• Review Article •

Characterization of and advanced diagnostic methods for ocular tuberculosis and tuberculosis

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Abstract

● Tuberculosis (TB) is an airborne infection caused by *Mycobacterium tuberculosis* that usually affects the lungs. Timely treatment of active TB, diagnosis and prevention of latent TB are very important. However, extrapulmonary TB affects almost any tissues around the eye and orbit, and it then requires a high degree of suspicion to accurately diagnose. Diagnostic delays are common and may lead to morbidity. For ophthalmologists and infectious disease specialists, it is important to work together to accurately diagnose and treat ocular tuberculosis (OTB) to prevent vision loss. This review reports the latest advanced diagnostic methods for active TB and latent TB as well as various known manifestations of OTB. Important elements of diagnosis and treatment are also reviewed.

● **KEYWORDS:** latent tuberculosis; ocular tuberculosis; diagnosis

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INTRODUCTION

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (Mtb), which causes a substantial amount of chronic disease and death throughout the world. Approximately one-fourth of the world's population is estimated to be infected by latent tuberculosis infection (LTBI), and approximately 3%-5% of these individuals develop active TB at some stage during their lifetime[1]. In addition, the recurrence of TB as a major public health problem increases the likelihood that ophthalmologists may encounter an increasing number of eye complications. TB with ocular manifestations can affect all parts of the eye, and without proper treatment, it may cause severe vision loss. Early diagnosis and prompt treatment are the keys to saving the sight of patients with ocular manifestations of TB. Despite intensive research efforts and great progress, it is still difficult to diagnose ocular tuberculosis (OTB) and active TB and LTBI rapidly and accurately, and predicting who is at risk of reactivation is even more difficult. The present review aimed to highlight the clinical spectrum of intraocular TB and more recent findings in the area of diagnosing OTB, active TB and LTBI and predicting reactivation in individuals. Meanwhile, the latest screening with prevention strategies has been introduced to contribute to furthering the research and treatment of TB.

OCULAR TUBERCULOSIS

TB is a multisystem disease that primarily affects the lungs in 80% of patients, while in the remaining 20%, TB may affect other organs, including the eye. The clinical manifestations of OTB are nonspecific and protean. In primary OTB, there are no other systemic lesions, and almost all primary ocular infections are confined to the conjunctiva and cornea. Secondary OTB is defined as an infection caused by contiguous spread from adjacent structures or blood-borne spread. Intraocular TB is usually associated with systemic disease and is therefore considered a secondary infection[2]. The most common OTB in patients with TB is choroiditis, anterior uveitis, retinal vasculitis, vascular occlusion, dense vitritis, and papillitis[3]. OTB is a major public health problem, and if not treated properly, TB with ocular manifestations may enter various parts of the eye and cause severe vision loss[2]. However, distinguishing noninfectious inflammatory eye disease from OTB is difficult, and current diagnostic techniques are not sufficiently accurate. As a consequence, OTB relies heavily on clinical diagnosis, the key points of which are the accuracy and timeliness of clinical concern.

Ocular Tuberculosis Epidemiology Mtb infection mainly occurs in Asia, of which India and China account for 38% of the world's TB infections and 5% in Europe[4]. In most cases, OTB occurs consecutively to TB reactivation many years after contamination and is caused by impaired immunity, such as that found in the elderly, patients with chronic renal failure or those on immunosuppressive therapy. Since the intraocular inflammatory process is mainly immunological, clinical improvement can be observed during steroid therapy, leading to misdiagnosis of OTB and frequent tuberculin skin test (TST) and interferon-gamma release assays (IGRA) examinations of steroid patients. Active extraocular TB with bacterial lesions is rare, especially in areas with low TB prevalence, resulting in very low sample collection. The diagnosis of TB-related ocular inflammation is mainly speculative. Tuberculous uveitis (TBU) is the most common ocular manifestation of the disease. It has been reported that 10.5% of all cases of uveitis are present in high-risk areas and between 1% and 7% in low-risk areas and are on the rise[5]. Furthermore, TBU is a vision-threatening disease that inevitably leads to blindness if not properly diagnosed and treated[6]. In the first 6mo of infection with OTB, up to 75% of patients have moderate-to-severe visual impairment, which means that timely and accurate diagnosis of OTB is a clinical challenge[5]. The pan-LOndon tuberculosis Pathway (LOOP) was developed by different eye hospitals in London to outline the management of patients with OTB[7]. Collaborative Ocular Tuberculosis Study (COTS) guidelines were generated based on the evidence from published literature, and it will be useful as a basis for prospective clinical studies to evaluate the role of Anti-Tubercular Therapy (ATT) in different phenotypes of TBU[8]. Moreover, large-scale international retrospective studies analyzed that the sensitivity of polymerase chain reaction (PCR) of aqueous or vitreous humor in the diagnosis and prognosis of specific ocular phenotypes is limited[7]. However, there is no prospective data available to inform the results of different OTB phenotypes, and there are still no agreed guidelines for the management of possible OTB.

Characterization of Ocular Tuberculosis

Orbit The performance of OTB can be divided into five clinical groups: 1) orbital periostitis, which presents as erythema and edema of the lids and conjunctiva with involvement of the spongy vascular tissue of the outer margin of the orbit. It is the most common type of orbital TB and can lead to the formation of a chronically discharging fistula. 2) orbital soft tissue TB without bone destruction; 3) orbital TB with bone involvement. Tuberculomas of the orbit present as a painless proptosis with or without involvement of the bones; 4) orbital spread caused by paranasal sinus; and 5) lacrimal gland inflammation[9].

Skin of the eyelids and periorbital area Eyelid TB is a chronic form of accessory lupus vulgaris pulmonary TB that affects the eyelid skin and occurs in patients who are sensitive to tuberculin antigens[10]. The lesion is single, small, reddish brown, usually involving the head and neck, and has a gel-like consistency[10]. It can manifest as chronic blepharitis or nodules. TB can also affect the eyelid secondary to skin involvement, which manifests as subepithelial nodules, plaques or ulcers. According to reports, for typical TB treatment, the diffuse infiltration of eyelid-like cellulose-like cells actually worsens, which ultimately requires systemic steroid therapy[9].

Lacrimal system The lacrimal glands are usually affected in the form of nonspecific dacryoadenitis with or without abscess formation. There are two manifestations of chronic dacryocystitis: attenuated sclerotic form and caseous granuloma[9].

Conjunctiva Conjunctival TB may present as conjunctivitis, subconjunctival nodules, tuberculoma or ulcer. However, TB, conjunctival ulcers and nodules are very rare[10].

Cornea Patients with corneal involvement may develop interstitial keratitis, disciform keratitis, and phlyctenular keratoconjunctivitis[9].

Sclera Scleritis and scleral nodules have been reported as ocular manifestations of TB. Due to the reaction with mycobacterial proteins, episcleral nodules may form. Scleritis can be nodular or diffuse, of which nodular scleritis is the most common manifestation. Necrosis of the lesion can also cause the sclera to thin or even perforate[9].

Uvea OTB also manifests in uveitis, which varies from tuberculous anterior uveitis (can be divided into unilateral and bilateral uveitis) and tuberculous intermediate uveitis TB (vitreous inflammation appears moderate to severe in the vitreous cavity cellular response) to tuberculous posterior uveitis (represented as posterior uveitis or hypersensitivity retinal vasculitis)[10].

Diagnosis of Ocular Tuberculosis The diagnosis of OTB can be very challenging because it can affect almost any part of the eye. Whether the patient's infection is due to TB infection of the eye or inflammation associated with TB infection is difficult to diagnose. Therefore, a complete workup is necessary to exclude other causes. Accurate diagnosis requires a high degree of suspicion, reexamination of relevant systemic signs and symptoms, and laboratory tests. Although there are no clear diagnostic criteria for diagnosing OTB, several factors that support the diagnosis still exist.

Acid-fast bacteria and histology To prove TB infection, Mtb must be detected from the eye. However, the use of Ziehl-Neelsen staining to detect acid-fast bacilli (AFB) has a low yield from the aqueous or vitreous region. Cultures have low yield and may take approximately 6-8wk to complete[4]. Even

more rare is the histopathological evidence of necrotizing granulomatous inflammation from ocular biopsy, which needs to be large enough to support evidence of OTB or Mtb DNA in the presence of AFB[11]. As a consequence, unlike pulmonary TB, OTB is less frequently diagnosed by microbiological or histological evaluation, and the OTB cases confirmed are relatively rare[12].

Polymerase chain reaction The nucleic acid amplification assay may be another method of detecting Mtb DNA from the eye, and it is rapidly becoming the preferred method for detecting TB DNA; however, it lacks comparison with culture, which is the gold standard, and does not distinguish between active infection and latent TB. Moreover, this technique does not make it easier to obtain intraocular specimens from inflamed eyes[12]. In addition, these tests are affected by sample volume, DNA extraction methods, the number of amplification targets, and the presence of inhibitors in the sample.

Mantoux skin test and interferon gamma testing The TST has been an immunodiagnostic test for the detection of LTBI since the 1940s. The principle is to measure the local immune reaction after intradermal injection of purified protein derivative from the Mtb organism. TST sensitivity and specificity for tracking OTB range from 92% to 95% and 72% to 90%, respectively[10]. However, for patients with immunodeficiency, there may be no response to protein, resulting in false-negative results. Patients who have previously received Bacillus Calmette-Guerin (BCG) vaccines or have been exposed to different mycobacteria may have false-positive reactions[10]. Therefore, IGRAs, including enzyme-linked immune absorbent spot (ELISpot) and whole-blood ELISA, have replaced TST as methods for detecting infection by Mtb. By detecting INF-γ, which is the proinflammatory cytokine released by memory T cells in response to an antigen, it is possible to determine the presence of TB infection. IGRAs are more specific than TST. IGRAs incorporate antigens such as ESAT 6 and CFP10, which are strong targets for Th1 T cells in Mtb infection and are removed from all BCG strains and most environmental mycobacteria. Thus, T cell responses to these antigens are not confused with previous BCG vaccination compared to TST and are therefore more specific markers of TB infection. It is particularly true for OTB in which TB-proven cultures are uncommon[13]. The diagnosis of uveitis associated with LTBI is indirect, and its pathogenesis is unclear. Hypothesized explanations include low-level TB infections within the eye or a tissue-destroying immune reaction provoked by persistent immune TB antigens in the eye or other parts of the body[14]. The accuracy of IGRAs is reliable for immunocompromised patients who present with uveitis and do not respond to TST results, especially when using steroids[14]. Positive IGRAs are useful for uveitis in nonendemic countries because the positive IGRAs in patients with uveitis of unknown origin can benefit from ATT, especially those with sight-threatening uveitis[15].

Chest X-ray Chest X-ray (CXR) is the most common imaging test for TB infection. It provides evidence of active infection and healed/primary or reactivated tubercular lesions. However, it is worth noting that OTB can occur in the absence of pulmonary TB, and the CXR is normal in up to 70% of these patients[9].

Although there is still no consensus on the clinical approach to OTB diagnosis, a reasonable explanation for each diagnosis of OTB, particularly its advantages and disadvantages in the clinical setting, allows them to be judiciously combined in an individual environment. Thus, clinical assessment plays an important role in the overall evaluation.

Ocular Tuberculosis After Immunosuppressive Therapy

With the development of organ transplantation and the increasing rate of diagnosis in the range of neoplastic and inflammatory diseases that respond to immune modulatory therapy, the number of patients receiving immunosuppressive therapy has increased. Long-term use of immunosuppressive agents impairs the immune function of the body to varying degrees, which increases the susceptibility of patients to various pathogenic microorganisms. Cell-mediated immune responses play an important role in the control of TB. Patients with previous immunosuppressive therapy develop TB more frequently than patients without this treatment. The main source of TB infection is the reactivation of TB in the latent state that develops into active TB[16]. Patients receiving immunosuppressive therapy who have LTBI and nontuberculous inflammatory eye disease may have the risk of reactivation of LTBI at any site in the body. TB has a tendency to reactivate under relatively immunodeficient conditions. The term immunosuppression implies a medical cause, while the term immunodeficiency is less specific about the cause and implies a ‘natural’ cause, such as older age, malnutrition, *etc*. This explains the high incidence of LTBI with extreme age and more pronounced rates in patients receiving antitumor necrosis factor for inflammatory diseases, including uveitis[12]. Therefore, the screening for LTBI has become mandatory before these patients begin effective treatment with immunosuppressive therapy, such as anti-TNF therapy[17]. Although IGRAs, the tests used to detect LTBI, perform well in healthy individuals, they have limited performance in immunocompromised patients. There is still uncertainty about the impact of immunosuppressive therapy on IGRA performance. Therefore, the risks and benefits of treatment must be carefully considered before treatment. Every suspected TB case requires consultation with infectious disease specialists—not only for treatment recommendations but also for epidemiological considerations of TB risk[12].

ADVANCED DIAGNOSTIC METHODS FOR ACTIVE TUBERCULOSIS

Due to the limitations of existing diagnostic methods, the diagnosis of TB lacks speed and accuracy. Smear microscopy is associated with strong specificity but less sensitivity. Culture is the gold standard but is time-consuming and laborious[1]. Sputum microscopy is less sensitive in children with extrapulmonary or HIV coinfection[18]. Therefore, tuberculosis biomarkers (TB-BMs) have been explored as new tools for measuring infection status and predicting TB infection, which will be discussed in the following section.

Transrenal DNA Mtb transrenal DNA (trDNA) can be detected in urine samples using standard molecular techniques[19]. Studies have shown that trDNA fragments are highly specific to Mtb. Although it is not a substitute for sputum-based PCR methods, it may be an aid to smear- and PCR-negative individuals. Mtb trDNA is a promising diagnostic tool for TB and may also be an attractive diagnostic marker for the treatment of a small number of pulmonary and extrapulmonary manifestations, especially in children, and the monitoring of anti-TB treatment reactions[20]. Moreover, PCR has been employed in the diagnosis of TBU in several reports published in the literature. The data form the Collaborative Ocular Tuberculosis Study (COTS)-1 was analyzed to understand the frequency and reliability of experts using PCR as a diagnostic tool when determining the initiation of treatment based on a positive PCR result. It is thought that a positive PCR report confirms that the uveitis is a manifestation of the infective and immune response due to the presence of the bacilli. PCR-positive results can then be used by clinicians for treatment to prevent recurrence. However, PCR still has some shortcomings, including a lack of standardization, low sensitivity, and delayed results[21].

Antigen 85 Complex The antigen 85 complex, including Ag85A (31 kD), Ag85B (30 kD) and Ag85C (31.5 kD), is the main secretory antigen of Mtb and has a significant role in the pathogenicity of Mtb. Recent research has shown that the Ag85 complex can be detected in serum from TB patients by indirect enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies (mAbs) against the purified Ag85 complex[22]. The presence of these antigens has been confirmed in the sputum of patients with TB, and because the antigens are not present in the non-Mtb complex, this method does not give false-positive results for other nontuberculous mycobacteria[23].

P-10 Among the candidate biomarkers, interferon gamma (IFN-γ)-inducible protein 10 (IP-10) is secreted by antigen presenting cells, which are proinflammatory chemokines that transport activated T lymphocytes to the site of inflammation. The IP-10 level is higher in unstimulated serum, plasma and urine of patients with active TB than in those with no active TB. IP-10 levels decrease dramatically after the completion of anti-TB treatment[24]. Studies have shown that blood and urine IP-10 levels are correlated in patients with active TB.

Lipoarabinomannan The development of serological tests for patients with TB based on antigenic biomarkers is a new direction for studying TB. ELISA is one of the serological techniques commonly used to detect the TB antigen. Lipoarabinomannan (LAM) is a thermostable carbohydrate antigen containing glycosidic bonds. Therefore, LAM can be cleared by the kidneys and detected by sensitive techniques such as ELISA[25]. LAM is an important immunodiagnostic target for detecting TB infection in patients with HIV-1 coinfection and is thought to mediate many functions that promote infection and disease progression[26]. Evidence supports that the concentration of LAM in urine decreased after 2mo of anti-TB treatment. LAM detection in urine may be a promising diagnostic alternative to existing techniques[27]. For patients with disseminated TB and those who are immunocompromised, such as patients with HIV, LAM detection in urine has the best performance in the rapid diagnosis of active TB. LAM detection in urine offers some advantages because it is easier to collect a sample *via* urine than *via* sputum. Rapid urine LAM test results are expected to improve childhood TB management as a point of care test, as current childhood TB diagnosis is often delayed for several days waiting for microbiological and TST results[25].

Volatile Organic Compounds The WHO called for the development of a rapid nonsputum-based biomarker test that does not require bacterial isolation to detect active TB[28]. A rapid and noninvasive analysis of exhaled gases is likely to meet these requirements. The human body produces a large number of volatile organic compounds (VOCs) as part of normal metabolism. However, pathogenic infections in the human body alter the amount and composition of VOC production. It has been found that different pathogenic species produce a characteristic distribution of VOCs by virtue of their different metabolism. Characteristic VOC profiles that can be detected in the headspace of cultures grown *in vitro* are produced by different pathogens because of their unique metabolism[29]. The test for breath-based Mtb infection diagnosis may provide a significant impact on diagnosing patients, especially in the case of low quality or inability to produce[30]. In addition, some studies found that exhaled gas samples from all patients with TB contain Mtb-associated biomarkers (1-methylnaphthalene and 1,4-dimethy1 cyclohexane), which can be detected from *in vitro* cultures. Other studies on headspace VOCs from cultured mycobacterial species *in vitro* revealed several niacin metabolites, four of which are believed to be specific for Mtb and *M. bovis* strains, including methyl phenylacetate, methyl anisate, methyl nicotinate, and o-phenylindole. High levels of methyl

nicotinate were also detected in the exhaled breath of patients with smear-positive pulmonary TB[31]. Gas chromatography-mass spectrometry (GC-MS) is currently considered the gold standard for separating, detecting, identifying, and quantifying VOCs. Using GC-MS analysis can isolate active TB from inactive TB. The 1,3,5-trimethylbenzene is a biomarker for active TB, whereas 1,2,3,4-tetramethyl benzene can be diagnosed in inactive TB[32]. However, this method still has some limitations. Few studies have compared individuals with active disease and other disease stages, such as active TB versus latent TB. Similarly, there are few data on the impact that coinfections (*e.g.*, TB and HIV coinfection) may have on the range and type of detectable VOCs. Targeted studies are still needed to adequately characterize VOC disease characteristics and to further assess the accuracy of these biomarkers in diagnosis in patient samples[33].

DIAGNOSIS OF LATENT TUBERCULOSIS INFECTION

In countries with high rates of TB, almost the entire population has LTBI, and early detection and treatment of active TB infections take priority[1]. Conversely, in countries with low rates of TB, accurate detection and treatment of LTBI are the basis for the control of TB infection[34]. Moreover, early diagnosis of LTBI is important to reduce the incidence of TB in those people who may progress from LTBI into active TB, especially immunocompromised individuals and children[35]. There is no gold standard for the diagnosis of LTBI, and the existing tests can only determine the probability of LTBI based on the immune response against Mtb bacilli. However, the limitations of IGRAs still exist, that is, a lack of sensitivity in children, especially those who are younger than 5 years of age, and the ability to distinguish active TB and latent TB[35]. Meanwhile, TB is re-emerging due to the increasing use of immunosuppressive drugs in chronic immune-mediated inflammatory diseases and transplant patients, which makes screening for LTBI increasingly important[34]. Therefore, LTBI screening should be performed before high-risk patients begin immunosuppressive therapy in anticipation of treatment escalation[36]. INF-γ is used as a key element in IGRAs and acts as a useful tool for specific detection of LTBI. However, studies have shown that many immunosuppressive agents are potent inhibitors of T cells and may impair INF-γ responses; thus, the sensitivity of IGRAs is negatively affected by immunosuppressive therapy[17]. IGRAs may be unreliable in the diagnosis of LTBI in immunosuppressed patients, including those receiving steroids, oral immunosuppressants (*e.g.*, azathioprine), and biotherapeutic 'biologic' agents[17].

PREDICTING REACTIVATION RISK

According to the WHO, the number of LTBI worldwide was approximately 1.7 billion in 2017, and the LTBI rate was 23%. However, only a small number of people will develop active TB. If clinicians can better predict who is at risk of reactivating LTBI, then they could target prevention chemoprophylaxis only for patients with LTBI who truly need it. Transcriptomics has the potential to be a useful tool for predicting progression from latent to active TB[37]. Early studies reported that patients with active TB have differences in their blood gene expression profiles compared with those of healthy patients with LTBI[38]. The blood transcriptional signature is dominated by interferon-inducible genes in the whole blood of patients with active TB but not in most asymptomatic individuals who are latently infected with Mtb. The major characteristic of active TB is the interferon-inducible gene, which includes genes downstream of IFN-γ and type I interferons and is reduced in expression after successful treatment[39]. Meanwhile, the downregulation of genes that encode B and T cell functions also occurs[38]. Blood transcriptomics analysis of patients with TB has shown that the decreased expression of an interferon-induced gene after successful treatment is expected to improve diagnostic capabilities and therapeutic surveillance, which are critical for eradicating TB. However, some limitations of blood transcriptomics remain. Transcriptomics cannot capture those patients who are destined to progress from latent TB to active TB but can identify the different stages of pathogenesis in those who are already on the path to developing active TB. Therefore, it is still necessary to determine the underlying reason for the progression of latent TB to active TB[37]. The use of blood transcriptomics to improve the diagnosis of TB can be applied using existing methods, including clinical symptoms, sputum smear positivity, and TB by culture or Nucleic Acid Amplification techniques (NAAT)[38]. This is especially useful for cases that are negative or culture negative and not always easily determined through traditional tests. The pathway controlled by histone deacetylase is associated with resistance to Mtb infection and suggests that the function is critical in early innate immune responses to infection[40]. Although recent transcriptomics studies have found that histone deacetylation is a key event in Mtb infection resistance in monocytes, transcriptomics has been shown to be less effective at distinguishing healthy people from patients with LTBI[37]. These results indicate that as a new research approach, transcriptomics has shown promise in the diagnosis, prognosis, and detection of response to drug therapy of TB, which will be important for the eradication of TB.

CONCLUSIONS

In summary, despite the emergence of many new diagnostic tools for TB and OTB, such as molecular techniques for TB biomarker detection and immunological tests, there is still no gold standard diagnostic test. The clinical diagnosis of TB is still complex, and the clinical manifestations of different

types of TB are variable. Prior to obtaining a better diagnostic method, the high probability of clinical suspicion and early presumptive treatment is critical to achieving beneficial outcomes for patients with OTB and LTBI.

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