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REVIEW

Advances in the Diagnosis of Latent Tuberculosis Infection

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Abstract: Latent tuberculosis infection (LTBI) is a critical stage of tuberculosis infection in which Mycobacterium tuberculosis (MTB) is dormant and does not cause active disease. Traditionally, the most commonly used clinical methods for diagnosing LTBI have been the tuberculin skin test (TST) and the interferon-gamma release assay (IGRA). Recently, however, novel skin tests, molecular biology techniques, and cytokine biomarkers have been developed. This review summarizes the latest research on the diagnosis of LTBI, highlighting new tools and methods to improve detection and differentiation from active tuberculosis(ATB).

Keywords: latent infection, tuberculosis, diagnosis, public health, review

Introduction

TB, an infectious disease caused by MTB, is a serious public health problem with TB as the main form. China is one of the high-burden countries in the world for TB, ranking third in the incidence of TB and second in the number of reported cases.[1] In the process of TB prevention and control, the problem of latent infection has received more and more attention. The prevalence of LTBI is notably high in the Middle East, certain regions of Asia, and sub-Saharan Africa, China has a large LTBI population, and studies show that China and India have the highest burden of LTBI, with the LTBI rate in China being about 26%, the relatively low prevalence of LTBI in the United States and in Europe, 5–10% of the LTBI population may develop ATB during their lifetime, representing a potential "reservoir" of TB patients.[2–4] Diagnosis of LTBI is a key component of TB prevention and control, helping to identify and treat patients at an early stage, thereby limiting the spread of TB. There is no accepted gold standard for the diagnosis of LTBI. The immune response of LTBI patients differs significantly from that of ATB patients, but the difficulty in accurately differentiating between LTBI and ATB with available testing techniques, coupled with the lack of specific markers, makes it very difficult to diagnose and monitor LTBI. In recent years, with the rapid development of molecular biology technology, some new detection methods with higher sensitivity and specificity, rapidity, simplicity, and ease of operation, such as RNA sequencing and proteomics, are gradually being applied to the diagnosis of latent infection. RNA sequencing has revealed the complexity and diversity of gene expression during latent infection, and proteomic technologies have improved diagnostic accuracy by finding specific protein markers, providing a basis for understanding the pathogenesis of tuberculosis and developing new diagnostic and therapeutic strategies. This paper reviews the diagnostic methods of LTBI to explore new ways for TB prevention and control.

Definition of LTBI and Diagnostic Criteria

The diagnostic issue of LTBI is also contingent upon the definition and diagnostic criteria for latent infection. Currently, there are some discrepancies in the definition and diagnostic criteria for LTBI.

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Definition of LTBI

In our "Classification of Tuberculosis" - (WS196-2017) standard LTBI patients are defined as a body infected with Mycobacterium tuberculosis in the organism but have not developed clinical tuberculosis and have no evidence of active tuberculosis in terms of clinical bacteriology or imaging.[5] The World Health Organization (WHO) defines LTBI as a state of sustained immune response to previously acquired Mycobacterium tuberculosis antigens with no evidence of active tuberculosis and no clinical manifestations.[6] The US Centers for Disease Control and Prevention defines LTBI as a person who is infected with Mycobacterium tuberculosis but has no clinical signs or symptoms of active tuberculosis.[7] Differences in definitions may lead to different selection of target populations and thus affect clinical management.[8–10]

Diagnostic Criteria for LTBI

The diagnosis of LTBI in china is based on the "China Technical Specifications for Tuberculosis Prevention and Control (2020 edition)": 1) Mycobacterium tuberculosis infection was considered in children under 5 years of age without a history of BCG vaccination, who were HIV-positive, who had received immunosuppressive drugs for >1 month, and who had close contact with pathogenetically positive TB patients who reacted to hard nodules ≥ 5 mm with a tuberculin-pure protein derivative (PPD); 2) Those with a history of BCG vaccination were considered to be infected with Mycobacterium tuberculosis by PPD reaction of hard nodules ≥10 mm; 3) a positive recombinant bacillus binding fusion protein (EC) test that indicates infection by Mycobacterium tuberculosis (a reaction with an average diameter of ≥5mm is considered a positive reaction); 4) a positive γ interferon release test indicates Mycobacterium tuberculosis infection; 5) no clinical symptoms other than the above Mycobacterium tuberculosis infection described above.[11] It is important to note that different threshold criteria (eg, ≥5 mm vs ≥10 mm) might lead to underdiagnosis or overdiagnosis in certain populations.[12,13] At a threshold of ≥5 mm for the response, sensitivity is reduced. Some individuals with weaker immune responses may have PPD responses below 5 mm, thus missing out on early diagnosis or even under-diagnosis. Conversely, individuals with stronger immune systems may have stronger responses to PPD, even in the absence of MTB infection, leading to over-diagnosis. A response at a threshold of ≥10 mm enhances specificity, reducing false positives but potentially leading to underdiagnosis by misclassifying some individuals who are actually infected as negative. Overdiagnosis may occur in high-risk or high-exposure settings. The diagnosis by WHO of LTBI is that individuals at high risk are asked about symptoms related to tuberculosis, if asymptomatic, TST or IGRA is conducted, those who are given a result have a positive chest X-ray, and if they do not have any abnormalities, they are considered to have LTBI.[14] The TST test is prone to false-positive results due to BCG inoculation. In this case, IGRA is usually selected and does not depend on the skin reaction. The diagnosis of LTBI in the United States is based on information gathered from medical history, tuberculosis testing, chest radiographs, physical examination, and in some cases, sputum examination, and, according to the Centers for Disease Control and Prevention Latent Tuberculosis Infections: A Guide for Primary Health Care Providers, 1) there are no symptoms or physical findings suggestive of tuberculosis; 2) the results of a tuberculosis blood test or TST are usually positive; 3) chest radiographs are usually normal; and 4) if testing is performed, respiratory specimens are smear and culture negative; 5) no transmission of TB organisms to others; 6) treatment of LTBI should be considered for TB prevention.[15]

The disparate definitions and diagnostic criteria that exist across different countries have an impact on the diagnosis and treatment of LTBI. It is, therefore necessary to further harmonize and coordinate these criteria and guideline policies to improve diagnostic and treatment outcomes on a global scale. Countries should actively participate in the development and updating of international guidelines for the diagnosis and management of LTBI, and establish a unified framework for diagnosis and treatment through international cooperation; develop consensus guidelines through cross-border data sharing and cooperation to coordinate actions across countries.

LTBI Diagnostic Techniques

Diagnostic Techniques in Immunology

In the field of medicine, the term "gold standard" is typically employed to denote the most accurate and reliable reference standard for the evaluation of novel diagnostic tests, procedures, or methods.[16] Notably there is currently no

internationally recognized gold standard for the diagnosis of LTBI and the two methods recommended by the WHO are the tuberculin skin test (TST) and the gamma-interferon release assay (IGRA).[4]

Tuberculin Skin Test (TST)

The TST is a traditional diagnostic method based on the principle of a type IV allergic reaction. It is an in vivo skin test that uses pure protein derivatives of tuberculin to induce a hypersensitivity reaction. Following infection with MTB, the human body will develop corresponding sensitized lymphocytes. Upon encountering the mycobacterial proteins of MTB once more, the sensitized lymphocytes will be activated and secrete a variety of soluble lymphokines. This will lead to an increase in vascular permeability and result in the accumulation and infiltration of macrophages in the local area.[17,18] All of our TSTs employ the use of PPD.[19] By injecting tuberculin into the subcutaneous and observing whether the injection site produces a red and swollen reaction, hard nodules are expressed as half the sum of the maximum transverse diameter and the maximum longitudinal diameter, according to the "Diagnosis of pulmonary tuberculosis WS288-2017", the criteria for determining LTBI are: a) those with an average diameter of hard nodules <5mm or no reaction are negative; b) those with an average diameter of hard nodules ≥5mm are positive; c) those with an average diameter of hard nodules 5–10mm are generally positive; d) those with an average diameter of hard nodules 10–15mm are moderately positive; and e) those with an average diameter of hard nodules ≥15mm or with the presence of localized double circles, blisters, necrosis and lymphangitis are strongly positive.[20]

The two principal skin tests employed in China are the BCG-PPD (Bacillus Calmette-Guerin Pure Protein Derivative) and the TB-PPD (Tuberculin Pure Protein Derivative).[21] The BCG-PPD preparation is derived from the culture filtrate of BCG and an accelerated stability test has demonstrated that BCG-PPD exhibits satisfactory long-term stability.[22] TB-PPD is a protein extracted from the human-type pathogen MTB.[23] The two methods differ in terms of their sensitivity and specificity, the study conducted by Huimin Zhang demonstrated that the overall positivity rate of the TB-PPD reagent, as well as the positivity rate of sputum smear-negative specimens, was markedly higher than that of the BCG-PPD reagent, while Qiang Liu's study showed that BCG-PPD in TB screening of students showed high and strong positivity rates. The decision regarding the use of BCG-PPD or TB-PPD in clinical practice should be based on a comprehensive assessment of the relevant factors. The TB-PPD approach is currently the preferred option for school-based TB screening.[24,25]

The TST is a widely utilized diagnostic tool in regions with a high prevalence of TB due to its cost-effectiveness and simplicity. However, the interpretation of TST results may be influenced by various factors, including non-tuberculous mycobacteria (NTM) infection and BCG vaccination. Additionally, subjective factors may also contribute to the variability in results, potentially leading to false-positive or false-negative outcomes.[18]

Given the limited specificity of the TST, there has been a push to develop alternative tests. This has led to the introduction of new skin tests, including the C-TB reagent developed in Denmark, the Diaskintest reagent developed in Russia, and the recombinant Mycobacterium tuberculosis fusion protein (EC) developed in China.[26] These reagents operate on the same fundamental principle. They are administered via injection into the dermis of the subject's forearm. In the event of a specific immune response to these proteins in the subject's body, a hard nodule will form at the injection site. The size of this nodule is then measured to ascertain whether the subject is infected with MTB.[27] The C-tb reagent is a mixture of ESAT-6 and CFP-10 in a 1:1 ratio, whereas the Diaskintest reagent is a recombinant ESAT6 and CFP10 fusion protein produced by transgenic E. coli BL21(DE3)/pCFP-ESAT.[28] EC is available in April 2020 and consists of ESAT-6 and CFP-10 to form a fusion protein in sequence, judged by the length of the transverse and longitudinal diameters of the erythema and sclerosis.[29] No erythema and no nodule or erythema and nodule average diameter <5mm is negative, erythema or nodule of the transverse diameter and longitudinal diameter of the average diameter of ≥5mm is positive, where there is blistering, necrosis, lymphangitis are strong positive reaction.[30] EC has been shown to improve specificity, up to 99.3% in one clinical trial, and is a candidate due to its simplicity, lack of laboratory facilities, low cost of the test, good stability and validity.[30,31] Mass treatment of LTBI may be less cost-effective in low-resource settings.[32]

Interferon-Gamma Release Assay (IGRA)

The IGRA is an in vitro assay that determines the presence of a bacterial infection based on the host immune response to specific antigens of MTB. This is achieved by measuring the level of gamma-interferon (IFN-γ) released by activated T-lymphocytes.[18] IGRA is comprised of two principal assays: enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot assay (ELISPOT), both employ early secretory antigen target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) as specific antigens. ELISA methods are employed to ascertain infection status by quantifying the amount of IFN-γ released by sensitized T cells. This is exemplified by the QFT-GIT assay. In contrast, ELISPOT methods utilize a different approach, whereby the number of sensitized T cells releasing IFN-γ is measured. This is exemplified by the T-SPOT.TB assay.[33,34] A positive IGRA result is indicative of the presence of an MTB infection. However, the definitive diagnosis of LTBI necessitates a comprehensive assessment that considers the clinical presentation and the exclusion of several NTM, including Mycobacterium kansasensis, Mycobacterium maritimus, and Mycobacterium sulphuricum.[35] The interpretation of IGRA results is contingent upon the patient's immunosuppressive status. Consequently, these results must be assessed on a patient-by-patient basis. In the event of inconclusive results, it indicates that the presence of effector T cells specific for MTB cannot be determined. In such instances, the experiment must be repeated. False-positive results can lead to unnecessary investigations and therapeutic measures, such as the use of anti-TB drugs, which not only increase the financial burden on the patient, but can also lead to an increase in drug side effects and drug resistance, in addition to neglecting other underlying diseases.[36,37] False negatives can lead to denial of appropriate treatment, worsening of the patient's condition and risk of complications, and can increase the complexity and difficulty of treatment, resulting in a wider spread of TB. It may also increase the complexity and difficulty of treatment, leading to more widespread transmission and, in some cases of severe TB, delayed treatment leading to higher mortality and poorer prognosis.[37–39]

The specificity and sensitivity of IGRA are superior to those of TST. The sensitivity of TST is generally within the range of 85% to 94%, with a maximum of 95% in selected studies, while its specificity ranges from 27.6% to 82.1%.[40–42] In contrast, the sensitivity of IGRA is typically within the range of 85% to 90%, with a high specificity of up to 96%.[43] There are significant variations in the use of IGRA across different regions and populations. In foreign countries, particularly in high-income countries such as the United States and some countries in Europe, IGRA has become the preferred diagnostic method for LTBI.[44] High-income countries tend to have more stringent TB prevention and control measures, IGRA is not affected by BCG vaccination.[45] High-income countries tend to have better health systems and infrastructure; IGRA does not require recalls and testing procedures are standardized, which greatly improves the efficiency of screening; and despite the high initial cost of IGRA, its high accuracy effectively reduces the need for unnecessary follow-up testing and treatment. IGRA can provide more accurate testing to identify immigrants who may be carrying TB infection for appropriate public health interventions.[46] In China, however, the higher cost and more complex laboratory operations associated with IGRA have limited its application, with TST remaining the primary method for diagnosing LTBI.[47] This discrepancy may be attributed to the high cost and complex operational constraints of IGRA, as well as the relatively low level of interest in LTBI within the country. A study from China showed that when other examination costs are the same, just calculating the sum of reagent and test costs, the lowest cost of TST is no more than one hundred, the EC cost is in the middle, and the highest cost of IGRA is ten times that of TST.[48] In low-income countries, TST is the most cost-effective choice, and in high-income countries or regions, the cost-effectiveness of IGRA is usually superior to that of TST, but for high-risk immigrants when screening, the savings of TST will be greater than that of IGRA.[46] The combined use of TST and IGRA strategy is considered the most cost-effective in some country regions, and this two-step combined screening method utilizes the low-cost advantage of TST upfront and improves specificity later by IGRA.[49,50]

Both TST and IGRA depend on the activation of T lymphocytes and are only capable of detecting the production of an immune response specific to MTB. They are unable to determine the presence of active mycobacteria within the body. It is challenging to differentiate between patients with LTBI and those with ATB based on positive test results. Additionally, it is difficult to distinguish between recent infection and long-term persistent infection, which requires careful consideration in clinical practice.[51] This means that even if the test result is positive, there is no certainty whether

the person has LTBI or has developed TB, which may lead to missed or misdiagnosed cases in the clinic; if a person with TB is not detected in time, there may be an increased risk of transmission; and if a person with LTBI is misdiagnosed as having TB, unnecessary anti-TB treatment leads to unnecessary drug side effects and treatment costs. People with LTBI are generally not infectious and need to be treated with prophylaxis, while those with TB need to be started immediately on standard anti-TB treatment and isolated until infectiousness is eliminated. Physicians must combine positive results with other clinical information, such as chest x-ray and sputum, to make more accurate diagnoses and treatment decisions.

Molecular Biology Testing Techniques

RNA Sequencing

In recent years, RNA sequencing has been increasingly employed for the diagnosis of latent infections, particularly in the context of long-stranded non-coding RNA (lncRNA) expression profiling, thereby facilitating the identification of novel biomarkers for the diagnosis of. It was demonstrated that the high-throughput detection of lncRNA expression profiles in the plasma of patients with LTBI using lncRNA microarray technology can facilitate the establishment of gene expression profiles in individuals with latent infection.[52] By analyzing exosomal RNA expression patterns in blood, molecular markers associated with latent infection can be identified.[53] Furthermore, the presence of LTBI can be discerned through the identification of specific microRNAs in serum. These microRNAs have the potential to serve as biomarkers for the diagnosis of latent infection.[54] However, the complexity of the experimental operation and the high equipment requirements and costs limit the popularity of large-scale clinical applications.[55] In addition, the laboratory operation is susceptible to sample contamination, and the degradation of RNA affects the accuracy of the technical operation, the analysis of the results requires the use of bioinformatics tools, which undoubtedly increases the complexity and time cost of the study.[56]

The current status of RNA sequencing of LTBI demonstrates advancements in research at the lncRNA and miRNA levels. However, the RNA sequencing methods currently employed are less clinically relevant, and the veracity of the relevant studies remains to be verified.

Proteomics

The application of proteomics technology offers a novel methodology for the diagnosis of LTBI. This approach involves the detection and identification of proteins and their interactions that are closely associated with the development of disease.[57] The prolonged carriage of pathogens in individuals with latent infection may result in alterations to the expression of associated proteins. These altered proteins may serve as potential biomarkers for diagnosis and differential diagnosis.[58] In a study, non-tagged proteomic techniques were employed to identify differential proteins in the plasma of patients with TB and those with LTBI, with a sensitivity and specificity of 75.0% and 96.1%, respectively. It was observed that α-1-antitrypsin, α-1-acid glycoprotein 1, and E-calcimucil exhibited greater capacity to differentiate between patients with ATB and those with LTBI.[59]

This technique can detect the occurrence and progression of LTBI at the protein level, offering the benefits of high sensitivity and good specificity.[60,61] However, this technology requires expensive equipment and complex experimental procedures that require specialized personnel for operation and data analysis, which undoubtedly increases the economic and labor costs of the experiments.[62] Proteomics technologies may be more cost effective than RNA sequencing in LTBI, especially in mass screening, due to their lower cost and relative ease of operation.[63] Currently, there is a paucity of research examining this technique for the diagnosis of LTBI, and further experimentation is required to elucidate its potential.

Although molecular biology techniques have shown promise in the diagnosis of LTBI, the lack of relevant research may result in limited practical clinical applications and potential clinical risks.

Advances in Biomarker Cytokine Research

The LTBI represents an intermediate state in the process of TB infection and a manifestation of long-term latency of MTB in the organism. MTB stimulates macrophages to produce cytokines, which are associated with the activation and

differentiation of T lymphocytes and mediate the immune response. The main cytokines identified thus far are interferons (IFNs), interleukins (ILs), tumor necrosis factors (TNFs), and chemokines.[64,65] To date, over a hundred IL analyses have been conducted, with the most prevalent investigations focusing on IFN-γ, IL-6, IL-4, IL-2, and other related cytokines. IL is an important factor regulating the immune system, affecting the progression of LTBI by regulating immune responses and cellular functions and its mechanisms in immune evasion are mainly immunosuppressive cytokines, regulatory T cells, etc.[66] The role of IFNs in promoting the development of LTBI is mainly manifested in their regulation of the immune system and the ability to pathogen inhibitory ability to achieve immune evasion through inhibition of interferon signaling, alteration of host cell phenotype, and so on.[67]

Interferon-Gamma (IFN-γ)

Immunogenicity and protective efficacy of MPT83 protein vaccine evaluated in study, the MPt 83 antigen, which measures IFN-γ, was found to be an effective method for differentiating TB from LTBI, thereby enhancing the sensitivity and specificity of LTBI screening.[68] The IFN-γ release assay demonstrates superior predictive capacity in comparison to the conventional TST.[69] Serum IFN-γ levels of 246 subjects in the three groups were examined, and the results showed that serum IFN-γ levels were abnormally higher in the TB patients than in the LTBI group, and higher in the TB and LTBI groups than in the control group.[70] This indicates that elevated levels of IFN-γ may be more closely associated with ATB disease. It can thus be concluded that IFN-γ is not a sufficient criterion for diagnosing LTBI. The immune responses induced by MTB exposure are not limited to the release of IFN-γ, and the differentiation between active TB and LTBI can be enhanced by measuring other cytokines, such as IL-2, induced by MTB antigens.[71]

Interleukins (IL)

Interleukin-6 (IL-6)

During bacterial infection, IL-6 functions as a pivotal signaling molecule, orchestrating the initiation and sustenance of the immune response. Changes in IL-6 levels were strongly associated with the onset and progression of LTBI, in a study in which 259 subjects were recruited as TB patients, household contacts, and healthy controls and blood samples were taken to measure their IL-6 levels, and in a study in which changes in the Th2-type cytokine IL-6 were measured in TB, LTBI, and healthy populations, with lower levels in patients with LTBI compared to those with ATB, and higher levels in the LTBI group compared to healthy controls.[72,73] Cytokine analysis of TB patients and asymptomatic contacts in Ghana demonstrated the efficacy of IL-6 in particular in improving the detection of MTB infection and the categorization of TB patients and healthy contacts.[74] In a study recruiting smear-positive patients and healthy subjects to measure plasma IL-6 levels by ELISA Cytokine, also found that plasma IL-6 expression was significantly higher in TB patients than in healthy controls and the LTBI group.[75] A meta-analysis provided a more accurate estimate of the association between IL-6 gene polymorphisms and the risk of tuberculosis.[76] These findings indicate that IL-6 may serve as a potential biomarker for differentiating between ATB and LTBI. However, it is possible that there may be some overlap in the expression levels of IL-6 across different groups, suggesting that combining it with other cytokines may enhance diagnostic accuracy.

Interleukin-4 (IL-4)

In their study of IL-4, Yang Xiaoyue et al detected changes in the levels of the Th2-type cytokine IL-4 in the TB, LTBI, and healthy populations and found that the levels of IL-4 increased in all three groups in a sequential manner and observed that IL-4 levels were lower in patients with ATB than in those with LTBI and in the control group. Furthermore, IL-4 levels in the LTBI group were also lower than in the control group, representing a distinct pattern of change from that observed for IL-6 and IFN-γ.[73] Measurement of blood samples from 245 TB patients, 80 people with LTBI and 100 healthy controls showed that IL-4 levels were higher in the TB group and lower in the LTBI group, which may affect TB versus LTBI.[77] A systematic review and meta-analysis showed differences in serum IL-4 between healthy individuals and patients with TB, and that patients with TB may also have higher levels of IL-4.[78] In instances where non-specific symptoms, such as fever, night sweats, lethargy, and malaise, manifest in individuals with LTBI, the presence of LTBI can be ascertained through the detection of IL-4 levels. Nevertheless, research findings have yet to reach a consensus

regarding the significance of IL-4 in the context of tuberculosis. Further comprehensive studies are required to elucidate the mechanism of action of IL-4.[79,80]

Interleukin-2 (IL-2)

IL-2 is capable of acting directly on MTBs and stimulating their growth. Additionally, it is capable of activating the complement system and stimulating the immune response and differentiation of lymphocytes. Based on a systematic review and meta-analysis to assess the diagnostic potential of IL-2, the serum expression of IL-2 in individuals with LTBI is markedly elevated in comparison to the control group, which may be attributed to the augmented secretion of IL-2 by T cells in the patient's body.[81] However, the T cells exhibit diminished capacity to produce IL-2 in comparison to macrophages, resulting in a compromised immune response. Consequently, IL-2 is regarded as a reliable indicator for the diagnosis of LTBI. In the absence of a clearly defined gold standard, in addition to IGRA, IL-2 release assays have the potential to enhance the capacity of IGRA to identify individuals with LTBI.[82] A foreign study showed that the accuracy of the test in LTBI subjects was 90% sensitivity and 97.5% specificity, and that IL-2 measurements help to identify patients at different stages of the disease.[83] The utilization of combined assays has the potential to markedly enhance the diagnostic efficacy of a given test when compared to the use of single cytokine assays. Of these, the combination of IL-2 and IFN-γ is the most commonly utilized.[84] Results of a study in which 190 healthcare workers were screened for LTBI suggest that long-term IFN-γ and IL-2 responses may help differentiate patients with LTBI from those without evidence of LTBI.[85] Sensitivity in confirmed TB (72.1%) was significantly higher than in clinically diagnosed TB (65.8%), with the highest sensitivity in parallel combinations at 87.9% and overall specificity at 79.8%.[86]

The presence of confounding factors may result in differences in cytokine assay results. Diabetic patients co-infected with LTBI show significant reductions in the levels of systemic and antigen-specific cytokines such as IFN-γ and IL-2, which may be related to the suppressive effect of diabetes on immune system function.[87] HIV patients co-infected with TB increase the levels of IFN-γ and IL-6, and the levels of these cytokines decrease with prolonged treatment.[88]

The immune system of infants and children is less mature in its immune response than that of adults, so their cytokine response is usually weaker, with lower levels of IFN-γ and IL-4, and conversely higher levels of IL-6, and older adults may have lower levels of IFN-γ, IL-2, IL-4, and often higher levels of IL-6 than younger adults due to the phenomenon of immune decline.[89] The immune system of HIV-infected individuals is usually in a state of sustained immune activation, and positive individuals have low levels of IL-2 and IFN-γ.[90,91] Areas with a high burden of TB tend to have high levels of IL-6, while levels of IL-4 may be influenced by parasitic infections or the nature of the immune response (eg, anaphylaxis), and levels of IL-4 are comparatively low in areas with a low burden of TB.[72,92]

Biomarker research is evolving rapidly, with a recent exploratory study demonstrating that latency-associated antigens improve the diagnostic ability of several cytokines for TB infection and that their combination with TB virulence factors significantly improves the ability of Th1-type cytokines to discriminate between ATB and LTBI.[93] A study of whole blood co-stimulated with ESAT-6 and CFP-10 demonstrated the potential of IL-2 in the diagnosis of LTBI by stimulating CD4 and CD8 T cell memory responses and the expression of certain biomarkers that can be used to differentiate between LTBI and healthy individuals.[94] A study developed and validated a new diagnostic biomarker, CP19128P, using immunoinformatics to explore the potential of these differentially expressed cytokines to serve as diagnostic biomarkers of MTB infection following CP19128P stimulation. ROC curves were performed and this marker was well characterized and thermally stable.[95]

Conclusions and Future Directions

Although TST and IGRA are widely used in students and other populations, they provide limited value in distinguishing LTBI from ATB. There is an urgent need to develop new diagnostic techniques for LTBI that can provide targeted screening for different characteristics and take into account patients with comorbidities. This will improve the specificity and sensitivity of the results. The diagnosis of LTBI needs to take into account a wide range of factors, including the patient's condition, underlying disease, and immune status. A diagnosis of LTBI necessitates the consideration of numerous factors, including the patient's condition, underlying disease, treatment effect, and immune status, among others. Despite the ongoing development of new diagnostic techniques, such as RNA sequencing and proteomics, which

offer significant advantages in the diagnosis of LTBI, numerous challenges remain to be addressed with urgency. The objective of future research will be to determine how these new technologies can be combined with traditional diagnostic methods to create a more comprehensive diagnostic method system. There is considerable variation in the screening and diagnostic methods for LTBI employed in different developed countries and regions. There is currently no unified standard or guidance, which also increases the complexity of prevention and treatment. There is an urgent need to strengthen coordination to establish a common standard. Further exploration of the individual and combined applications of cytokines is warranted, although the current evidence base is inconclusive. There is an urgent need for in-depth discussions to clarify the optimal biomarkers for screening and combination to enhance diagnostic efficacy in clinical practice.

Despite the numerous challenges inherent to the diagnosis of LTBI, it is anticipated that technological advancements, international collaboration, and policy optimization will facilitate enhanced innovation and ultimately contribute to the eradication of TB.

Author Contributions

All authors made a significant contribution to the work reported, whether in conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; participated in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; agreed to submit the article to the journal; and agreed to take responsibility for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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