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## SILICOSIS AND SILICOTUBERCULOSIS

### SILICOSIS

#### Introduction

Silicosis is an occupational lung disease which remains a major public health problem in both industrialized and developing countries. Silicosis is a fibrotic lung disease due to inhalation of crystalline silica. Tuberculosis contributes significantly to the morbidity and mortality in patients with silicosis.

#### Etiology

Silicosis, the oldest known occupational pulmonary disease, is caused by inhalation of tiny particles of silicon in the form of crystalline "free" silica (usually quartz) or, less commonly, by inhalation of silicates, minerals containing silicon dioxide bound to other elements, such as talc. Workers at greatest risk are those who move or blast rock and sand (miners, quarry workers, stonecutters) or those who use silica-containing rock or sand abrasives (sand blasters; glass makers; foundry, gemstone, and ceramic workers; potters). Coal miners are at risk of mixed silicosis and coal workers' pneumoconiosis.

Factors that influence the likelihood of progression to silicosis include duration and intensity of exposure, the form of silicon (exposure to crystalline form poses greater risk than bound form), surface characteristics (exposure to uncoated form poses greater risk than coated form), and rapidity of inhalation after the dust is fractured and becomes airborne (exposure immediately after fracturing poses greater risk than delayed exposure).

#### Types of Silicosis

Workers may develop any of the three types of silicosis, depending on the concentration of airborne silica:

- **Chronic silicosis** which usually occurs after ten or more years of exposure to crystalline silica at relatively low concentrations
- **Accelerated silicosis** which results from exposure to high concentrations of crystalline silica and develops five to ten years after the initial exposure
- **Acute silicosis** which occurs where exposure concentrations are the highest and can cause symptoms to develop within a few weeks to four or five years after the initial exposure

#### Radiographic features

Radiological findings in simple silicosis include presence of widespread nodules measuring 2-5 mm in diameter, with predominance in the middle and upper lung zones. Other manifestations include presence of reticular and small nodular opacities in these lung zones and large round opacities (more commonly on the right side). The latter represent conglomerate nodules. Eggshell calcification of the mediastinal

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lymph nodes may also be apparent, most notably in the regions of the right paratracheal and aortopulmonary window. Silicosis with progressive massive fibrosis shows large, conglomerate nodules in the middle and upper lung zones. Peripheral hyperlucency represents emphysematous lung tissue secondary to central migration of the large nodules. It may be associated with evidence of volume loss in both upper lobes. The term complicated silicosis is used when conglomerate masses are greater than 1 cm in diameter. The masses can reach as much as 10 cm in diameter and in such cases, there may be associated cicatrization atelectasis of the upper lobes, hilar retraction, bibasilar hyperexpansion and emphysema. The masses may undergo ischemic necrosis and cavitation. Computed tomography (CT) is useful in further characterizing the disease and can determine the presence of early coalescence and emphysema better than plain chest radiographs.

### **Pathophysiology**

Alveolar macrophages engulf inhaled free silica particles and enter the lymphatics and interstitial tissue. The macrophages cause release of cytokines tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1), transforming growth factor- $\beta$  (TGF- $\beta$ ) and oxidants stimulating parenchymal inflammation, collagen synthesis, and ultimately, fibrosis. When the macrophages die, they release the silica into the interstitial tissue around the small bronchioles, causing formation of the pathognomonic silicotic nodule. As they mature, the nodule centers become a dense ball of fibrotic scar with a classic onion-skin appearance, surrounded by an outer layer of inflammatory cells. In low-intensity or short-term exposures, these nodules remain discrete and cause no compromise of lung function (simple chronic silicosis). But, with higher-intensity or more prolonged exposures (complicated chronic silicosis), these nodules coalesce and cause progressive fibrosis and reduction of lung volumes, and sometimes forming large conglomerate masses (also called progressive massive fibrosis).

### **Symptoms, Signs**

Chronic silicosis patients are often asymptomatic but many eventually develop dyspnea on exertion that progresses to dyspnea at rest. Productive cough, when present, may be due to silicosis, coexisting chronic occupational (industrial) bronchitis, or smoking. Accelerated silicosis patients experience the same symptoms as those with chronic silicosis but over a shorter period. Similar pathologic lesions and radiographic abnormalities often develop over months to years. Acute silicosis patients experience rapid progression of dyspnea, weight loss and fatigue, with diffuse bilateral crackles. Respiratory failure often develops within 2 years of onset of symptoms. Conglomerate (complicated) silicosis, the advanced form of chronic or accelerated disease, characterized by widespread masses of fibrosis, typically

occurs in the upper lung zones. It causes severe, chronic respiratory symptoms.

### **Treatment**

Whole lung lavage can reduce the total mineral dust load in the lungs of patients with chronic silicosis. Case series have shown short-term reduction in symptoms after lavage, but controlled trials have not been performed. Anecdotal evidence supports the use of oral corticosteroids in acute and accelerated silicosis. Lung transplantation is an option for patients with advanced lung disease and respiratory insufficiency. Patients with evidence of airflow obstruction may be treated empirically with bronchodilators and inhaled corticosteroids. Patients should be monitored and treated for hypoxemia to forestall the onset of pulmonary hypertension. Pulmonary rehabilitation may help patients to perform activities of daily living.

### **Prevention**

The most effective preventive interventions occur at an industrial rather than clinical level and include dust suppression, process isolation, ventilation, and use of non-silica-containing abrasives. Respiratory masks provide imperfect protection and, although helpful, their usage is not an adequate solution. Surveillance of exposed workers with respiratory questionnaires, spirometry, and chest radiographs is recommended. Physicians must be alert to the risk of pulmonary tuberculosis (PTB) and nontuberculous mycobacterial (NTM) infections in silica-exposed patients, especially miners. People exposed to silica but without silicosis have three times the risk of developing PTB compared with the non-exposed general population. Miners with silicosis have a more than 20-fold risk of PTB and NTM infections compared with the general population and are more likely to develop both pulmonary and extra pulmonary manifestations. Patients exposed to silica with a positive tuberculin test and negative sputum TB cultures may be considered for isoniazid chemoprophylaxis in areas that are not endemic for TB. Relapse is more common in patients with silicotuberculosis, sometimes necessitating longer courses than are usually recommended

### **SILICOTUBERCULOSIS**

Tuberculosis is regarded as one of the most common complications in patients with silicosis. It is generally accepted that silicosis facilitates the development and propagation of tuberculosis, because the prevalence of tuberculosis in silicotics is much higher than in the general population. Tuberculosis accounted for 11.8% deaths in patients with silicosis. In India, tuberculosis occurs in as many as 28.6% of patients with silicosis. Overall, it appears that tuberculosis occurs in as many as 20-25 % of all silicosis patients in their lifetime.

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## Pathogenesis

Silicosis and tuberculosis may develop in the lungs fully independently or in close topographic relation. On the other hand it is clear than tuberculosis is more frequently combined with advanced forms of silicosis. The cause of aggravation of tuberculosis in dust-exposed people is still unclear. Most probably the defense mechanisms of the lung are impeded by massive dust deposits, particularly by blocking the lymph drainage and thus hindering the elimination of invaded bacteria. Furthermore, the high prevalence of tuberculosis in dust-exposed people is probably caused by alteration of the macrophages. Quartz reduces the ability of macrophages to stop the growth of tubercle bacteria.

## Clinical features

Clinical symptoms of silicotuberculosis in chronic forms are similar to pure silicosis. The clinical symptoms depend on the stage of silicosis and on the extent of tuberculosis. Chest examination in the presence of active tuberculosis may reveal much more abnormalities than those seen in pure silicosis. Presence of hemoptysis in a patient with silicosis, in addition to tuberculosis, may also result from non-specific colliquation of silicotic scars, perforation of silicotic lymph nodes into the vascular and bronchial system, bronchiectasis or development of a scar carcinoma.

## Diagnostic Problems

The symptoms of silicosis and silicotuberculosis are often overlapping. Cough, wheeze, expectoration, dyspnea and vague chest pains are common in both pure silicosis and silicosis complicated by tuberculosis. On this account, they may be frequently ignored by the patient and occasionally overlooked by the treating physician. The interpretation of the chest radiographs is also difficult in the presence of silicotic nodules.

## Diagnosis

Pure silicosis is characterized by symmetric, small round opacities, less frequent in the apical and basal lung fields, whereas superimposed tuberculosis is mostly asymmetric and exhibits rapid increasing alveolar opacities. In advanced silicosis, the hilar nodules are mostly small, but in tuberculosis they are enlarged. Generally the more active the tuberculous process, the faster are the radiologic changes. Cavitations are often early findings in tuberculosis while they tend to occur rather late in pure silicosis. Silicotuberculous cavitations are more often irregularly shaped because of the mechanical effects of shrinking fibrotic masses.

Establishing the diagnosis of silicotuberculosis by bacteriological methods is often difficult. Therefore, more frequent sputum examination for acid fast bacilli (AFB) is recommended. The need for AFB culture is more important in areas where there is high prevalence of atypical

mycobacteria. It is suggested that sputum examination, fiberoptic bronchoscopy, bronchoalveolar lavage and transbronchial lung biopsy should be considered in an attempt to facilitate an accurate and early diagnosis.

## Treatment

Due to the pathological and physiological peculiarities of silicotuberculosis, the results with anti-tubercular treatment may not as good as in pure tuberculosis. Silicotuberculosis affects not only the lung parenchyma but also the vasculature which may impede blood circulation to the affected areas. Moreover, tuberculous cavities often occur inside silicotic nodes, where concentrations of chemotherapeutic drugs may not be optimal. Finally the fibrotic scars can prevent the collapse and scarification of a cavity.

As in pulmonary tuberculosis, most of the relapses in patients with silicotuberculosis occur within the first two years after stopping the drugs but the risk of relapse may continue indefinitely after completion of treatment. Short-course chemotherapy is sufficient to treat the patient with silicotuberculosis, but a few studies have suggested the need for a longer duration of antitubercular treatment. Hence the initial two months of intensive phase should consist of standard four drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) while continuation phase with two drugs (rifampicin and isoniazid) should be given for a period of four to seven months. Long-term follow-up is recommended to diagnose possible relapse after the completion of treatment.

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## INTERFERON GAMMA RELEASE ASSAYS FOR DIAGNOSIS OF TUBERCULOSIS : CURRENT STATUS

Infection with *Mycobacterium tuberculosis* (MTB), in most individuals, is contained by the host immune defenses, and the infection therefore remains latent [latent tuberculosis infection (LTBI)]. MTB bacilli in such individuals can subsequently undergo reactivation and cause active disease with a predicted lifetime risk of around 10%. Currently, it is difficult to predict exactly who among the latently infected will develop the disease and when. The World Health Organization estimates that one third of the world's population is infected with MTB bacilli and it is from this enormous reservoir that new cases emerge which presents a major hurdle for global tuberculosis (TB) control.

In high burden countries such as India, diagnosis and treatment of active TB receives greater priority; testing for LTBI is usually done only in selected high risk groups such as children, household contacts, and individuals with Human Immunodeficiency Virus (HIV) infection. For the effective and efficient control of TB in developing countries, rapid diagnosis and treatment for active TB patients is the mainstay of the TB control program. However, acid-fast staining of sputum has a sensitivity of only 50 to 60%, and mycobacterial culture usually requires 6 to 8 weeks to be interpretable. A rapid diagnosis is crucial not only for patients, but also for TB control in the community. In addition to difficulties in isolation of MTB from infected subjects, humoral responses to MTB in these individuals are weak. These factors have resulted in difficulties in developing clinically useful serological tests.

In low TB endemic countries, targeted testing for LTBI and treatment is a key part of the TB control strategy. It is based on identification and treatment of persons infected by MTB bacilli who are at high risk for progression to active disease. This strategy is powerful because preventive treatment of latently infected people diminishes the risk of subsequent development of active TB by about 90%. The determinants of increased risk of progression to disease are recent infection with MTB and several host-related factors, all of which are associated with an impaired cell-mediated immune response. These include

### Physiologic factors

- Young age, especially children aged under 5 years

### Pathologic factors

- HIV coinfection
- Chronic renal failure

### Iatrogenic immunosuppression

- Antitumor necrosis factor- $\alpha$  agents
- Organ transplantation
- Systemic corticosteroids

People included in these vulnerable groups have more

severe forms of TB that are often disseminated and fatal if untreated or treated late.

### TUBERCULIN SKIN TEST

The classic diagnostic tool for LTBI is the tuberculin skin test (TST), also known as the intra-dermal Mantoux test since 1910. It is the oldest diagnostic test in use in modern medical practice, and its limitations constitute the weakest element in the strategy of targeted testing of LTBI. TST is a simple test with low material costs and can be performed without the need for a specialist laboratory. This is important in high burden, resource-limited countries where even quality sputum microscopy may be difficult to access.

The TST is based on the fact that infection with MTB produces a delayed-type (cell-mediated) hypersensitivity reaction to certain antigenic components of the organism that are contained in extracts of culture filtrates called "tuberculins." Tuberculin Purified Protein Derivative (PPD) is isolated from a culture filtrate of tubercle bacilli by protein precipitation. A batch of PPD (lot 49608) called PPD-S, which was produced by Seibert and Glenn in 1939, has continued to serve as the international standard. The standard 5-tuberculin unit (TU) dose of PPD-S is defined as the delayed skin test activity contained in a PPD-S dose of 0.1 mg/0.1 ml. The standard test dose of a commercial PPD preparation is defined as the dose of the product that is biologically equivalent to that contained in 5 TU of PPD-S (i.e., it elicits reactions of equivalent size  $\pm$  20%).

The reaction to intracutaneously injected tuberculin is the classic example of a delayed (cellular) hypersensitivity reaction. T cells sensitized by prior infection are recruited to the skin site where they release lymphokines. These lymphokines induce induration through local vasodilatation, edema, fibrin deposition, and recruitment of other inflammatory cells to the area. Features of the reaction include

- delayed course reaching a peak more than 24 hours after injection of the antigen
- indurated character
- occasional vesiculation and necrosis

The test is administered by injecting 0.1 ml of 5-TU PPD intradermally (Mantoux method) into the volar or dorsal surface of the forearm. A discrete, pale elevation of the skin (a wheal) 6 to 10 mm in diameter should be produced when the injection is done correctly. Tests should be read between 48 and 72 hours after injection, when the induration is maximum. Tests read after 72 hours tend to underestimate the true size of induration. The absence of induration should be recorded as "0 mm", not "negative".

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The PPD skin test has a reported false-negative rate of 25% during the initial evaluation of persons with active TB. This high false-negative rate appears to be due to poor nutrition and general health, overwhelming acute illness, or immunosuppression.

Factors causing false negative tuberculin skin tests are :

- Factors related to the person being tested
  - ★ Infections
    - ✓ Viral (measles, mumps, chicken pox, HIV)
    - ✓ Bacterial (typhoid fever, brucellosis, typhus, leprosy, pertussis, overwhelming TB, tuberculous pleurisy)
    - ✓ Fungal (South American blastomycosis)
  - ★ Live virus vaccinations (measles, mumps, polio, varicella)
  - ★ Metabolic derangements (chronic renal failure)
  - ★ Low protein states (severe protein depletion, afibrinogenemia)
  - ★ Diseases affecting lymphoid organs (Hodgkin's disease, lymphoma, chronic leukemia, sarcoidosis)
  - ★ Drugs (corticosteroids and many other immunosuppressive agents)
  - ★ Age (newborns, elderly patients with "waned" sensitivity)
  - ★ Stress (surgery, burns, mental illness, graft-versus-host reactions)
- Factors related to the tuberculin used
  - ★ Improper storage (exposure to light and heat)
  - ★ Improper dilutions
  - ★ Chemical denaturation
  - ★ Contamination
  - ★ Adsorption (partially controlled by adding Tween 80)
- Factors related to the method of administration
  - ★ Injection of too little antigen
  - ★ Subcutaneous injection
  - ★ Delayed administration after drawing into syringe
  - ★ Injection too close to other skin tests
- Factors related to reading the test and recording results
  - ★ Inexperienced reader
  - ★ Conscious or unconscious bias
  - ★ Error in recording

On the basis of the sensitivity, specificity, and the prevalence of TB in different groups, three cut points have been recommended for defining a positive tuberculin reaction. For individuals who are at great risk of developing TB disease if they become infected with MTB, a cut point of  $\geq$

5 mm is recommended. A cut point of  $> 10$  mm is suggested for individuals who have normal or mildly impaired immunity and a high likelihood of being infected with MTB but are without other risk factors that would increase their likelihood of developing active disease.

Major drawback of TST is its low specificity. Because PPD is a culture filtrate of tubercle bacilli containing over 200 antigens shared with the bacille Calmette-Guerin (BCG) vaccine and most nontuberculous mycobacteria (NTM), individuals vaccinated with BCG but not infected with MTB can test falsely positive using the tuberculin test. The proportion of individuals with a prior BCG vaccination who have a positive TST result has been reported to vary from 0% to 90%. Subsequent reactivity can vary depending on dose, manufacturer of the vaccine, age when vaccinated, and the interval between vaccination and testing. Even large TST reactions in adults living in low-prevalence areas can be due to a prior BCG vaccination, and a meta-analysis by Wang et al. showed that BCG administration increases the likelihood of false-positive TST results for up to 15 years after vaccination.

Despite these limitations, the TST is still widely used because of its ability to predict active disease in latently infected individuals, and the fact that trials have shown that treatment of LTBI, diagnosed on the basis of TST results, reduces the risk of active disease by about 60%. This strong experimental evidence has resulted in targeted skin testing and LTBI treatment programmes in developed countries. A major advantage of the TST is its low material cost, and the fact that it does not require any laboratory infrastructure.

## INTERFERON-GAMMA ASSAYS

Interferons (IFNs) are classified into type I and type II according to receptor specificity and sequence homology. The type I IFNs are comprised of multiple IFN  $\alpha$  subtypes (14 –20, depending on species), IFN  $\beta$ , IFN  $\omega$ , and IFN  $\gamma$ , all of which are structurally related and bind to a common heterodimeric receptor. Although type I IFNs are secreted at low levels by almost all cell types, hematopoietic cells are the major producers of IFN  $\alpha$  and IFN  $\omega$ , whereas fibroblasts are a major cellular source of IFN  $\beta$ . IFN  $\beta$  is also produced by macrophages under appropriate stimulus (discussed later). Viral infection is the classic stimulus for IFN  $\alpha$  and IFN  $\alpha$  expression.

IFN  $\gamma$  is the sole type II IFN. It is structurally unrelated to type I IFNs, binds to a different receptor, and is encoded by a separate chromosomal locus. IFN  $\gamma$  is secreted by CD4+ T helper cell type 1 (Th1) lymphocytes, CD8+ cytotoxic lymphocytes, natural killer (NK) cells, B cells and other antigen-presenting cells (APCs). IFN  $\gamma$  secretion by NK cells and possibly other APCs is likely to be important in early host defense against infection, whereas T lymphocytes become the major source of IFN  $\gamma$  in the adaptive immune response.

An immunological diagnostic test is directly related

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to the immune response of the patient. Therefore, the advantage of an immunological test lies in its capacity to demonstrate whether the patient has been previously sensitized to the mycobacterium and confirm an infection, without the need to detect the bacillus in sputum or any other biological sample of the patient.

For nearly a century, there were no alternatives to the TST for diagnosing LTBI. In vitro methods for measuring cell-mediated immune reactivity offer potential advantages and may avoid some limitations of the TST. Because of advances in immunology and genomics, for the first time, an alternative has emerged in the form of T cell based interferon- $\gamma$  (IFN- $\gamma$ ) assays, a new generation of in vitro tests of cellular immunity. Immune-based rapid blood tests exploit the fact that the predominant host response to MTB infection consists of antigen-specific memory T cells releasing interferon (IFN- $\gamma$ ) in response to previously encountered mycobacterial antigens. These assays measure cell mediated immune response by quantifying IFN- $\gamma$  released by T cells in response to stimulation by MTB antigens.

These new tests use two proteins encoded by a unique genomic segment termed "Region of Difference 1," which is absent from all strains of *M. bovis*, BCG and the vast majority of NTM but is present in all clinical isolates of MTB. These proteins, ESAT-6 (early secretory antigenic target protein 6) and CFP10 (culture filtrate protein 10), are major targets for IFN- $\gamma$  secreting T lymphocytes in MTB-infected individuals. ESAT-6 and CFP-10 are deleted from BCG Region 1 (RD1), and are not present in most NTM (exceptions are *M. kansasii*, *M. szulgai*, and *M. marinum*).

Interferon gamma based blood tests have an internal positive control (i.e., a sample well stimulated with a potent nonspecific stimulator of IFN- $\gamma$  production by T cells); this controls the results of the test for technical errors, including failure to add viable functioning cells to the well. Although a negative TST in immunosuppressed individuals can be a false negative, the failure of the positive control in the blood tests provides the important information that the test's result cannot be reliably interpreted because it may reflect an underlying *in vivo* immunosuppression, negatively affecting T-cell function in the *in vitro* stimulation.

The antigens used to illicit an IFN- $\gamma$  response define the main types of existing commercial tests: assays based on PPD and those based on RD-1 specific antigens. Three commercial IFN- $\gamma$  release assays (IGRAs) have been developed, the QuantiFERON-TB assay, the T SPOT-TB assay and the Quantiferon Gold assay.

### **Interferon-gamma assays based on PPD**

The first IGRA approved as an alternative to the TST by the US Food and Drug Administration was the QuantiFERON-TB assay. This assay measures IFN  $\gamma$  production with ELISA, after in vitro stimulation of whole

blood cells with PPD from MTB and control antigens. The whole blood ELISA IFN- $\gamma$  assay is comparable with TST in its ability to detect LTBI. Disadvantages of a PPD based assay such as the whole blood ELISA is, that it can give false-positive results in BCG vaccinated people and that it does not discriminate between most of the NTM and MTB

### **Interferon-gamma assays based on RD-1 specific antigens**

Assays based on RD1-specific antigens have shown to cause less confounding by BCG vaccination than TST and are therefore more reliable to use in BCG vaccinated individuals. ESAT-6 and CFP-10 share the same messenger RNA transcript, which suggest that CFP-10 and ESAT-6 may interact with one another and serve a common function in detection of MTB. All stimulated T-lymphocytes secrete IFN- $\gamma$ , but ESAT-6 and CFP-10 assays can only detect IFN- $\gamma$  secreted from T-lymphocytes produced as a result of exposure to ESAT-6 and CFP-10 antigen. In vivo and in vitro experiments have shown that the combination of ESAT-6 and CFP-10 has a higher sensitivity and specificity than PPD in diagnosis of TB infection.

There are two commercial assays available incorporating the two RD1-based antigens ESAT-6 and CFP-10. The T-SPOT.TB assay is an ELISPOT assay, whereas QuantiFERON-TB Gold Test is an ELISA test.

### **QuantiFERON-TB Gold Test**

QuantiFERON-TB Gold Test (QFT-G) measures the IFN- $\gamma$  released by sensitized white blood cells after whole blood is incubated with antigen. The test consist of a negative control (a nil well [i.e., whole blood without antigens or mitogen]), a positive control (a mitogen well [i.e., whole blood stimulated with the mitogen photohemagglutinin]), and 2 sample wells (i.e., whole blood stimulated with either ESAT-6 or CFP-10). Whole blood specimens are incubated for 18 hours (overnight) at 37°C in a humidified atmosphere. The IFN- $\gamma$  level of the nil well is considered to be the background value and is subtracted from the values for the mitogen well and the antigen-stimulated wells. The test result is considered to be positive if the IFN- $\gamma$  level in the sample well after stimulation with ESAT-6 and/or CFP-10 is  $\geq 0.35$  IU/mL (after subtraction of the value for the nil well), irrespective of the result for the positive control well. The test result is considered to be negative if the IFN- $\gamma$  level is  $< 0.35$  IU/mL and if the IFN- $\gamma$  level of the positive control well (after subtraction of the value for the nil well) is  $\geq 0.5$  IU/mL. The test result is considered to be indeterminate if the IFN- $\gamma$  level is  $< 0.35$  IU/mL in both antigen wells and  $< 0.5$  IU/mL in the positive control well.

As with a negative TST result, negative QFT-G results should not be used alone to exclude MTB infection in persons with symptoms or signs suggestive of TB disease. The presence of symptoms or signs suggestive of TB disease increases the likelihood that MTB infection is present, and

these circumstances decrease the predictive value of a negative QFT-G or TST result. Medical evaluation of such persons should include a history and physical examination, chest radiograph, bacteriologic studies, serology for HIV, and, when indicated, other tests or studies.

The performance of QFT-G, in particular its sensitivity and its rate of indeterminate results, has not been determined in persons who, because of impaired immune function, are at increased risk for MTB infection progressing to TB disease. Impaired immune function can be caused by HIV infection or acquired immunodeficiency syndrome (AIDS); current treatment with immunosuppressive drugs including high-dose corticosteroids, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) antagonists, and drugs used for managing organ transplantation; selected hematologic disorders (e.g., myeloproliferative disorders, leukemias, and lymphomas); specific malignancies (e.g., carcinoma of the head, neck, or lung); diabetes; silicosis; and chronic renal failure. Each of these conditions or treatments is known or suspected to decrease responsiveness to the TST, and they also might decrease production of IFN- $\gamma$  in the QFT-G assay. Consequently, as with a negative TST result, negative QFT-G results alone might not be sufficient to exclude MTB infection in these persons.

#### Interpretation of QFT-G result from IFN- $\gamma$ concentration in test samples

ESAT-6 nil or CFP-10 nil or both	Nil	Mitogen nil	QFT-G Results	Interpretation
$\geq 0.35$ IU/ml & $> 50\%$ above nil	Any	Any	Positive	<i>Mycobacterium tuberculosis</i> infection likely
$< 0.35$ IU/ml	$\leq 0.7$	$\square 0.5$	Negative	<i>M. tuberculosis</i> unlikely but cannot be excluded especially when illness is consistent with TB disease and likelihood of progression to TB disease is increased
$< 0.35$ IU/ml	Any	$< 0.5$	Intermediate	QFT-G results cannot be interpreted as result of low mitogen response
$\leq 50\%$ above nil	$> 0.7$	Any	Intermediate	QFT-G results cannot be interpreted as a result of high background response.

#### T-SPOT.TB Test

T-SPOT.TB Test assay is designed for the detection of effector T cells that secrete the cytokine in response to stimulation by antigens specific for MTB.

Peripheral blood mononuclear cells (PBMCs) are separated from a whole blood sample, washed (to remove background cytokine) and counted (to adjust for low T cell titers in the immunocompromised) and are added to microtitre wells backed with a membrane pre-coated with

an antibody to the cytokine. The T-SPOT.TB assay requires  $2.5 \times 10^5$  viable PBMCs per well with a total of four wells for each patient sample.

The antigens are incubated with the PBMCs to allow stimulation of any activated T cells present. Secreted cytokine is captured by specific antibodies on the surface of the membrane and the cells and other unwanted materials are removed by washing. A second antibody, conjugated to alkaline phosphatase and directed to a different epitope on the cytokine molecule, is added and binds to the cytokine captured on the well surface. Any unbound conjugate is removed by washing. A soluble substrate is added to each well; this is cleaved by bound enzyme to form a spot of insoluble precipitate at the site of the reaction. Each spot represents the footprint of an individual cytokine-secreting T cell, and evaluating the number of spots obtained provides a measurement of the abundance of MTB sensitive T cells in the peripheral blood.

#### Characteristics of the TST and Interferon Gamma Assays

	TST	QuantiFERON-TB Gold	T-SPOT.TB
<b>Antigens</b>	PPD	ESAT-6 and CFP10	ESAT-6 and CFP10
<b>Positive internal control</b>	No	Yes	Yes
<b>Uniformity of methods &amp; reagents</b>	No	Yes	Yes
<b>Potential for boosting effect</b>	Yes	No	No
<b>Return visit</b>	Yes	No	No
<b>Time for results</b>	48–72 h	16–24 h	16–20 h
<b>Setting of test</b>	<i>In vivo</i>	<i>In vitro</i>	<i>In vitro</i>
<b>Interpretation</b>	Subjective (operator-based)	Objective (instrument-based)	Objective (instrument-based)
<b>Readout units</b>	Millimeters of induration	International units of IFN- $\gamma$	IFN- $\gamma$ spot-forming cells
<b>Technological platform</b>	NA	ELISA	ELISpot
<b>Test's substrate</b>	NA	Whole blood	PBMC
<b>Outcome measure</b>	NA	Serum concentration of IFN- $\gamma$ produced by T cells	Number of IFN- $\gamma$ producing T cells
<b>Readout system</b>	Palpable induration	Measurement of optical density values using an automated reader	Enumeration of spots by naked eye, magnifying lens, or automated counter

For high burden countries, improving prompt diagnosis and treatment of active disease remain the immediate priorities. However, better diagnosis of TB infection by ELISPOT could help TB control in high burden countries in three ways : (1) by improving diagnosis of asymptomatic infection (and active TB) in children; (2) by improving diagnosis in HIV infected individuals; and (3) by enhancing

epidemiological surveys to assess the effect of TB control measures. Thus, although the greatest impact of ELISPOT will initially be on TB control in the developed world, it is likely that countries with a high burden of TB and HIV will also stand to benefit from this new approach to spotting TB infection.

Kang et al have recently shown that high negative predictive values of QFT-G and T-SPOT.TB for the diagnosis of active TB may have a supplementary role of these tests for the diagnostic exclusion of active TB. However, the low positive predictive values limits their usefulness in routine clinical practice in countries, where the prevalence of LTBI is considerable. Mazurek et al have demonstrated that TST, QFT, and QFT-G have similar sensitivity in persons with culture-confirmed infection. However, as with the TST, negative QFT and QFT-G results should not be used to exclude the diagnosis of TB in persons with suggestive signs or symptoms. Mori et al have shown that the whole blood IFN- $\gamma$  assay using CFP-10 and ESAT-6 was highly specific and sensitive for MTB infection and was unaffected by BCG vaccination status.

Published studies categorically demonstrate that T-SPOT.TB and QuantiFERON-TB Gold are more specific than the TST for the diagnosis of LTBI in BCG-vaccinated populations. T-SPOT.TB, in addition, seems to be more sensitive than the TST in immunocompetent people with LTBI and in patients with active TB, including those with impaired cellular immunity at high risk of false-negative TST results. QuantiFERON-TB Gold probably has sensitivity similar to the TST in immunocompetent people with LTBI. As with T-SPOT.TB, QuantiFERON-TB Gold has a higher sensitivity than TST in immunocompetent patients with active TB.

In addition to their improved diagnostic accuracy, the blood tests have operational advantages over TST, including lack of inter-individual variability in administration of the test, a more objective read-out, and a result by the next day. One significant practical drawback of the blood tests is the fact that they need to be processed within 6 hours from venipuncture and the reliability of results declines after this time point.

Replacement of the skin test with the blood tests will not change the principles of targeted testing, which are based on identifying those groups at highest risk of progression to active TB. Higher specificity will reduce or eliminate false-positive test results in BCG-vaccinated people, thus avoiding the costs associated with unnecessary chemoprophylaxis and its associated toxicity. Higher sensitivity would, on the other hand, identify more infected persons among those with a false-negative TST result. More true-positive results in infected people would increase the rate of diagnosis and treatment of LTBI in the most vulnerable populations before progression to active TB.

Because of the lack of a gold standard for latent

infection, it is impossible to accurately determine the sensitivity and specificity of IFN- $\gamma$  assays for the diagnosis of LTBI. Therefore, several studies have evaluated the agreement (concordance) between TST and IFN- $\gamma$  assays. This approach avoids the use of TST, an imperfect test, as the gold standard. Most studies reported modest to high agreement (60% to 80%) between the two tests.

## CONCLUSIONS

The diagnosis of LTBI requires not only a positive TST or positive IGRA results, but also that active TB be ruled out by a careful history taking, appropriate evaluation of symptoms, and radiographic examination of the chest. Selecting which test to use among the two blood tests or between a blood and the skin test in a given epidemiologic situation depends on the population being tested, the purpose of testing, and the resources available. In high-burden and resource-limited settings such as India, where even access to simple technology such as sputum microscopy may be poor in some areas, the TST might continue to serve a useful purpose.

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