure extended periods without replication, utilizing various immune-evading strategies [61,62]. Conditions that foster this low metabolic state include factors such as oxygen deprivation and fluctuations in nitric oxide levels.

The accumulation of evidence, although at times conflicting, linking specific latency antigens with cytokine responses has yielded the observations reported in Table 4 [63-65].

Contribution of antibodies in diagnosing latent tuberculosis infection

A prevalent viewpoint in the medical field suggests that the role of the human antibody response against *M. tuberculosis* in protecting against TB is relatively limited, especially when compared to the significance of cell-mediated immunity. This perspective has been reinforced by two key observations: the presence of elevated antibody levels in individuals with active TB, implying that antibodies do not provide substantial protection [66], and the seemingly unchanged risk of TB reactivation in patients treated with rituximab, a human/mouse chimeric anti-CD20 antibody known to swiftly deplete normal CD20-expressing B cells [67].

However, emerging evidence indicates that as the metabolism of *M. tuberculosis* changes during infection, the expression of immunodominant antigens should reflect these alterations. This, in turn, results in variations in the antibody profile between individuals with LTBI and those with active TB. These distinctions in antibody profiles hold potential for diagnostic applications [68].

Several noteworthy observations include the following.

Mycobacterial proteins with molecular weights of 36, 25, and 23 kDa, found in membrane vesicles, have been exclusively identified in the sera of TB patients, not in healthy controls. Additionally, the titers of these antibodies are lower in individuals with LTBI [66].

Immunization with BCG leads to the production of immunoglobulin G (IgG) antibodies against Ag85A, which have been linked to a reduced risk of developing active TB [69].

LTBI individuals exhibit notably higher levels of specific IgG antibodies against the transmembrane protein Rv1733c when compared to TB patients [70]. Conversely, TB patients in endemic regions display significantly higher antibody levels against specific *M. tuberculosis* proteins in contrast to healthy individuals living in the same areas [70].

Individuals with established LTBI demonstrate elevated plasma levels of anti-Rv2626c IgG compared to recently infected individuals and patients with active TB [71].

Identifying the onset of active tuberculosis progression

The progression from LTBI to active TB is influenced by various factors. These factors encompass aspects related to the bacteria, such as strain virulence and inoculum size, as well as host-related factors like the state of the immune response, treatment with steroids, the use of biologic agents such as antibodies targeting TNF-α, solid organ or hematological transplantation, HIV infection, and the individual's age. Environmental factors like smoking and occupational exposure, particularly in healthcare workers, also play a role in this progression.

Furthermore, in the context of differentiating active TB from LTBI patients, a specific subset of PPD-specific CD4 T-cells has been identified, which secretes TNF-α but not IFN-γ or IL-2. These cells possess a differentiated effector memory phenotype, characterized by the absence of CD45RA, CCR7, and CD127. This particular subset has shown promise as a useful marker for

Table 3. Comparison of tuberculin skin test and interferon-γ release assay for the diagnosis of latent tuberculosis infection.

Aspect	Details
TST *vs.* IGRA for LTBI diagnosis	TST: *in vivo* assessment of delayed-type hypersensitivity using purified protein derivative from tuberculosis bacilli. IGRA: *in vitro* examination of the cell-mediated immune response, measuring interferon-γ production by circulating effector memory cells [57].
Antigen diversity and immune response variations	Antigen diversity contributes to variations in specificity between TST and IGRA. Genetic diversity and individual immune response differences impact test performance. IGRA shows higher specificity in low-risk, BCG-vaccinated individuals and greater sensitivity in HIV-infected patients [58,59].
Discrepancies in results and test accuracy	Discrepancies between TST and IGRA results are common in individuals with LTBI. IGRA accuracy can be enhanced by extending the incubation period and measuring interleukin-2 levels, particularly QuantiFERON Gold In-Tube.
Involvement of T-cell subsets and local prevalence	Positive results in both TST and IGRA associated with an increased number of regulatory T cells (CD4CD25 high CD39+ cells). Correlation between TST and IGRA results varies based on regional tuberculosis incidence, Bacillus Calmette-Guérin vaccination, environmental mycobacteria exposure, and risk of reinfection.
Limitations of TST and IGRA	Low precision when screening immune-compromised individuals for LTBI. Neither test highly effective in predicting the progression to active tuberculosis.

TST, tuberculin skin test; IGRA, interferon-γ release assay; LTBI, latent tuberculosis infection.

Table 4. Summary of various antigens affecting immune response in latent tuberculosis and active tuberculosis.

Antigen	Immune response in LTBI	Immune response in active TB	Comparison with healthy controls
Rv2628 [61]	Higher IFN-γ response in remote LTBI compared to recent infection	-	-
Rv2031c [61]	Lower IFN-γ, TNF-α, and IL-10 in active TB compared to controls	Some studies found no differences in IFN-γ response	-
DosR antigens [62]	Disparities in IFN-γ responses in healthy contacts *vs.* TB patients	-	Study involved multiple DosR antigens
Rv1737c, Rv2029c	Increased IFN-γ or TNF-α-producing CD4 and CD8 T-cells in LTBI	-	Stimulation of PBMC with specific antigens
RV2004 [63]	Robust proinflammatory response in LTBI *vs.* active TB and controls	-	Elevated TNF-α, IL-8, IL-1b, IL-12 levels
DosR antigens [62]	Extensively studied antigens with potential for distinguishing LTBI from active TB	-	Rv0081, Rv1733c, Rv1737c, Rv2029c, Rv2031, Rv2628

LTBI, latent tuberculosis infection; TB, tuberculosis; IFN-γ, interferon-γ; IL, interleukin; TNF, tumor necrosis factor; DosR, dormancy survival regulon; PBMC, peripheral blood mononuclear cells.

distinguishing individuals with active TB from those with LTBI [72]. Additionally, recent research involving the stimulation of blood cells from patients with active TB or LTBI using PPD or ESAT-6/CFP-10 revealed that the CD4+CD27−CCR4+ T-cell subset was induced to a greater extent in subjects with active TB compared to those with LTBI. This suggests that investigating the expression of CD27 and CCR4 may hold potential as valuable immunodiagnostic markers for TB [73].

Policy statement and guidelines

The 2018 WHO policy on the use of IGRAs states that either a TST or IGRA can be used to test for LTBI; however, the availability and affordability of the tests will determine which will be chosen by clinicians and program managers [6]. IGRAs or the TST should be used for the diagnosis of active TB [74]. IGRAs also cannot distinguish between LTBI and active TB, and therefore, the specificity of TB diagnosis will always be poor in countries with high TB burdens [56,75]. LTBI testing by TST or IGRA is not a requirement for initiating preventive treatment in people living with HIV or child household contacts aged <5 years [6]. As per guidelines for programmatic management of TPT from India, all household contacts of pulmonary TB, if asymptomatic and age ≥5 years, should be given TPT, if they have positive IGRA/TST or unavailable with normal or unavailable chest X-ray after ruling out active TB. Also, other high-risk groups should have a negative symptom screening to rule out active TB and should only receive TPT if IGRA/TST is positive and chest X-ray, if available, is normal [76].

Way forward

LTBI constitutes a concealed facet of the broader global health issue of TB. Achieving a dependable diagnosis and effective treatment for individuals with LTBI is of utmost importance in TB control, as they harbor the potential to progress to active TB. TST has traditionally been the most widely used method for LTBI diagnosis due to its simplicity and the *in vivo* evidence it provides for anti-mycobacterial cellular immune responses. Nevertheless, it is compromised by false positives in BCG-vaccinated individuals. The introduction of IGRA has improved specificity, and the new QuantiFERON-TB version holds promise for distinguishing between active TB and LTBI. Despite these advancements, the quest for a reliable biomarker of LTBI and the assessment of drug therapy efficacy in LTBI patients remain ongoing challenges.

This review summarizes key strategies and proposed targets, or immunological markers, developed over the past decade for distinguishing between LTBI and active TB and for evaluating the effectiveness of LTBI treatment. These strategies include analyzing cellular profiles, such as the proportion of TNF-α-only effector T-cells with an effector memory phenotype (CD45RA-CCR7-CD127-), which has been associated with a higher risk of progressing to active TB in immunocompetent adults. Another approach involves investigating a diverse population of immature myeloid-derived suppressor cells, which have been linked to both active TB and recently acquired LTBI. Additionally, the cellular response to mycobacterial latency-associated antigens, particularly those encoded by the DosR regulon, has shown promise in identifying individuals with LTBI or active TB.

Other potential candidates for differentiation include the specific antibody response to distinct *M. tuberculosis* antigens, the identification of specific miRNA, and molecular signatures observed in blood transcriptome analysis, particularly those related to IFN-γ signaling. Challenges ahead encompass the validation of these tests across diverse populations and their suitability for low-income countries where TB remains a significant public health concern. Overcoming these challenges may herald a transformative approach to tackling the disease.

Conclusions

Both TST and IGRAs are acceptable, but both have advantages and disadvantages. There are situations where neither test is appropriate (*e.g.*, active TB diagnosis in adults) and scenarios where both tests may be necessary to detect *M. tuberculosis* infection (*e.g.*, immunocompromised populations), and there are scenarios where one test may be preferable to another. Both TST and IGRAs have reproducibility challenges. The ability of both IGRAs and TST is limited in regard to finding the beneficiaries of LTBI therapy. Neither of the tests can predict the subsequent development of active TB in subjects with LTBI with certainty. In resource-limited and high TB burden countries, TST should remain as the mainstay of LTBI testing due to low cost, ease of applicability, no requirement for technical expertise, sophisticated labs, and venous puncture. In the future, highly predictive and accurate biomarkers need to be identified that have minimal limitations. Although both tests are valuable screening tools, their results should never be used alone. Careful clinical evaluation with emphasis on risk stratification should always precede diagnostic and therapeutic modalities.

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