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MINIREVIEW

Gamma Interferon Assays Used in the Diagnosis of Tuberculosis

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Tuberculosis (TB) is an ancient disease that has infected humans for thousands of years. However, despite diagnostic tests that detect the disease and effective therapy, there are still millions of people worldwide who are infected with TB. The first TB test used to detect infected patients was a skin test that identifies individuals actively infected with TB. This test used a mix of proteins from culture filtrates of the bacteria *Mycobacterium tuberculosis*. Recently, two new diagnostic tests have been introduced; these two new tests can detect TB infection in patients by challenging peripheral blood cells with specific TB proteins. These assays measure the cellular immune response to these proteins. This minireview evaluates the new assays and compares them to the use of the TB skin test. The use of these new assays may have some advantages in detecting individuals with active tuberculosis. However, there is still a role for the use of the skin test, especially in young patients.

Active or latent tuberculosis (TB) is found worldwide in approximately one-third of the human population. TB is commonly spread person to person from coughing, which causes the *Mycobacterium tuberculosis* bacteria to become airborne. The inhalation of just a few of these bacteria can lead to infection. The WHO reported that in 2013, 9 million people were ill with tuberculosis and that 1.5 million patients died from the disease (1). However, tuberculosis can be detected and successfully treated by using reliable diagnostic tests that identify individuals with TB.

When individuals become infected with TB, their symptoms may be marginal and might not alert the patient that an active infection is occurring. Many patients with TB do not always immediately seek medical care and unfortunately will transmit the infection to others during this period of infection.

The testing methods for screening patients who may have tuberculosis have changed recently. The TB Mantoux skin test was introduced in the 1890s as a skin test to determine whether a person is infected with TB. This test used a tuberculin precipitate known as purified protein derivative (PPD), which is essentially proteins obtained from culture filtrates of *Mycobacterium tuberculosis*. This test was named for the French physician, Charles Mantoux, who introduced this test in 1907. Recently, two new TB tests have been introduced that can test patient blood for reactions to specific TB antigens *in vitro* (2, 3). These assays use TB-specific proteins to measure the activation of the patient's immune system to detect and measure the patient's response to the TB proteins. Both the PPD test and the new TB tests are considered valid for diagnosing active TB infections.

HISTORY OF TUBERCULOSIS

Hippocrates recognized and described TB around 460 BCE (*be*fore the *C*ommon *E*ra). During that time, TB disease was highly prevalent in human populations. Currently, TB is found worldwide; however, half of all TB cases are in South-East Asia and Western Pacific regions of the world. The WHO estimates that 9.0 million individuals developed TB and 1.5 million people died from this disease in 2013 (1).

Tuberculosis was first described in the seventeenth century, and several different names have been used to describe tuberculosis disease, such as consumption, Pott's disease, phthisis, and scrofula. In 1882, Robert Koch introduced staining methods that detect the *M. tuberculosis* organism in patient samples. This ability to detect *M. tuberculosis* in patient specimens helped to prevent *M. tuberculosis* transmission. However, since the discovery of *M. tuberculosis*, it is estimated that a third of the world's population continues to be infected with *M. tuberculosis* (1).

DIAGNOSTIC TESTING OF INDIVIDUALS FOR TUBERCULOSIS

Diagnostic tests for tuberculosis infections are used worldwide to detect individuals with active disease and prevent the spread of TB. Currently, there are two types of tests that can screen individuals who may have active tuberculosis. These tests are the tuberculin skin test (TST) and two different assays that measure gamma interferon (IFN-γ) from the patient's lymphocytes after exposure to specific TB antigens. Both of these tests measure the immunological response of the host to specific TB proteins. The first assay used was the TST. This test has been used for over 100 years and was developed by Charles Mantoux in 1908. He based this test on the work of Robert Koch (4).

The TST involves an intradermal injection of purified protein derivative (PPD). Individuals who show an obvious local induration at the inoculation site after 48 to 72 h of exposure to the specific TB antigens confirm a delayed-type cell-mediated immunity to TB, indicating active or past TB infection (5). Despite its long-term use, the TST has several known limitations. False-positive results occur due to reactions induced by nontuberculous mycobacterium (NTM) infection. Additionally, individuals with a prior *Mycobacterium bovis* BCG vaccination often have a positive TST result (5–7).

Some patients have had false-negative TST results, especially those who are immunosuppressed, such as individuals on immunosuppressive treatment or individuals with uncontrolled HIV infection or malnutrition (8). In addition, false TST results can be caused by inappropriate storage of the tuberculin antigen, incor-

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rect inoculation of the tuberculin, or inaccurate interpretation of the induration at the test site. Most concerning is that false-negative TST results can occur in patients with active tuberculosis or with HIV infection or immunosuppressed patients. These false-negative results are due to a diminished immune response due to active infections (9).

TEST SYSTEMS

Recently, two new Food and Drug Administration (FDA)-approved assays were introduced. These two assays detect *in vitro* a specific immune response to *M. tuberculosis*. These tests are the QuantiFERON-TB Gold In-Tube (Cellestis/Qiagen, Carnegie, Australia) and the T-SPOT.TB assay (Oxford Immunotec, Abingdon, United Kingdom). Both assays use whole blood from the patient and measure the production of IFN-γ after the whole blood is exposed to specific antigens from *M. tuberculosis*. These tests are based on the knowledge that IFN-γ is a product of an active cell-mediated immune response induced by *M. tuberculosis* (10, 11).

The first assay available for detection of *M. tuberculosis* infection (QuantiFERON-TB) was approved by the FDA in 2001 (12).

Subsequently, in 2005, a newer version of this assay was introduced as the QFT Gold In-Tube which simplifies the assay by adding the specific tuberculosis antigens into one of the specimen collection tubes.

As with the first assay, this new version uses peripheral blood to detect specific immune responses to *M. tuberculosis*. Three *M. tuberculosis*-specific antigens are used in this new assay; the early secreted antigenic target 6 (ESAT-6), culture filtrate protein 10 (CFP 10), and the TB7.7 protein. A sample of the patient's blood is collected directly into a set of three tubes, and the specimen is then incubated. One tube is the control and contains no TB antigens or mitogen. The second tube contains the three *M. tuberculosis*-specific antigens, and the third tube contains the mitogen phytohemagglutinin (PHA). During incubation, the T cells respond to these TB antigens. The specific response is measured by the detection of IFN-γ. This change expedited the process of testing because the blood is collected directly into three sealed tubes. Thus, this test contains all the testing system, including patient-specific controls (QuantiFERON-TB Gold package insert; Cellestis/Qiagen, Carnegie, Australia).

The other commercial interferon gamma release assay (IGRA) is the T-SPOT.TB assay (Oxford Immunotec, Abingdon, United Kingdom). This assay uses an immunospot assay that detects the number of IFN-γ-producing cells on coated plates (13). The T-SPOT.TB assay uses peripheral blood mononuclear cells (PBMCs) and exposes the cells to two tuberculosis-specific antigens, ESAT-6 and CFP-10. This assay requires that peripheral blood mononuclear cells be isolated away from the whole blood before being tested. This assay is an enzyme-linked immunospot (ELISPOT) assay and requires that PBMCs be separated and counted before adding them to the wells. This assay incorporates a positive-control well that contains PHA and a negative well with no TB antigens or PHA. The result is reported as the number of IFN-γ-producing T cells as recognized by spots on the filter paper. An individual is considered positive for *M. tuberculosis* infection if the TB antigen well(s) has six or more spots (after the background is subtracted from the antigen well). Likewise, the test would be considered negative if there are five spots or less (after the background is subtracted from the count on the antigen well).

ADVANTAGES

Several publications have shown the accuracy of the TB IGRA (14–17). These assays are now used in a number of countries worldwide (17–19). There are several advantages for using a TB-specific IGRA compared to the tuberculin skin test (TST). The IGRA does not require the patient to follow up with a second visit as is needed for the TST. Additionally, the IGRA tests do not have cross-reactivity with BCG; thus, patients vaccinated with BCG will not have the complication of a false-positive result.

An additional advantage of this assay is the standardization of the number of cells exposed to antigens and mitogen. Both IGRAs use very specific TB antigens (ESAT-6 and CFP-10, and one has TB7.7) which are not present in strains of BCG or most other mycobacteria. The use of these specific proteins results in improved specificity compared to the use of TST to detect active TB disease. Additionally, studies show the use of IGRAs in screening for active tuberculosis is cost-effective, especially when screening high-risk populations, such as immigrants, close contacts of individuals with active tuberculosis, and health care workers (12, 14, 20–23).

LIMITATIONS

There are some limitations to using TB IGRAs. The test requires a blood draw, which may be difficult in young children and some adults. The test kits need to be refrigerated during storage and transportation. This may be difficult for clinics in remote areas. Additionally, after the specimens are collected, they require specific handling, such as shaking the tubes and incubating the blood samples within a specific time period. These tests also need to be collected by phlebotomists who are trained in handling the tubes.

A positive result for *M. tuberculosis* infection will have an IFN-γ response to TB antigens above the test cutoff (background IFN-γ response in the negative control). These assays are designed to detect the specific cellular immune response to specific *M. tuberculosis* antigens. The proteins used in the assay are not present in BCG or most nontuberculous mycobacteria; thus, there is no response in individuals vaccinated with BCG. To avoid cross-reactivity, these tests use antigens encoded in the region of difference 1 (RD1), a portion of the *M. tuberculosis* genome that is absent from the genome of BCG and many NTM (22, 24). Both tests depend on cell-mediated immunity (memory T-cell response); however, neither test can accurately distinguish between latent tuberculosis infection (LTBI) and active TB disease (15, 20, 24).

Testing for LTBI is useful when the risk of disease is higher, such as an individual with close contact to a person known to have an active TB infection, patients who might not be able to contain the infection due to immunosuppression, or those patients who are young or very old. Testing for LTBI in individuals who are healthy is not appropriate unless they have a high risk of infection such as working with TB-infected patients or working in environments that have high-risk individuals, such as hospitals or prisons. The predictive value of testing patient populations with a low risk of TB indicates that this practice is not productive (25).

The largest limitation of using the TB IGRA tests compared to the TST is that the cost of the TB IGRA is higher. The average cost of a TB IGRA test is about $37, while the average cost of the TST is approximately $9.80 (26). However, a major advantage of the TB IGRAs is the high negative predictive value; thus; it excludes tuberculosis in most patients (22).

Alternately, the positive predictive value of both the TST and the TB IGRA is related to the prevalence of TB in the patient population that is tested. Thus, there may be false-positive results from both tests. However, several publications show strong evidence that the use of the TB IGRA was cost-effective, especially when testing high-risk individuals, such as health care workers, immigrants from countries where tuberculosis is endemic, and individuals with close contact with infected individuals (household contacts) (27, 28).

RECOMMENDATIONS REGARDING TESTING FOR TUBERCULOSIS

The CDC has noted that the number of tuberculosis cases in the United States has decreased in the past few years. In 2013, there were a total of 9,582 TB cases in the United States which represents a 4% decline in cases (http://www.cdc.gov/tb/statistics/). However, there has been an increase in the number of multidrug resistance (MDR) in the tuberculosis isolates. In 2013, the percentage of MDR was 1.4 of all tuberculosis cases. Most of these antibiotic-resistant bacteria (89%) were isolated from patients born outside the United States.

The primary focus of the WHO is on preventing the transmission of TB worldwide. It is encouraging to note that in the last few years, the number of cases of TB worldwide has been slowly declining. However, in 2013, there were still 9 million people in the world with active TB, and during this time, 1.5 million patients died from TB. The largest number of TB cases occurs in Asia, Western Pacific area, and Africa. Additionally, more than half a million children worldwide have active TB, and approximately 15% of these patients died from the infection. Another concern as mentioned above is the development of multidrug-resistant TB. At this time, there are approximately 480,000 people worldwide infected with multidrug-resistant TB (29).

TESTING FOR TUBERCULOSIS IN SPECIAL POPULATIONS

When testing patients for tuberculosis, an accurate test depends on the risk factors associated with the patient being tested. However, the interpretation of results from the IGRA test is the same for all patients, and results are reported as positive, negative, or indeterminate regardless of the underlying condition or age of the patient. A number of studies have evaluated the use of IGRAs in specific types of patients to determine the reliability of using this test on patients with underlying conditions or situations.

PREGNANCY

Disease due to tuberculosis in women who are pregnant is influenced by the stage of pregnancy, the severity of TB disease, and coinfections, such as HIV or hepatitis infections. However, despite the complications, pregnant patients with active TB should be treated with appropriate therapy. The drugs used to treat tuberculosis can cross the placenta, and fortunately, they do not have adverse effects on the baby (30, 31). However, complications have been noted in pregnant women with tuberculosis. These complications include a higher rate of spontaneous abortion, low weight gain during pregnancy, preterm labor, and an increase in neonatal mortality (32–34).

HEALTH CARE WORKERS

There have been reports from around the world of the transmission of TB to health care personnel. Health care workers have become infected with TB in every country of the world. TB is easily aerosolized in droplet nuclei and then can be inhaled by a caretaker. A recent study showed that health care workers have a higher risk of acquiring TB than the general public (35). Thus, acquisition of TB by health care workers is a serious occupational hazard. Transmission in health care workers can be prevented with practices, such as using an N95 respirator and changing gloves frequently when tending to a TB patient. It is also critical that health care workers recognize the signs and symptoms of tuberculosis so that TB-infected patients can be quickly isolated from other patients. All health care facilities should have a TB infection control plan that works closely with the infection control program. This plan should consider using methods to rapidly detect TB-infected patients so that airborne precautions and appropriate treatment can be initiated in patients with confirmed TB.

CORRECTIONAL INSTITUTIONS

The transmission of TB in correctional institutions in the United States accounts for approximately 3 to 6% of all TB cases in the country (36). Transmission of TB is problematic in correctional facilities because individuals from different backgrounds and communities are housed in close proximity for various periods of time. Most prison facilities will have inmates that are often moved in and out of several correctional facilities. This movement can interrupt TB therapy, leading to the return of symptoms and possibility of transmission to other individuals. It can also expose more inmates to tuberculosis if the disease is not detected. To maintain an effective TB control program in correctional facilities, there must be a method to quickly identify and monitor individuals with active TB disease. It is also necessary to have a successful treatment program with reliable follow-up and contact investigation. These practices should be coordinated with the local public health systems in order to manage the movement of prisoners in and out of incarceration. Continuing education of inmates, detainees, and correctional facility staff is necessary to successfully prevent the spread of TB in correctional facilities.

CHILDREN

In 2013, the WHO predicted that more than half a million new cases of tuberculosis in children would occur each year worldwide. They also estimated that currently there are more than one million cases of active TB in patients younger than 15 years old each year. This report also noted that approximately 80,000 children worldwide die each year from these *M. tuberculosis* infections (http://www.who.int/tb/challenges/children/en/).

Children and infants usually acquire TB from an adult with active, infectious TB. Children younger than 4 years old are more susceptible to TB than adults are. Thus, the discovery of TB in a child should lead to an investigation of the family and living conditions (37). After infection with TB, children are more likely to develop symptoms rapidly. Additionally, young children often develop disseminated tuberculosis that may invade the central nervous system, leading to serious, life-threatening disease.

Due to the high consequences of active tuberculosis in children, a prompt response to any positive result is critical (38). Diagnosing and confirming TB in children can be difficult because it is challenging to collect sputum especially in very young children (14). In young children, frequently the only sign of a TB infection is a positive TB skin test or a positive TB blood test. Thus, suspected TB in young children may require confirmation of dis-

ease based on a history of contact with a TB-infected person, positive chest X-ray patterns that suggest TB disease, as well as a positive TB skin test. In young patients, the TB skin test is considered more reliable than the TB blood tests (14, 15).

Children with a high risk for acquiring tuberculosis are those who live in a household with a person with active TB, children born in a country with a high prevalence of tuberculosis, those who do not have regular medical services, and children who are immunosuppressed due to HIV or medications. A major concern regarding tuberculosis in children is that the disease is frequently asymptomatic. Thus, the only sign of an active TB infection might be a positive reaction to the TB skin test or a positive TB IGRA (38).

Detection and diagnosis of TB in children are more difficult than adults. This presents an additional challenge in detecting tuberculosis in children. Thus, the use of accurate tests for tuberculosis infection is important. There are a few studies that have evaluated the use of IGRAs in children (15, 39).

These studies found that there is an increase in indeterminate test results when evaluating children with the QFT Gold In-Tube (QFT-G-IT) that can be as high at 32% (15). One issue of concern was that approximately 25% of the positive tests for children using QFT-G-IT were negative after confirming results on the same specimen (39). Because of this issue, the CDC recommends the use of TB skin testing in children less than 5 years of age (http://www.cdc.gov/tb/topic/populations/tbinchildren/default.htm).

DISCUSSION

TB is a global disease, and individuals in all areas of the world are infected with this disease. In 2013, the WHO reported that there were 6.1 million cases of TB worldwide. Approximately 5.7 million of these cases were recently diagnosed. In this time period, the WHO estimated that there were approximately 1.5 million deaths worldwide due to TB infection. They have established a goal to reduce deaths associated with tuberculosis by 75% by 2015 and to reduce the incidence of TB by 50%. The ultimate goal is to eventually eliminate TB disease around the world (http://www.who.int/tb/strategy/en/).

TB is spread predominantly by person-to-person interactions. TB-infected individuals can infect others by simply talking, coughing, or sneezing during contact with other individuals. Patients with active TB disease are clearly sick and usually have fever, night sweats, cough, and weight loss. Individuals who live in close quarters with TB patients have a higher chance of acquiring the TB infection. This initial infection can spread throughout the body and infect the lungs, kidneys, brain, and sometimes the bones, especially the spine. However, some individuals exposed to TB may not become sick but can harbor the bacteria that have invaded tissues or organs. These patients have latent TB infections and do not often spread the bacteria to other individuals. TB can infect people of all ages; however, some individuals are more susceptible to TB infection, such as those individuals that are immunosuppressed, such as transplant patients, HIV-infected patients, pregnant women, and children.

In order to eliminate TB in the world, an accurate test should be used to detect those individuals with active or latent TB. The most common way to evaluate individuals for TB is to screen for the disease using one of the tests available for detecting active infection. For many years, the only method to screen for TB was the tuberculin skin test (TST). The TST has been used since the 1930s and is still used in many situations. The TST consists of an extract of *M. tuberculosis*, *M. bovis*, and *Mycobacterium avium* and is injected intradermally to detect active disease. The results of the TST must be interpreted 48 to 72 h after administration, which can be problematic if patients cannot return in that time period. Additionally, the reading of the TST varies based on the state of the patient's immune system and their age or occupation. Individuals with a healthy immune system would be considered to have a positive TST if the induration is 15 mm or larger (38, 40). However, if the patient is a known intravenous (i.v.) drug user, the patient would be positive with an induration of 10 mm or larger. An induration of 5 mm is considered positive if the patient is a child younger than 4 years old, an individual who works with mycobacteria in laboratories, or an immunosuppressed patient (41). In order to preserve the potency of the tuberculin, the test material should be kept refrigerated and stored in the dark. This is sometimes a disadvantage for clinics performing TST testing in remote areas of the world. However, both of the new TB IGRA assays require sophisticated laboratory testing that may not be easily available in some countries without the resources to provide equipment for incubation of specimens and detection of results.

In conclusion, both the TST and TB IGRA tests are valuable methods for screening individuals for latent and active tuberculosis. These tests play a valuable role in the detection of active tuberculosis. An advantage of the TB IGRA is that the patient does not need to return to have the test interpreted as the TST requires. The disadvantage of the IGRA is the increased cost and the limitations of testing specific patient populations, such as children and immunosuppressed patients. However, a diagnosis of tuberculosis still requires a clinical assessment and laboratory cultures to confirm active disease. Thus, additional studies in various patient populations are still needed to verify the use of the IGRA in a variety of patient populations.

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