

Analysis of different detection methods for pulmonary tuberculosis

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Abstract. Tuberculosis is classified as a chronic respiratory disease with its pathogen being *Mycobacterium tuberculosis* (MTB). It poses a significant health threat due to its highly contagious nature and is difficult in treatment. Various methods are employed to test for tuberculosis, including pathological, immunological and molecular biological tests. Each test has its unique principles and all are different in effects, and are widely used in clinical practice. In this research, principles, development and effects of each type of tests in testing pulmonary tuberculosis will be explored respectively. Additionally, the review will provide insights into the optimization of tuberculosis testing by comparing and contrasting different testing methods. Pathological tests represent the most traditional approach to testing for tuberculosis. Bacterial culture, a pathological test, is the gold standard of tuberculosis test. Nevertheless, it suffers from a long detection time. Meanwhile, immunological tests and molecular biological tests benefit from a fast detection, while the accuracy of the tests can be influenced by multiple factors. Thus, it will accelerate the detection speed while enhancing the precision to combine three different kinds of tests in clinical practice.

Keywords: Tuberculosis test; MTB; Pathological test; Immunological test; Molecular biological test.

1. Introduction

Tuberculosis is triggered by *Mycobacterium tuberculosis* (MTB), being the most fatal infectious disease among all the infectious diseases with single causes, the second lethal killer after Covid-19, the major cause of death with HIV co-infection and the major cause of death related to antibiotic resistance. It has brought the world a huge number of casualties and profound economic losses. Meanwhile, the prevention and the treatment of tuberculosis are extremely difficult. MTB is able to survive in critical environments, for it is resistant to acid, base and aridity.

MTB is primarily transmitted through the respiratory tract, with highly contagious droplets produced by tuberculosis patients. Within a year, an individual infected with tuberculosis without prompt diagnosis and treatment can infect 10 to 15 people on average. What is particularly concerning is that patients infected with MTB do not necessarily develop clinical symptoms, with 90% of the infected people remaining asymptomatic throughout their lifetime while having MTB constantly lying dormant in their bodies. MTB will not be eliminated by the hosts, but will be waiting for the hosts' suffering from low immunity or malnutrition to reactivate, replicate and escape. This unique mechanism of infection and latency has resulted in one-third of the population suffering from MTB infection worldwide. Unfortunately, there is no effective vaccine for lifelong protection against tuberculosis, with the only vaccine available in clinical practice, Bacille Calmette-Guerin (BCG), only preventing 20% children from tuberculosis infection and only preventing them from severe infection. Additionally, BCG's effectiveness only lasts for 10-20 years, thus no protective effect will be expected when the recipients reach their adulthood. What's more, MTB's acquiring of an increasing drug resistance since the last century and the side-effects of anti-tuberculosis drugs will hinder patients' prognosis profoundly. Therefore, the effective detection of tuberculosis is crucial to the prevention of tuberculosis's spreading. Based on the aforementioned points, it can be inferred that the effectiveness of detection is paramount in improving the cure rate and prognosis of tuberculosis. Clinical practice predominantly employs pathological, immunological molecular biological tests for diagnosis. Pathological tests encompass bacterial culture and chemical staining. Immunological tests

encompass Interferon Gamma Release Assay (IGRA), tuberculosis skin test (TST) and assay of immune molecules. Biological tests encompass screening specific DNA or RNA fragments of MTB, or screening specific RNA fragments in one's peripheral blood to determine if an individual has been infected by MTB.

Pathological tests screen for one at risk of tuberculosis by identifying the existence of MTB in one's samples. Bacterial culture, a kind of pathological test, which is the gold standard in clinical practice, separates and culture MTB in patients' samples. Staining, another kind of pathological test, is also widely used for its convenience and its low cost. Nevertheless, the testing time of pathological methods can be as long as 2-4 weeks due to the long growth cycle of MTB. Immunological tests screen for one at risk of tuberculosis by assessing the immune response triggered by the host's infection of MTB or by examining the immune molecules produced by the interactions between the host and MTB. IGRA, one specific type of immunological test, benefits from testing without culturing bacteria, which enhances the efficiency by eliminating the need to endure long growth cycle of MTB during the testing procedure. Meanwhile, the low MTB content in patients' samples does not impact the accuracy of the experiment since the test does not screen bacteria directly. Nevertheless, certain immunological methods are demanding in terms of lab equipment and the laboratory skills of technicians, making them unrealistic for a number of hospitals. Additionally, BCG vaccination can lead to false positive results in immunological methods such as skin tests. Molecular biological tests in detecting tuberculosis are relatively recent methods. The methods screen for one at risk of tuberculosis by identifying MTB DNA or RNA, or specific RNA fragments in patients, thus providing a highly specific means of testing. Compared to traditional pathogenic tests, molecular biology tests detect target sequences by employing PCR technology, enabling faster and more sensitive testing. Nonetheless, low bacterial content of patients' samples could result in decreased accuracy in test results.

The aforementioned tests vary in principles and steps, each with its own advantages and disadvantages, and all are widely used in practice. These ways of testing are principally combined in clinical practice to compensate for their respective weaknesses. While testing methods of tuberculosis has been improving, its detection speed and positive detection rate remain inadequate for clinical needs. As a result, shortening detection time, simplifying detection steps and improving detection efficiency remain challenges for researchers worldwide. This research provides an analytical examination of the advantages and disadvantages of pathological tests, immunological tests and molecular biology tests respectively, as well as an exploration of the research process and development prospects of tuberculosis testing technology.

2. Different detection methods of pulmonary tuberculosis

2.1. Pathological tests

Pathological tests of MTB are based on isolation and cultivation of MTB from patient samples. By means of isolating MTB from samples before morphological observation or chemical staining, it is possible to identify the presence of MTB in samples. The testing efficiency is heavily reliant on the growth of MTB and the long growth cycle of MTB makes the aforementioned tests endure a long testing procedure, for the bacteria needs 2 to 6 weeks of growth on solid culture media before detection can be achieved. Pathological tests encompass bacterial culture and staining, and the review will explore the principles underlying these methods and their corresponding methodological innovations.

Bacterial culture method of MTB is a technique for isolating and culturing MTB from patient samples such as sputum, alveolar lavage fluid or tracheal biopsy specimens. The culture medium contains nutrients necessary for the growth of MTB as well as antibiotics that inhibit the growth of other bacteria. This method is the recognized means of identification of MTB as well as assessment of drug resistance and infection status. Bacterial culture is the gold standard for laboratory testing of

tuberculosis, which is widely used in the diagnosis and research of tuberculosis. It can provide highly specific and sensitive testing result for clinical practice.

Based on the culture medium used, the bacterial culture media of MTB can be classified as either solid or liquid culture media. Solid culture media is applied at a wider range and has a higher detection than that of liquid media. Nevertheless, MTB is a slow-growing bacterium with a growth cycle lasting up to 5 to 8 weeks, which poses a great challenge to the fast diagnosis and treatment of tuberculosis. MTB culture in liquid culture media benefits from convenient operation and a shorter culturing time, taking about a month to clarify the culture results. Nonetheless, given the clinical urgency for prompt detection of MTB, the detection speed of liquid culture media is still falling short of meeting the needs of clinical practice. Despite the high sensitivity of bacterial culture as the gold standard for MTB detection, there are still 15%-20% patients diagnosed with tuberculosis or receiving tuberculosis treatments lack bacterial culturing evidence for MTB infection. It is notably that there is no statistically significant difference in treatment failure rate and recurrence rate between patients with negative and positive bacterial culturing results. However, the mortality rate of patients with negative bacterial culturing results is significantly higher than those with positive results [1]. The exact mechanism behind this phenomenon remains clear, but it is evident that the bacterial culture method still has certain defects in the detection accuracy, presenting great challenges to the prompt diagnosis and treatment of tuberculosis.

MTB is acid-resistant and this characteristic enables it to be differentiated from other bacteria through acid-fast staining. The acid-fast property of MTB and the acid-fast staining method were analyzed in 1882. Phenol was used for staining purpose in the 1890s, while the main dyeing agent can be altered to carbolic acid fuchsin, resulting in the widely used Ziehl-Neelsen (ZN) staining method. The acid resistance of MTB allows it to retain its dyed color after the decolorization step, thus the stained MTB shows a characteristic red color under the microscope. The testing method benefits from its convenience and its low cost. Until recently, the method is still widely used in clinical diagnosis of tuberculosis. The method is often used to detect sputum or alveolar lavage fluid smears of patients. After fixing the sample cells on a slide, dyeing, decolorization and counterstaining, the specimen will be observed under microscope to determine the result. Nevertheless, non-tuberculous mycobacteria such as *Mycobacterium leprae* and *Mycobacterium bovis* can trigger false positive result in acid-fast tests. What's more, MTB is able to transform its form from being positive in acid-fast tests to non-replicating negative in acid-fast tests during the course of infection. The specific mechanism behind its acid resistance lost is not yet clear, but the phenomenon can cause false negative results in experiments [2]. The acid-fast staining involves two classic dyeing techniques, which are conventional Ziehl-Neelsen (ZN) staining and Gabbett cold staining. The former staining technique combines MTB with carbolic acid fuchsin under heating conditions to form a complex. When counterstained with methylene blue, acid-fast MTB will appear red, while other bacteria or other substances in the background will appear blue. Freed from the heating step, hydrochloric acid ethanol is used in Gabbett staining for separation after carbolic acid-fuchsin staining, which is followed by contrasting staining using methylene blue.

Recent advancements in acid-fast staining technology mainly focus on optimizing stain configuration and parameters of microscope hangings. Certain improved staining methods have eliminated the decolorization and counterstaining steps, thereby simplifying the experimental process [3]. Nevertheless, it should be noted that some improved staining methods have only been assessed for their accuracy in the laboratory for their accuracy in detecting MTB, and have not yet to be validated in clinical practice [4].

2.2. Immunological tests

MTB primarily enters the host through the lungs, with pulmonary symptoms being the major symptoms of MTB infection. Upon infection, Macrophages together with dendritic cells will ingest MTB, leading to the formation of the Ghon complex. The penetration of phagocytes into lung parenchyma triggers inflammation, while the accumulation of inflammatory cells can form

granulomas. T cells are able to stabilize granuloma formation as well as contacting, differentiating and activating phagocytes. MTB can thus remain dormant within granulomas for decades without triggering symptoms in the host. However, MTB can be activated and proliferate in the granulomas, triggering various symptoms in the host body when the host's immunity is compromised. Immunological examinations diagnose the patient's condition by observing the host's immune response to MTB or by observing corresponding substances in MTB, or by detecting the immune molecules produced during the interaction between pathogenic bacteria and the host. The immunological examination methods include IGRA, skin test and immune molecule detection. It's a common approach to utilize patients' peripheral blood samples in immunological testing, as it effectively mitigates the possible inaccuracies in test results caused by indirect sputum discharge and substandard quality of sputum specimens in pathogenic testing. Additionally, it accelerates the long testing time caused by MTB growth cycle. Nevertheless, it's noteworthy that immunological testing may not possess sufficient sensitivity to differentiate active pulmonary tuberculosis from controlled, latent pulmonary tuberculosis. In this regard, the following review will delve into the significance of three immunological testing in tuberculosis: IGRA, skin test and immune molecule detection.

The IGRA test offers a means to detect immune response to MTB by analyzing IFN- γ secretion from specific anti-MTB effector T cells in the patient's peripheral blood. Prior to the processing procedures, the samples of the IGRA test requires stimulation and incubation. There are two detection methods for qualifying IFN- γ in the samples: ELISA or ELISPOT technology. The IGRA test based on ELISA detection method is QuantiFERON-TB Gold (QFT) and the IGRA test based on the enzyme-linked immunospot technology detection method is T-cell immunospot test for tuberculosis infection. Compared with pathological tests, IGRA benefits from a shorter detection time with test results available within only 12 to 18 hours. Research have revealed that second-generation IGRAs technology is highly effective in ruling out active tuberculosis in low burden settings [5]. Additionally, Chloé Wyndham-Thomas et al. also found that HBHA-IGRA, an interferon-gamma release assay for the MTB latency antigen, is able to serve as a complementary option to the QFT test when screening latent tuberculosis in HIV infected patients [6].

Known also as the tuberculin purified protein derivative (PPD) test, TST utilizes tuberculin as its antigen and is injected into patient's skin to observe for any delayed allergic reactions to it. Patients infected with MTB will have a delayed allergic reaction to tuberculin by produced sensitized T cells and increased permeability of blood vessel, leading to macrophages accumulation at the injection site and triggering positive reactions like redness, swelling, and induration. The tuberculin used in TST is obtained from the growth, lysis, and purification of *Mycobacterium bovis*, *Mycobacterium avium*, or other mycobacteria. TST has a long history of over 100 years and a wide range of applications. Nonetheless, vaccination of BCG may trigger false-negative results. Some studies have compared the efficiency of IGRA with TST and found that IGRA has higher specificity in detecting latent tuberculosis and HIV combined tuberculosis infections [7].

In the process of MTB-host interaction, immune molecules are produced. The presence of these immune molecules in the patient's serum can serve as a reliable indicator for diagnosing tuberculosis infection. The immune system employs various immune cell subsets, such as cytotoxic T lymphocytes, macrophages, NK cells, dendritic cells, B lymphocytes and T helper cells to combat tuberculosis infection. The cytokines produced by the aforementioned cell subsets and molecular inhibitors from them may function as crucial molecular indicators of the immune response against tuberculosis, aiding in the identification of different phases of MTB infection or the assessment of anti-tuberculosis treatment efficacy, and as targets of pharmacotherapy.

The current existing testing methods rely on the identification of a cytokine namely interferon- γ that is generated by T lymphocytes as an anti-tuberculosis response to MTB. Studies have revealed that the aforementioned testing methods exhibit a higher degree of sensitivity and are not interfered by BCG vaccination or other environmental mycobacterial infections [8]. Additionally, researchers have identified two novel MTB complex-specific candidate agents, Rv2653 and Rv2654, which are highly specific in animal experiments and in the diagnosis of tuberculosis patients. Importantly, the

diagnostic results are not affected by BCG vaccination [9]. The study offers a viable approach to diagnosing pulmonary tuberculosis by detecting specific molecules produced by MTB infection.

Based on the points mentioned above, it appears that serologic testing for MTB has a high negative predictive value, allowing it to be used to screen for and rule out active tuberculosis. Nevertheless, when conducting serologic testing to samples of patients with HIV-combined tuberculosis infections, the sensitivity and negative predictive value are significantly reduced, failing to meet clinical requirements for tuberculosis diagnosis. The positive expected value of serological tests is also relatively low among people with latent pulmonary tuberculosis at a high infection rate, reducing its diagnostic specificity of active tuberculosis.

2.3. Molecular biological tests

MTB is a Gram-positive bacillus with haploid chromosome being its genetic material. Molecular biology methods benefit from shorter detection time, simpler operation steps and increasing detection sensitivity. The aforementioned methods are utilized as replacements for a number of traditional pathological testing methods to varying degrees, or as a supplement to traditional detection methods. Molecular biology tests include DNA detection of MTB and detection of specific RNA sequences in the patient's blood; the role of the aforementioned two molecular biology testing methods in the diagnosis of tuberculosis will be discussed below respectively.

DNA sequencing of MTB employs molecular biological technology to detect specific DNA sequences in MTB for the identification of MTB in patient samples. In addition, nucleic acid amplification testing (NAAT) has provided an approach with higher sensitivity as well as specificity in tests for the purpose of diagnosing tuberculosis and of reducing the testing cost for resource-poor countries that are heavy in disease burden. The detection of active MTB infection in patients with contraindications to invasive surgery can be a challenging task. Nevertheless, PCR can provide a low-invasive and high-sensitivity method for detection with the use of digital PCR (dPCR), sensitivity can be improved for the detection where target molecules are small in copy numbers and amplification of targeted sequences of MTB complex can be showed effectively by assay of circulating cell-free DNA in patients. Additionally, immuno-PCR (I-PCR) can label DNA fragments through streptavidin-biotin or covalent binding and amplify them through PCR. The technique effectively overcomes the shortcomings of traditional immunology tests, which are triggered by low antigen-antibody concentrations.

The detection of tuberculosis infection through RNA sequencing of MTB involves the utilization molecular biological technology to identify specific RNA sequence fragments in the patient's whole blood. These RNA sequence fragments can either be non-coding RNA or RNA secreted during host's anti-tuberculosis immune infection. Researchers have revealed that measurements of long non-coding RNAs (lncRNAs) varied in expression, such as ENST00000497872, n333737 and n335265, can help diagnosing patients with negative results in microbiological tests, as shown in Figure 1. This approach facilitates the early detection of tuberculosis instances in suspected individuals with negative MTB microbiological testing results [10].

MTB's capability of eluding immune system of the host for the purpose of ensuring its continued existence in the host's cell is well-established. This is achieved through the alternation of key microRNAs expression, which governs the innate and adaptive immune response of the host against MTB. Interestingly, miRNA expression varies in treated tuberculosis, active tuberculosis as well as latent tuberculosis, indicating the potential for the discovery of new diagnostic molecular markers for pulmonary tuberculosis by studying the relationship between the expression levels of key RNAs in the host's immune response for anti-tuberculosis purpose and the developing stages of tuberculosis.

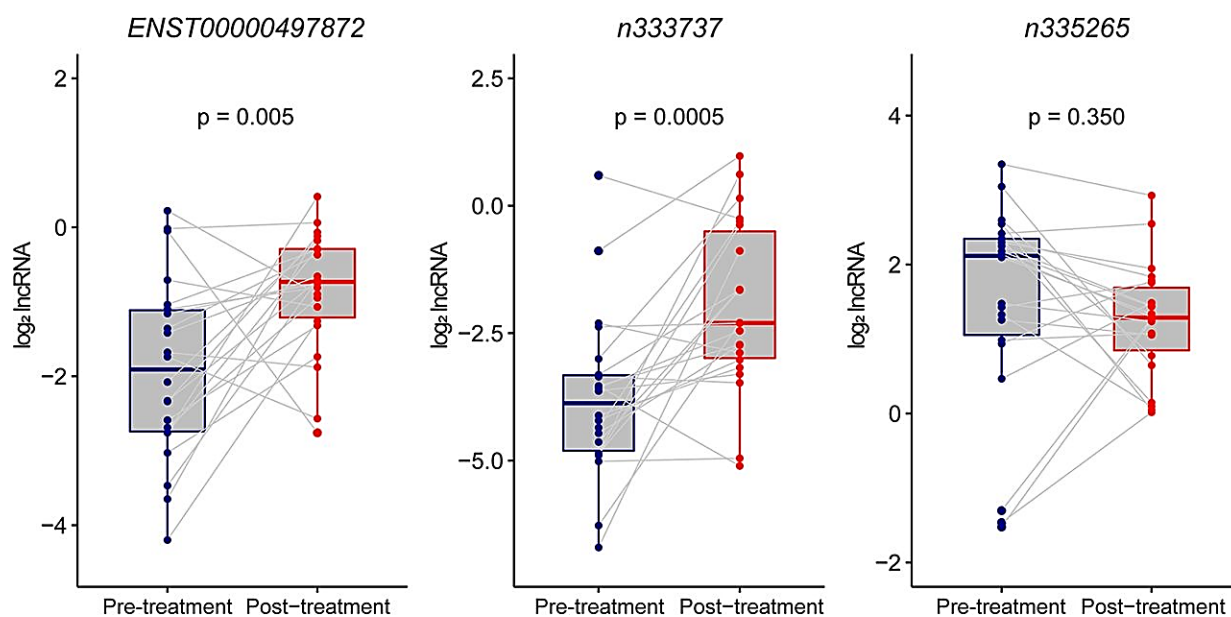


Figure 1. Changes in lncRNA before and after 2 months treatment [10].

According to research conducted by Myrini Kaforou et al., quantitatively analyze RNA of specific cells or peripheral blood can be achieved by microarray analysis or RNA high-throughput sequencing methods in order to reveal the host's anti-tuberculosis immune response and to provide insight into the occurrence and progression of the disease [11]. Furthermore, a study has identified 51 transcript signatures that can distinguish tuberculosis from other diseases, indicating that RNA sequencing can improve the specificity and sensitivity to accurately detect [12].

3. Conclusion

According to the aforementioned points on the detection methods of pulmonary tuberculosis, respective advantages and disadvantages of pathological tests, immunological tests and molecular biological tests are revealed while recognizing their characteristics complementing each other. Pathological tests, being more developed, cost-effective, and widely used in clinical practice, nonetheless suffers from false negative results as a result of low bacterial content in patient samples or failed culture. Immunological tests directly detect immune molecules in peripheral blood or conduct skin tests, thereby minimizing the possibility of interference from sample issues. Molecular biological tests, on the other hand, benefits from high specificity and sensitivity. Nevertheless, the mechanism of differential expression of some sequences in the host is not yet clear, necessitating further research to confirm the effectiveness of utilizing the specific part of the sequence as a detection sequence in the diagnosis of tuberculosis.

Pathological tests, immunological tests and molecular biological tests each have their own strengths and limitations. In clinical practice, it is essential to assess the advantages and disadvantages of various methods respectively. To ensure the accuracy of test results, it is often necessary to employ a combination of these methods. Among the aforementioned testing methods, bacterial culture of MTB is known to be the benchmark in the diagnosis of pulmonary tuberculosis. It is more advanced in terms of technical application and carries greater authority in terms of detection methods. Nevertheless, due to its long detection period, auxiliary detection such as immunological tests or molecular biological tests are required to provide a comprehensive assessment of the patient's condition through multiple detection results. This approach allows an accurate and timely diagnosis.

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