One Health Outlook

REVIEW

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Advancement in diagnostic approaches for latent tuberculosis: distinguishing recent from remote infections

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Abstract

Tuberculosis (TB) remains as a significant global health threat to date, with latent TB infection (LTBI) serving as a major reservoir for future active disease cases. A practical approach to an effective control and eradication of TB hence, requires an explicit identification of infected patient whom are at high risk of progressing from latent to active TB, particularly in those recently infected individuals. Current diagnostic tools however, including Tuberculin Skin Test and Interferon-Gamma Release Assays, are still lacking for their ability to critically distinguish between recent and remote infections, leading to insufficiency in optimizing targeted preventive treatment strategies. This review examines the limitations of current diagnostic tools and explores novel biomarkers to enhance distinction within the infection timeline in LTBI diagnostics. Advancement in immune profiling, dormancy antigen, along with molecular and transcriptomic approaches holds great promise to develop a diagnostic tools with better accuracy to differentiate recent from remote infections, thereby optimizing targeted interventions to improve TB control strategies. These underscores the need for further research into these emerging diagnostic tools to facilitate an effective public health strategies and contribute to the united efforts in End TB Strategy.

Keywords LTBI, LTBI diagnostics, Recent TB infection, Remote TB infection, IGRA, TST

Background

Tuberculosis (TB) is an airborne infectious disease caused by *Mycobacterium tuberculosis* (MTB). Despite substantial global efforts in combating TB, it remains as a major public health threat, placing it the world's second leading cause of death from a single infectious agent, killing 1.6 million people annually [1, 2]. One significant challenge in controlling TB is attributed to the bacteria's capability to decrease its metabolic activity upon infection, thereby persisting within the host in dormant state which is referred to as latent tuberculosis infection (LTBI) [3]. As defined by the World Health Organization (WHO), LTBI is characterized by a persistent immune response to MTB antigens which do not exhibit clinical symptoms of TB [4]. Globally, it is estimated that one-fourth of the population are latently infected with MTB [5], with higher prevalence rates of 31% from Southeast Asia and 28% from Western Pacific region [6].

Although individuals with LTBI do not manifest disease symptoms, these individuals harbour live bacteria which are capable of reactivation to active TB disease under certain health conditions [7]. While it is commonly agreed that the general public infected with LTBI carries a 5–10% lifetime risk of reactivation upon infection

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[8–10], studies has revealed that the reactivation risk are highest within the first few years of initial infection compared to established remote LTBI individuals [11, 12]. An estimation done by Houben and Dodd (2016) further projected an annual incidence of 16.5 per 100,000 TB reactivation cases from the latent pool in the year of 2035, which exceed the End TB Strategy target by WHO at 10 per 100,000, assuming the LTBI activation rate per annum is 0.15% [5]. The risk of reactivation hence highlights LTBI as a critical reservoir for future active TB cases, posing great challenge for TB control and elimination efforts [13]. Given these findings, it is imperative to develop a highly dependable diagnostic method, which identify LTBI in those at higher risk of TB reactivation such as recently infected individuals to facilitate targeted treatment in global TB eradication plan [4].

To date, there is still no gold standard for LTBI diagnosis despite LTBI having been recognized almost a century ago [14]. The currently available, primary diagnostic tools for detecting LTBI are Tuberculin Skin Test (TST) and Interferon-Gamma Release Assays (IGRAs). However, neither tests are able to give a direct measurement of live MTB bacteria within the host in LTBI state. Instead, they merely provide an indirect assessment of host immune response against TB in which the results rely heavily on the host's competent immune response in identifying LTBI individuals [15, 16]. Consequently, persistent positive results of TST and IGRA could be detected throughout the life of a latently-infected individual, demonstrating its inability to provide a temporal resolution for detecting recently infected individuals who are at higher risk of reactivation. In addition, results interpretation of the tests varies extensively based on factors such as history of BCG vaccination, immunosuppression and TB burden in a specific region leading to inconsistent and potentially unreliable diagnosis [17–20].

Collectively, these limitations pose significant challenge in clinical-decision making and public health interventions for TB preventive treatment (TPT) and contact tracing measures in the End TB Strategies which focuses on determining and treating higher risk individuals such as young children, the immunosuppressed and recently infected individuals [21, 22]. While it is clear that TB transmission often occurs in the general community in high-incidence settings [23], identifying individuals who have recently been infected which are at higher risk of reactivation could be a prudent strategy for controlling transmission. Fortunately, there are increasing research focused on identifying biomarkers and developing new technologies that can provide this critical information, such as advanced immunological assays and molecular techniques [24–29]. These innovative strategies hold promise in providing temporal resolution thereby enhancing predictive value in LTBI diagnostics, ultimately improving global TB control and elimination efforts.

This review address one of the most significant limitation of LTBI diagnostics in their inability to distinguish recent and remotely infected individuals and the public health implications for current issue. We also discuss the potential biomarker to differentiate recent and remote LTBI in hope to enhance targeted treatment and TB prevention strategies.

Current diagnostic test

Tuberculin is a term adopted by Robert Koch a few years after identifying MTB as the causative agent for TB, referring to the filtrate of tubercle bacilli grown in glycerol broth [30]. Although Koch's findings diverged from his initial intention in curing TB infection, they opened the door to a significant diagnostic breakthrough, enabling differentiation between infected and healthy individuals. His formulation was later refined to what we now know as purified protein derivative (PPD), which is an autoclaved mixture of protein precipitated from mycobacterial culture filtrate used in TST [31, 32].

The mechanism of TST involves a delayed-type hypersensitivity (DTH) reaction, which occurs in two distinct stages: the sensitization stage and the effector stage. During initial infection, MTB antigens are recognized by the immune system, generating sensitized T cells that specifically target MTB antigens. The subsequent effector stage occurs upon PPD challenge via intradermal injection, resulting in the infiltration of immune cells such as monocytes, T and B lymphocytes, initiated by Th1 cells under the skin [33, 34]. TST is performed by injecting 0.1 mL of PPD into the inner surface of the forearm, creating a slight wheal under the skin. Test results are read 48–72 h later by measuring the diameter of induration on the forearm [16, 35]. Interpretation of a positive TST result depends on established cut-off points, varying with TB burden, individual risk, and BCG vaccination history [36–39]. The reliance of TST on the in vivo immune reaction highlights its limitations, leading to false negative results in immunodeficient individuals, haemodialysis patients, and those undergoing anti-TNFα treatment, as well as false positive results in individuals previously sensitized to environmental mycobacteria or with a history of BCG vaccination [36, 40–42].

The introduction of interferon gamma release assay (IGRA) marks a new milestone in LTBI diagnostics. IGRA overcomes significant weaknesses in TST by greatly reducing false positivity in BCG-vaccinated individuals and minimizing cross-reactivity with the majority of environmental mycobacteria. The success of IGRA is tied to the identification of a specific region

of MTB DNA, Region of Difference 1 (RD1), absent in BCG strains and most environmental bacteria except *M. kansasii, M. szulgai* and *M. marinum* [43–45].

The two main types of IGRAs widely used are T-SPOT.TB and QuantiFERON-TB Gold In-Tube Test (QFT-GIT). Both tests measure the cell-mediated immune response, quantifying IFN-γ released from effector memory T-cells upon 16–20 h of stimulation in response to MTB specific antigens [46–48]. T-SPOT.TB utilizes early secretory antigen target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10) from RD1 as stimulating antigens, known to activate CD4 + T-cells to stimulate IFN-γ production [24, 49]. On the other hand, QuantiFERON-TB Gold In-Tube Test (QFT-GIT) includes the same antigens as T-SPOT.TB, with an additional antigen from RD11 known as TB7.7 (Rv2654). Studies suggest TB7.7 is highly specific against MTB and can stimulate higher IFN-γ levels in TB patients compared to BCG-vaccinated individuals [50, 51]. Despite the differences, both tests show concordance in diagnosing TB, [52–54], with excellent specificity in populations who had received BCG vaccination [55, 56].

Overall, the aforementioned tests have distinct advantages and limitations. While TST is inexpensive and simple to perform, its requirement for two healthcare visits to complete the test may result in loss of follow-up [57, 58]. On the other hand, although IGRA seem to provide better diagnostic value over TST in terms of sensitivity and specificity, their higher cost and need for specialized equipment can be a barrier in resource-limited settings [59]. Both methods however exhibit similar limitations as neither test are able to differentiate between active tuberculosis (ATB) and LTBI. Moreover, they lack diagnostic value in immune impaired individuals especially in HIV co-infected patient that have higher risk of reactivation. Additionally, these tests cannot reliably determine and distinguish recent and remotely infected individuals, which are attributable to their reliance on host's immunological memory, thus failing to provide a sufficiently accurate positive predictive value for this differentiation.

Differentiating remote and recent infection

Recently, there has been a growing trend to use the terms "recent" and "remote" to describe the timeline of TB infections, with "recent" referring to infections that occurred lately and "remote" referring to infections that happened years ago. However, these studies often lack specific timeline or a concrete definition for these terms, leading to diverse interpretations. Typically, the defining criteria are associated to the risk of disease progression. Research suggests that the first 2 years following primary infection carry a 15-fold higher risk for disease progression compared to more established infection (> 2 years) without known risk factors [60, 61]. Thus, many studies, including that from the Centre of Disease Control US, use the first two years to define a recent infection, while subsequent years are considered as a remote infection [12, 62, 63]. Nonetheless, within this definition it remains unclear for remote infection whether the infection persists or if bacterial clearance occurs over time.

Typically, in serial TST testing, individuals with a remote infection typically show a positive result in the initial test due to previous exposure to MTB antigen, while recently infected individuals are identified by conversion in the second TST, marked by an induration of at least 10 mm with an increase of at least 6 mm compared to the first test [42, 64]. For IGRA, recent infection could be inferred by a conversion from negative to positive result within a two-year period, regardless of the magnitude of change in IGRA results [65]. However, these lenient criteria likely overestimate conversion rates compared to those observed in reality. To more accurately differentiate between recent and remote infection using IGRA, a more stringent criteria was used in some studies [25, 66–68]. However, none of these criteria precisely establish the timeline of infection, as no gold-standard test for this purpose is currently available.

Differentiating between individuals with remote versus recent TB infections is critical and is typically addressed during screening and contact tracing programs. This distinction is particularly important because recently infected individuals are at a higher risk of progressing to active TB. Consequently, they are often considered for a TPT regimen, with decisions based on certain risk factors such as the intensity of exposure, the source of the disease, and the potential for adverse drug reactions [69]. WHO's operational handbook on TPT recommend using both TST and IGRA to diagnose LTBI before initiating TPT [58, 70]. Apart from that, a serial testing was also recommended by WHO as a surveillance program for those who might have occupational exposure such as healthcare workers [71–74]. This approach may involve various methods, including serial testing with the IGRA [24, 67, 68] or TST [42, 64] within a time frame or combined TST with IGRA which were tested sequentially [75]. Nonetheless, the best method for serial screening remained elusive and shall be further validated in countries with different TB burden.

Challenges in TB infection timeline diagnostics

To date, the precise T cell memory subset that provides immune protection against TB has not been determined. Nonetheless, the presence of a heterogeneous population of T memory cells is crucial in conferring a certain level of "immune protection" [76, 77], although most studies have suggested neither a previous TB infection nor

vaccination could provide protection against infection or re-infection [78–82]. Regardless of the theory, the existence of immunological memory presents a significant challenge in current diagnostic tests for LTBI. This complexity arises because the immune response generated by these memory cells persist within the body long enough to potentially interfere with the subsequent result interpretations, hence, complicating the diagnostic decision.

This concept holds true especially in TST, as small number of sensitized T-cells from the initial TB antigenic challenge may persist to become long-lived memory cells. Subsequent repeated challenges with PPD can result in a positive reaction for a long time, even after treatment [83, 84]. It is generally believed that the positive result of TST will persist for life [85]. Studies done around 1970s showed those who had previous positive TST for more than 1 year remained positive after one year or longer of isoniazid treatment [86, 87]. These findings suggest that reversion of TST results are unlikely, particularly for those with long term and established latent infection even after treatment. Thus, immune reactivity detected in such cases does not accurately reflect an ongoing infection at the specific time point especially during screening [88], leading to its inadequacy in determining recent and remote infections thereafter.

Despite its reliance on immunological memory, both TST and IGRA results are subject to fluctuations, leading to reversion and conversion phenomena that remain not fully understood, though it is more commonly seen in IGRA [89, 90]. This inconsistency hampers both tests' ability to accurately distinguishing between previous infection that may have resolved and a recent infection. The heterogeneity of immune response among individuals to TB infection is the most plausible explanation for reversion without prophylactic treatment. These differences can be attributed to varying host's pathogen clearance capability in reducing bacterial load over the course of infection related to immune aging, comorbidities, chronic illness and etc. [90–93]. Additionally, misclassification often occur in IGRA when test results appear to be in a borderline zone, leading to inconsistent outcomes in serial testing in the absence of a parallel control group [94, 95].

IGRA reversion is commonly observed in individuals who initiate prophylactic treatment. Theoretically, IGRA results should decline with treatment due to the reduction of effector memory T-cells, which are more active during acute infection, while long-term memory T-cells (central memory T-cells) persist. Since recalling central memory T-cells require a longer incubation period, the overnight incubation of around 16–20 h is often insufficient to activate these cells, leading to negative IGRA results [96, 97]. Based on this understanding, IGRA results are being explored for their potential use in monitoring the effectiveness of TB treatment [98, 99]. However, numerous studies suggested that the IFN-γ levels remained persistently elevated months after treatment, deeming IGRA unsuitable for treatment monitoring and limit their potential use in determining a relapsed recent MTB infection [93, 100, 101].

Briefly stated, TST and IGRA are not a reliable tools to provide a temporal resolution. The persistent elevation of both TST and IGRA following treatment, along with frequent reversion and conversion, can complicate the interpretation of test outcomes even if serial testing is implemented. Consequently, a robust historical medical data tracking system is needed to assist in interpreting TST and IGRA results over decades. However, these data are often unretrievable in low- and middle-income countries (LMICs) that experience high prevalence of TB [64, 102]. Even with proper tacking system in place, the lengthy timeline for retesting can lead to loss of follow-up, making serial testing less practical in public settings compared to its use among healthcare workers. Additionally, the implementation of serial testing is hampered by various practical challenge especially in resource limited, high TB burden settings. As a result, there is a critical need for supplementary diagnostic methods that can accurately assess TB infection status with a single test.

Implications of the inability to distinguish between remote and recent infection

Public health implications

From a public health perspective, the inability to distinguish between recent and remote infection using TST and IGRA presents substantial challenges for controlling outbreaks and implementing targeted interventions. TB screening and contact tracing aim to quickly identify potential active TB cases in preventing further transmission, and detect contact clusters to provide TPT before they progress to an active disease [103, 104]. Given that each active TB case can transmit the disease to approximately 10–15 contacts within a year if left undetected [105, 106], prompt and targeted action is critical in managing active cases and their contacts to control the spread of TB [107]. Additionally, recent infections can indicate ongoing transmission within a community [108], highlighting the urgent need for rapid intervention to break the transmission chains.

In high TB prevalence countries, differentiating between recent and remote infections using TST and IGRA is particularly challenging. A positive result in these tests often indicates the presence of immunological memory towards MTB antigens, but does not necessarily distinguish between recent and remote infections as discussed earlier. This limitation is critical

because recent infections are of particular concern for progression to active TB disease and subsequent transmission within the community [12]. The challenge is compounded by the fact that individuals in high TB burden settings frequently experience reinfection, even after successful treatment of a previous infection, unlike in low TB burden countries [79, 109]. Thus, a positive TST or IGRA result might reflect either a remote, previously treated infection or a recent reinfection, complicating the determination of an individual's current infection status [25, 110].

The lack of temporal resolution in these tests creates public health dilemma, hindering the ability to implement immediate and effective intervention strategies [106]. This limitation affects both short-term response and long-term TB control efforts. Without the ability to distinguish recent from remote infections, transmission chains may go undetected, particularly among individuals with recent infections who may evade appropriate intervention due to the ambiguity of their infection status. If these individuals eventually develop active TB, they can initiate new transmission chains, necessitating additional rounds of contact tracing and interventions. This cycle strains public health resources and complicates efforts to manage and control TB outbreaks effectively, as it requires diversion of resources from treating people with active TB as discussed above [111]. This challenge is further exacerbated by the COVID-19 pandemic, which has led to a tremendous increase in disease burden due to reactivation of latent infection in post-COVID infected patient, along with the diversion of health resources towards combating COVID-19. These factors have severely impacted ongoing TB control efforts [112–114]. Therefore, the development of diagnostic tools with strong predictive value in determining infection progression would greatly enhance TB control efforts, particularly during this syndemic period.

Clinical implication

TB preventive therapy (TPT) consists of a course of one or more anti-tuberculosis medicines designed for LTBI patient to prevent the development of TB disease. This treatment regimen is a cornerstone of the End TB Strategy, aimed at safeguarding both individuals and the communities from TB. Regardless of the type of regimen used, TPT is recommended for individuals who are recently exposed to TB and are at higher risk of developing active TB following exposure [111]. The detailed criteria for initiating TPT are outlined in WHO consolidated guidelines on TB preventive treatment [70]. These guidelines emphasized that individuals with recent contacts are one of the key targets for TPT intervention, as those recently infected individuals are at a heightened risk of progression to active disease and LTBI constitutes the largest reservoir of TB.

Prior to initiating a TPT regimen, a TST or IGRA is typically recommended as part of the a "test-to-treat" approach in TB screening and contact tracing measures [115], hence, this provides a reference to clinicians to inform their decision about TPT initiation in an individual. Positive TST and IGRA results strongly indicate the need for TPT, which can significantly reduce the risk of developing an active TB when used effectively [116]. A study in 2021 demonstrated that the risk of disease progression is threefold higher in TST converters, and schoolchildren who received TPT had a 79% lower risk of developing TB, with protection being particularly effective (93% risk reduction) in recent contacts [21]. However, TST and IGRA results present certain concerns because their positivity can persist for long durations and may potentially remain elevated for life. This persistent elevation may not necessarily indicate an active infection, and thus, do not accurately reflect the needs for TPT [116, 117]. Consequently, from a clinical perspective, these limitations can complicate decisions regarding TPT implementation, potentially leading to unnecessary treatment that expose individuals with adverse drug reactions or missed opportunities to prevent the development of active TB [118, 119].

The limitation of current diagnostic test, specifically their inability to distinguish between recent and remote TB infections, are often overlooked when evaluating treatment decisions versus resource allocation. Treating LTBI requires a lengthy course of antibiotics which is costly and burdensome for both the patient and healthcare system. Although studies have shown that providing TPT is cost-effective compared to treating TB disease in the future [120], the cost of scaling up TPT with contact investigation to all TB contacts can be substantial. In countries such as Congo and Pakistan, it represents more than 50% of total TB care budget [121]. Additionally, an estimation done in 2023 shows that the cost for TB tracing and TPT provision in high TB-burden countries amounts to 6.7 billion USD, far exceeding the total TB care funding of all countries combined, which is 5.4 billion USD [122]. This estimate however, does not include the costs associated with the mis-prescription of TPT due to inaccurate TST and IGRA result interpretation. If these cost were included, the total cost would far exceed the 6.7 billion USD estimate.

Apart from cost, the drug supply chain and availability further complicate resource allocation for TPT. When TPT is not precisely supplied to high-risk LTBI patients, it not only leads to ineffective TPT allocation but also results in significant resource wastage [123]. The challenge in drug supply and availability is universal and

particularly significant in resource-limited settings [124, 125]. Drug shortage is a common reason for discontinuing Isoniazid Preventive Therapy (IPT) in children, as observed in a community-based LTBI treatment study in Ethiopia. Isoniazid stockouts were also identified as the primary cause for low TPT uptake [126, 127]. These issues underscore the need for resource optimization in TPT delivery, which can be significantly improved with accurate diagnostic tools capable of identifying high-risk individuals, such as those who have been recently infected.

Promising strategies in overcoming the limitations

Host immune profiling

Immune profiling plays a pivotal role in advancing our understanding of host immune response dynamics over time, particularly in the context of TB and LTBI diagnosis [128, 129]. Current LTBI diagnostics are based on immunological principles, emphasizing the importance of immunological research in TB, though these tools are not yet optimal. Therefore, continued research into immune profiling remains crucial despite the limitations of current methods. By analysing the complex interactions between MTB and the host immune system, immune profiling can provide a detailed perspective on disease progression and treatment responses. This approach involves a comprehensive assessment of immune cells, cytokines, chemokines, and cell surface markers, offering insights into how these components vary at different stages of TB infection [130, 131]. Such detailed immune profiles have the potential to identify novel biomarkers for more accurate LTBI diagnosis, overcoming current limitations and facilitating earlier intervention and improved clinical outcomes.

Given the limitations of existing diagnostics in distinguishing recent from remote infections, researchers have tried to refine these methods. For example, a 2010 study by Krummel et al. improvised the ELISpot assay by measuring IL-2 production in T cells stimulated with ESAT-6 and CFP-10. This approach is based on the principle that CD4 + CD45RA- CCR7- effector memory cells rapidly produce IFN-γ in response to these antigens, while central memory T cells, which may persist post-treatment, predominantly produce IL-2 upon re-stimulation. [132]. Simultaneous measurement of IL-2 and IFN-γ could potentially identify individuals who were treated and then recently reinfected with TB, enabling rapid intervention [133]. Another study in 2020 identified that high proliferative CD4 + T-cell responses to CFP-10 and PPD, coupled with low responses to ESAT-6, are specific indicators of recent latent infection when measured early after exposure [134].

MTB is an intracellular pathogen primarily triggers a cellular-mediated immune response, with CD4 + T cells playing a crucial role in controlling MTB in the early phase of infection [135, 136]. This theory proved evident showing the loss of CD4 + T cells can result in progressive TB disease, reactivation of LTBI and enhanced susceptibility to reinfection [137–139]. Thus, extensive ongoing research focused on the mechanisms of CD4 + T cells such as cytokine co-expression profiles, T cell differentiation and T cell activation for their involvement in its dynamic immune response for controlling TB infection and their diagnostic applicability [140–142]. Recent studies highlights the value of CD4 + T cell activation markers, such as HLA-DR expression and ΔHLA-DR median fluorescent intensity (MFI), as promising biomarkers for distinguishing recent from remote MTB infections demonstrating high specificity and sensitivity [24, 25]. A multidimensional analysis incorporating machine learning has further confirmed by identifying HLA-DR expression on ESAT6 and CFP10 specific Th1 cells as a robust biomarker for this differentiation [26]. Additionally, CD4 + TEFF cells producing TNF-α, but not IFN-γ or IL-2, have been identified as a potential biomarker, with the ability to distinguish recent from remote infections with 100% sensitivity and 95% specificity [143].

Although CD4 + T cells were initially considered the primary mediators of immune defence against MTB infection, recent studies have demonstrated the significant protective role of CD8 + T cells. Depletion of CD8 + T cells in chronic infection has been shown to increase bacterial burden in murine models [144], highlighting their importance. Subsequent findings have further supported their role in conferring protection by producing IL-2, IFN-γ and TNF-α, which are crucial for controlling MTB infection [136, 145]. Given these insights, researchers have increasingly focused on the diagnostic potential of CD8 + T cells.

The recent FDA-approved QuantiFERON-TB Gold Plus (QFT-Plus) assay, which includes an additional tube to quantify IFN-γ production by CD8 + T cells upon antigen stimulation, claims to detect recent MTB exposure based on the premise that acute antigen load in early infection leads to an increase in CD8 + T cells [146, 147]. However, the test results vary; while some studies suggest its potential to differentiate between recent and remote infections [148–150], others find it inadequate to reliably distinguish infection stages [151, 152]. Despite this debate, the potential of CD8 + T cells remains promising. A study in 2013 demonstrated that combining flow cytometry and QFT testing might improve classification between the infection states, showing that CD8 + / CD69 + /IFNγ + T cells are significantly higher in recent infected individuals when stimulated with QFT antigens

compared to those with remote or active TB [149]. Building on this, the applicability of tetramer technology could also be explored to detect MTB-specific CD8 + T cells in distinguishing recent and remote infections as this method is highly specific and are able to detect antigen-specific T cells even with limited capacity to mount cytokine response, shedding light for a more accurate diagnosis in HIV/TB co-infected patient [153].

In short, the integration of multiple detection methods such as flow cytometry, QFT testing and tetramer-based assay for immune profiling on CD4 + and CD8 + T cells could potentially capture a more defined dynamics of immune response in early infection, ultimately enhancing infection stage detection and aiding in TB management.

Dormancy antigens

The diagnosis of LTBI has always been hinging on detecting immune responses to specific mycobacterial antigens, making the selection of these antigens critical for both identifying LTBI and developing assays that can differentiate between infection stages. A significant body of research has been dedicated to discovering mycobacterial antigens that are naturally expressed during the latent phase of TB infection with TB dormancy/latency antigens being one of the promising antigens of study. As mentioned earlier, initial TB infection are usually followed by a dormant or non-replicating state of this bacteria within the host known as LTBI. This capability is highly important in MTB to survive in the host for long duration and to evade the immune system [154, 155]. Studies have identified over 100 antigens associated with LTBI, categorized into six main groups: dormancy survival regulon antigens, reactivation antigens, nutrition starvation-associated antigens, resuscitation-promoting factor antigens, toxin-antitoxin system associated antigens and others [154, 156–158]. Among these, dormancy survival regulon antigen that are regulated by DosR regulon have gain particular attention as potential biomarker for LTBI diagnosis due to their role in facilitating the bacteria's transition into a dormant state [155, 159–161].

Recent studies suggest that using antigens from the DosR regulon as stimulating agents can reliably differentiate between active TB and LTBI individuals [160, 162, 163]. Building on these promising results, researchers have begun to explore their potential in distinguishing recent from remote infections. One example is the Rv2626c latency antigen, which has shown potential in differentiating recent and remote infections by analysing IFN-γ production following Rv2626c stimulation [27]. Additionally, Rv2628 has been found to induce a higher IFN-γ response in T-cells from individuals with remote infections or those cured of TB, compared to those with recent infections, regardless of the incubation period [28]. Although studies on the use of latency antigens to differentiate recent from remote infections are still limited and require further validation, the promising findings suggest that incorporating these antigens into contact screening could enhance clinical decision-making in the provision of TPT.

Molecular methods

Gene expression signatures have garnered significant attention in recent years due to advances in molecular techniques in infectious disease research, particularly for monitoring disease activity through blood transcriptomic studies [164]. Evidence suggests that the transcriptomic profiles of specific immune-related genes are altered in response to MTB infection and disease, with distinct patterns of up- and down-regulation documented in various reviews [165, 166]. Current research in blood transcriptomics largely falls into four major categories: diagnosing LTBI [167–169], triage testing for active TB [170, 171], monitoring treatment response [172–175], and predicting risk of progression to active TB [29, 166, 176]. These studies underscore the clinical potential of differential transcriptomic expression in providing valuable insights for TB management.

However, there is a relative paucity of research focused on identifying molecular biomarkers to distinguish between recent and remote TB infections. Some studies, though limited, have shown promise in this area. For instance, a 2022 study identified differentially expressed genes (DEGs) related to cytokine signalling, signal transduction, neutrophil degranulation and other genes among newly infected prisoners, compared to non-infected prisoners and those with active TB [177]. Although this study did not directly compare DEGs between recent and remote infections, the identified DEGs may hold potential for further investigation. Additionally, a 2020 study identified 186 gene signatures that were differentially expressed in recent TB-exposed household contact compared to those without recent exposure, highlighting the potential value of transcriptomics in contact tracing and differentiating between recent and repeated TB exposures, especially in high-burden settings where IGRA and TST positivity are high [178]. Another study in 2020 explored whole blood transcriptomic responses in mice, macaques, and human with recent and remote infections. It demonstrated promising findings in both mice and macaques, and identified six gene signatures in human capable of providing temporal resolution for the timing of TB infection [178].

As a summary, the promising findings suggest that blood transcriptomic analysis has the potential to offer temporal resolution, demonstrating significant utility for contact tracing and TB management especially in

countries with high TB burden. However, further validation with larger cohorts is necessary to confirm its clinical applicability (Fig. 1).

Conclusions

TB remains a significant global health threat, and accurately distinguishing between recent and remote infections is critical for timely disease management. This distinction is crucial to ascertain appropriate preventive treatment and to guide accurate public health interventions. Despite decades of efforts and progress in LTBI diagnostics, identifying the precise timing of infection remains challenging, as many patients cannot recall their exposure to MTB. The lack of a standardized definition for recent and remote infections across studies further complicates the search for effective biomarkers. This

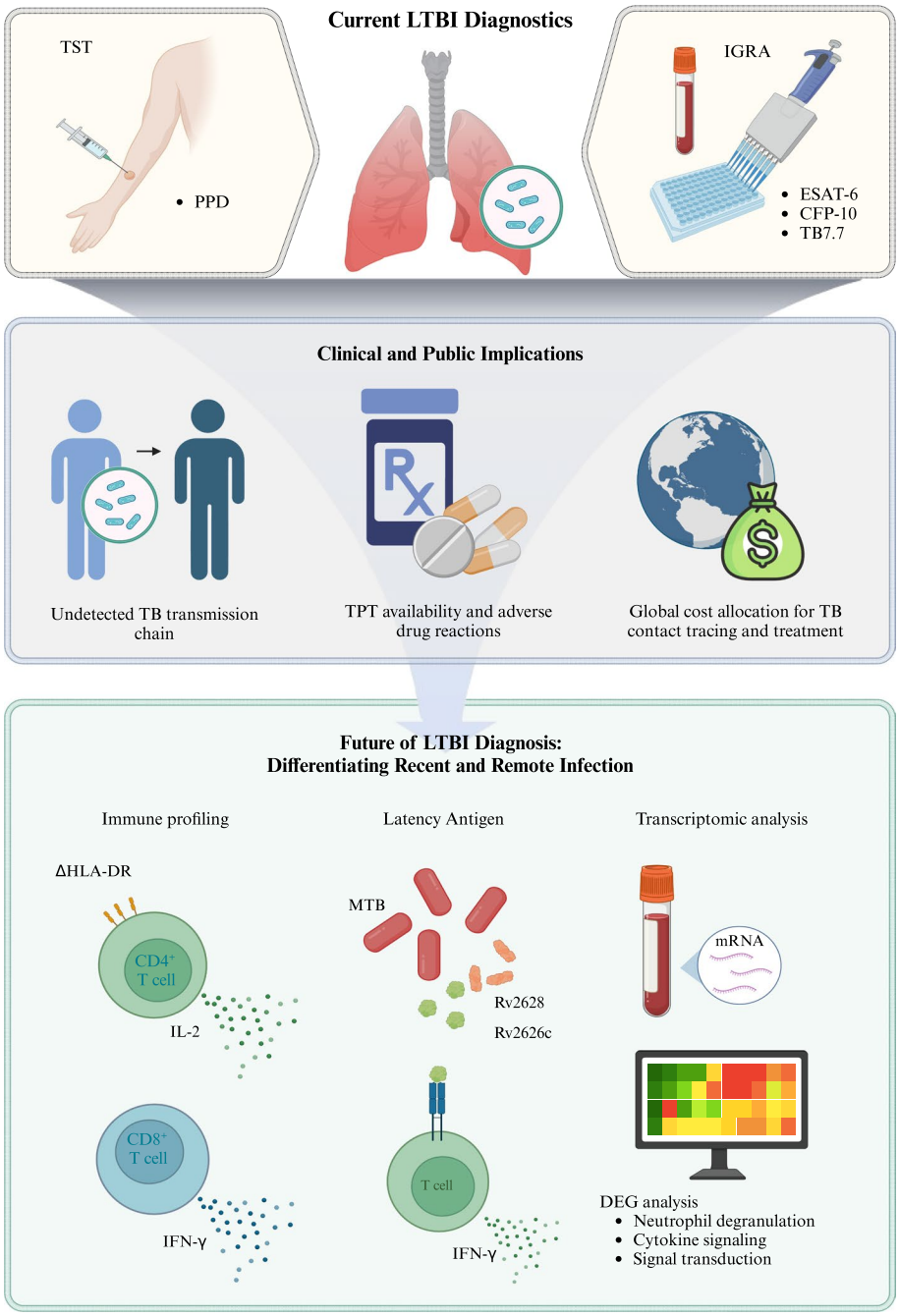

Fig. 1 Overview of current diagnostic tools for LTBI, highlighting existing challenges and future advancements for differentiating recent versus remote infections. Created in BioRender. Ding, Y. (2024) https://BioRender.com/h84i055

uncertainty impedes efforts to monitor disease progression and transmission, thus affecting timely TB control measures.

The pursuit for reliable biomarker through immune profiling has potential but is fraught with challenges as these biomarkers indirectly detects MTB through an individual immune response, hence are greatly influenced by individual variability against MTB. Similarly, dormancy antigens, which are used as stimulating antigens, rely heavily on indirect immune responses rather than direct pathogen detection [179]. The development of novel diagnostic biomarkers, particularly those utilizing transcriptomics technology, also encounter significant barriers. These tests, while promising, require extensive validation across diverse populations and are hindered by high cost and logistical challenges, including the need for sophisticated equipment and skilled technician. Hence, these tools might not be able to meet operational and pricing targets when translated into clinical use products with high cost and low point of care utility especially in contact screening and triage process [180]. Such requirements make these tests particularly challenging to implement in low- and middle- income countries where TB is most prevalent.

Addressing these challenges requires establishing well-defined standards for the terms "recent" and "remote" infections and standardizing cutoff points across countries with varying TB burdens. Such standardization will allow for more consistent interpretation of results across studies, reducing variations in cutoff points and enhancing the accuracy of biomarker utility assessments [180]. A multidisciplinary approach that integrates immunology, genomics, epidemiology, bioinformatics and machine learning is essential for advancing LTBI diagnostics [154]. Collaboration with public health experts is crucial to ensure that new diagnostic tools are both scientifically rigorous and practical for real-world application. Additionally, more longitudinal studies involving high-risk populations are vital for identifying reliable biomarkers and refining TB risk prediction. Through these concerted efforts, the global health community can achieve significant advancements in TB diagnostics and control.

In this review, we have summarized the significance of distinguishing between recent and remote TB infections in the context of TB control and elimination strategies. Current studies mainly focused on immune profiling, with the HLA-DR cell surface marker emerging as a promising biomarker for differentiating between recent and remote infections. Additionally, cellular responses to dormancy antigens and transcriptomic studies on cellular responses during infection have been explored for their potential to distinguish between recent and remote latent TB infections, with promising results. Continued research and refinement of these approaches are crucial for advancing TB diagnostics and improving disease management.

Abbreviations

TB	Tuberculosis
MTB	*Mycobacterium tuberculosis*
LTBI	Latent tuberculosis infection
ATB	Active tuberculosis
WHO	World Health Organization
TST	Tuberculin skin test
IGRA	Interferon-gamma release assay
BCG	Bacillus Calmette-Guérin
TPT	Tuberculosis preventive therapy
PPD	Purified protein derivative
DTH	Delayed-type hypersensitivity
RD1	Region of difference 1
QFT-GIT	QuantiFERON-TB Gold In-Tube Test
ESAT-6	Early secretory antigen target-6
CFP-10	Culture filtrate protein-10
HIV	Human immunodeficiency virus
IFN-γ	Interferon-gamma
Anti-TNFα	Tumour necrosis factor
LMICs	Low- and middle-income countries
IPT	Isoniazid preventive therapy
IL-2	Interleukin-2
MFI	Median fluorescent intensity
QFT-Plus	QuantiFERON-TB Gold Plus
FDA	Food and drug administration
DEG	Differentially expressed gene

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions
YED is responsible for conceptualization, writing the original draft, reviewing and editing. MTJW is responsible in review and editing. MNN is responsible for review and editing. VB is responsible in review and editing. GJT is responsible for conceptualization, funding acquisition, supervision, review and editing.

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