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The challenge of pediatric tuberculosis in face of new diagnostic techniques

Flavio R. Sztajnbok,[1] Neio L. Boechat,[2] Denise C. N. Sztajnbok,[3] Samantha Brum Ribeiro,[4] Sheila K. F. Oliveira,[5] Clemax C. Sant'Anna[6]

Abstract

Objectives: To present an updated review concerning new assays for diagnosing tuberculosis based on in vitro interferon-gamma production by host T cells, and to compare them with tuberculin skin test.

Sources: A literature review was carried out based on MEDLINE and LILACS databases (2000-2008) searching for the following keywords: tuberculosis, interferon-gamma, quantiFERON, ELISPOT and T-SPOT.TB.

Summary of the findings: These new assays proved to have, in general, equal or superior sensitivity and specificity than the tuberculin skin test not only in adults but also in children and immunosuppressed patients for the diagnosis of both latent tuberculosis infection or active disease, with some advantages such as less cross-reactivity as a result of previous BCG vaccination, less influence of anergy and better accuracy in small children. In the United States, these assays have been used instead of the tuberculin skin test and, although still very expensive, the World Health Organization will be making its economic viability a priority.

Conclusions: Always having in mind the importance of clinical and epidemiological histories, these new assays based on interferon-gamma release present promising results and should be considered in tuberculosis investigation procedures for all patients, however with a special concern in the risk groups (i.e., children and immunosuppressed patients).

J Pediatr (Rio J). 2009;85(3):183-193: Tuberculosis, interferon-gamma, quantiFERON, ELISPOT, children, immunosuppression.

Introduction

Tuberculosis (TB) remains a challenge for public health. In developing countries, sanitary and socioeconomic conditions facilitate its dissemination and make the populations susceptible to it. In developed countries, in addition to the problem of multiresistant TB, the disease reappearance is related to the large migratory flows and the epidemic of acquired immunodeficiency syndrome (AIDS). There are estimates that one third of the world population is infected with *Mycobacterium tuberculosis* and that, most of the time, this infection remains latent in individuals who have an efficient immune system.[1,2] Latent TB can persist for many years, with the risk of becoming an active disease in about 10% of the general population. TB is an important cause of morbidity and mortality, mainly in immunosuppressed patients, and it still remains as the most frequent cause of deaths due to infectious disease in adults.[3,4]

1. Professor assistente, Pediatria, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brazil. Responsável, Setor de Reumatologia, Núcleo de Estudos da Saúde do Adolescente, Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, RJ, Brazil. Professor adjunto, Reumatologia Pediátrica, Universidade do Grande Rio Prof. José de Souza Herdy (UNIGRANRIO), Rio de Janeiro, RJ, Brazil.
2. Professor adjunto, Clínica Médica, UFRJ, Rio de Janeiro, RJ, Brazil.
3. Professora assistente, Pediatria, UERJ, Rio de Janeiro, RJ, Brazil.
4. Responsável, Setor de Biologia Molecular, Laboratório Multidisciplinar de Pesquisas, Hospital Universitário Clementino Fraga Filho (HUCFF), UFRJ, Rio de Janeiro, RJ, Brazil. Mestranda, Clínica Médica, HUCFF-UFRJ, Rio de Janeiro, RJ, Brazil.
5. Professora associada, Pediatria, UFRJ, Rio de Janeiro, RJ, Brazil. Chefe, Serviço de Reumatologia Pediátrica, Instituto de Puericultura e Pediatria Martagão Gesteira, UFRJ, Rio de Janeiro, RJ, Brazil.
6. Professor associado, Pediatria, UFRJ, Rio de Janeiro, RJ, Brazil. Médico, Serviço de Pneumologia Pediátrica, Instituto de Puericultura e Pediatria Martagão Gesteira, UFRJ, Rio de Janeiro, RJ, Brazil.

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Diseases that affect immunosuppressed patients, such as AIDS, diffuse connective tissue diseases, myeloproliferative diseases, neoplasias, diabetes and other conditions (malnutrition, old age), as well as use of immunosuppressive drugs, may lead to a less effective immune response against TB. Then, there will be reduced production of cytokines, such as interferon-gamma (IFN-γ) and tumor necrosis factor (TNF-α), which play an important role in the immune response against *Mycobacterium tuberculosis*[5-9] and in the possible absence of response to the tuberculin skin test (TST), making it difficult to diagnose the disease. The TST might have lower sensitivity in immunosuppressed patients when compared to patients with efficient immune system, making the diagnosis of TB more difficult and delaying it.[10,11] Since there is not a confirmatory assay for the diagnosis of latent TB or culture-negative active disease, the TST is still considered the most appropriate tool. In spite of its several limitations, the gold standard for diagnosis of infection would be the confirmation of progression to active disease, which is only possible in longitudinal studies. The presence of positive cultures for *Mycobacterium tuberculosis* would be the gold standard for the diagnosis of the active disease.[12,13] With regard to the latent infection, it is not possible to predict which patients will progress to active disease.

In the pediatric age group, mainly regarding children younger than 3 years old, TB is often more severe than in adults and, in comparison, there is a higher percentage of extrapulmonary manifestation and disseminated forms of TB. Samples of material for culture in children are often difficult to be collected, and the results are frequently negative. The difficulty to establish a definitive diagnosis may lead to undiagnosed or wrongly diagnosed cases. The TST seems to have low sensitivity when used in the pediatric age group, and negative results do not rule out active disease. Children can progress to active disease short after acquiring latent infection and, therefore, early diagnosis is crucial so that preventive measures can be taken.[14]

Early and accurate diagnosis of TB is an important step while a new vaccine that can effectively prevent the disease is not available. Several new bacteriological and molecular diagnostic techniques have been studied, such as more sensitive polymerase chain reaction (PCR), assays for microscopic detection and drug susceptibility (Microscopic Observation for Detection and Susceptibility – MODS) and new culture media. There is a shortage of information about all of these assays in the pediatric field. *In vitro* whole-blood assays have been recently developed with the purpose of assisting the diagnosis of latent TB. These assays have proved to be quite promising for studies on the latent tuberculosis infection and its epidemiology, as well as the active disease.[13,14] Such assays are based on the production of IFN-γ by host T cells that undergo *in vitro* exposure to more specific antigens of *Mycobacterium tuberculosis* than the purified protein derivative (PPD) of the TST. The QuantiFERON-TB Gold® (Cellestis LTD, Carnegie, Victoria, Australia) and the T-SPOT.TB® (Oxford Immunotec, Oxford, United Kingdom) are available in the market. The QuantiFERON-TB Gold uses well plates, approved by the Food and Drug Administration (FDA), or tubes, not approved by the FDA, and measures the amount of IFN-γ produced by the patient's T cells mixed with specific antigens using the enzyme linked immunoabsorbent assay (ELISA). The T-SPOT.TB, also known as the ELISPOT (enzyme linked immunospot assay), counts the number of IFN-γ-producing T cells when these cells are also sensitized with specific antigens. It has been approved for use in Europe, as well as by the FDA.[11,15-17] These assays have good sensitivity and specificity for the diagnosis of TB, presenting advantages in comparison with the TST, mainly in patients with some degree of immunosuppression.[18-21]

Early diagnosis and treatment of tuberculosis infection, avoiding progression to disease, are challenges that need to be overcome so that worldwide control of TB can be achieved.[22] New diagnostic methods will be welcome, mainly when their aim is to aid the diagnosis of TB in special groups like children and immunosuppressed patients. By 2015, the World Health Organization (WHO) expects to reduce the prevalence and the mortality rate of the disease by less than 50% of the rates found in 1990.[23]

Tuberculosis epidemiology in Brazil and in the world

Human TB is caused by mycobacteria that belong to the so-called *Mycobacterium tuberculosis* complex, which includes *Mycobacterium bovis, Mycobacterium africanum* and *Mycobacterium tuberculosis*. Latent TB or TB infection can be defined as a clinical syndrome caused by the exposure to *Mycobacterium tuberculosis* followed by infection evidenced by the presence of positive TST, but without clinical and radiological signs and symptoms of active disease, since there is an efficient immune response to control mycobacterium growth. The bacillus remains quiescent in the infected tissue. There is decreased bacterial metabolism, but *Mycobacterium tuberculosis* is not always eradicated.[24] Progression of infection to active disease seems to be related to some risk factors such as age (elderly and children) and patients with immunosuppressive diseases or conditions (coinfection with HIV, autoimmune diseases, malnutrition, neoplasia, diabetes, chronic renal failure, among others) or even the use of drugs that affect immunity.[25-28] In most cases, the infection remains latent and the individual becomes a reservoir for future cases, creating an important epidemiological problem.[29] In the United States, there are estimates that between 9 and 14 million people are currently with latent infection.[30] The global estimates suggest that approximately 2 billion people are infected and, among these, about 8 million will progress to disease and 2 million

will die every year, and approximately 100,000 deaths will be in the pediatric age group. Around 95% of the new cases and 98% of deaths occur in developing countries, where the access to the health system is inappropriate and HIV infection is more frequent.[4,24,31]

Approximately 50% of the TB cases recorded in the Americas are found in Brazil and Peru. Brazil is the 15th among the 22 countries responsible for 80% of the total number of TB cases in the world. According to data from the Information System for Notifiable Diseases (*Sistema de Informação de Agravos de Notificação* – Sinam/Ministry of Health), there is notification of about 85,000 new cases per year (incidence ratio of 47/100,000 inhabitants), and between 1980 and 2002 there was a mean of 6,000 deaths/year. In 2001, Brazil had a mortality rate due to TB of 3.07 cases/100,000 inhabitants. The mean incidence ratio of TB in Brazil between 1990 and 2001 was 48 new cases per 100,000 inhabitants. Ten states had higher rates than the Brazilian mean. The incidence ratio per 100,000 inhabitants in 2001 was higher in the age group from 20 to 39 years, with an incidence ratio of 98.8/100,000, but, in the pediatric age group, mainly among adolescents, who may have an epidemiological behavior similar to that of adults, this ratio was not low (9.7 in the age group 0-4 years; 5.3 in the age group 5-9 years; 21.8 in the age group 10-19 years). Also according to data from Sinam, in 2000, 4.2% of the notified TB cases affected individuals younger than 15 years old and 6.8% affected individuals between 15 and 19 years old, that is, 11% of the notified cases occurred in children and adolescents. In 2005, these data were respectively 3.7 and 6%, that is, 9.7% of the notified TB cases affected individuals in the pediatric age group. These data highlight the importance of such disease in Brazil in terms of morbidity and mortality and reinforce the need for early and accurate diagnosis and treatment.[4,32]

Immunology and pathogenesis

Immune control of TB starts with innate immunity by means of physical barriers and the action of macrophages and dendritic cells. These cells define a cell response to mycobacteria. Such response is predominantly a Th1 type response with increase in the CD4+ (which induces the production of IFN-γ) and CD8+ population and it is fully active after 2 to 6 weeks of infection.[24] In general, there are important differences between adults and children in terms of TB immunopathogenesis. In children, there is functional impairment of macrophages and dendritic cells and a prevalence of Th2 type cell response, with lack of CD8+ cells and production of interleukin-4 and interleukin-5 by CD4+ cells.[33,34] This is a possible explanation for the fact that disseminated and meningeal forms of TB are proportionally more frequent in children younger than 1 year old (20% compared with less than 1% in older children and adults).[35] Very young children are more likely to develop the active disease due to the immaturity of their immune system (innate and adaptive) and because of the long-term contact with caregivers who are possible bacilli carriers. The increase in the susceptibility to TB in children seems to be related to factors such as impaired function of macrophages and reduced production of some cytokines by the macrophages (IL-10, TGF-β, TNF-α) and to the functional impairment of dendritic cells and CD4+ cell response.[34] Maternal lymphocytes usually do not pass through the placenta and its presence in the maternal milk is reduced. Newborns and nursing infants depend on their own cellular immunity, which tends to mature during the following years, to act against intracellular pathogens, as it is the case of TB.[14,36]

Transmission of *Mycobacterium tuberculosis* happens mainly through the airborne route. The first physical and anatomical barriers are cough and mucociliary transport, since they reduce the amount of bacilli reaching the distal alveoli. After reaching the distal alveoli, mycobacteria will find other innate defense mechanisms of the respiratory tract, such as defensins, collectins, lectins and complement, which perform several actions like, for instance, activation of complement pathways, stimulation of the production of cytokines and chemokines, permeabilization and damage of the germs' membrane, and agglutination or opsonization of bacteria. In case mycobacteria cross these barriers, they primarily infect macrophages and then activates them.

Activated macrophages will inhibit the intracellular growth of mycobacteria using unclear mechanisms. Probably there is apoptosis of the infected macrophages and production of cytokines like tumor necrosis factor (TNF-α) and interleukins (IL) 12 and 18, which somehow hinder the dissemination of bacteria.[24,37,38] TNF-α is also secreted by T cells, and interleukin-12 and interleukin-18 are released by dendritic cells. However, mycobacteria present defense mechanisms: inhibition of maturation of the endosomal compartment of the infected macrophage,[39] and stimulation to the production of cytokines by the infected macrophages. These cytokines, such as the transforming growth factor-beta (TGF-β), reduce the immune response (inactivation of macrophages, suppression of cell response to mycobacteria), stimulate the intracellular growth of bacteria and inhibit the production of TNF-α and IL-12 induced by mycobacteria.[40,41] In addition, the infected macrophages produce IL-10, a potent anti-inflammatory cytokine that reduces the cell response, partially because it decreases the production of IL-12.[42] IL-10 can convert dendritic cells into macrophage-like cells, which are able to inhibit the intracellular growth of mycobacteria, but are not very capable of producing antigen.[43] Another function of IL-10 is to inhibit the proliferation of CD4+ T cells stimulated by *Mycobacterium tuberculosis* and the production of IFN-γ by CD4+ T cells.[40] In short, TNF and IL-12 cytokines promote

the immunological control of the infection, while IL-10 and TGF-β inhibit (downregulation) the innate and acquired response in TB.

Dendritic cells also play an important role in the immunopathogenesis of TB and seem to be an important means of communication between innate and acquired immunities, since they are also antigen-presenting cells. Dendritic cells activation, at first, determines better efficacy of phagocytosis and macropinocytosis in their peripheral region, processing of antigen and exposure on the cells surface, achieving the potent activation of the cell response to mycobacteria. After these phases, there is migration to lymph nodes, where the dendritic cells lose their ability to perform phagocytosis; however, they become potent antigen-presenting cells that are able to stimulate the cellular immunity for a long time.[34] The dendritic cells located in the respiratory tract epithelium and in the interseptal areas of the lungs are essential to maintain the local immune homeostasis. It is necessary to maintain a non-inflammatory status in these areas, in spite of the constant arrival of new antigens. Dendritic cells can induce Th1 or Th2 cell response through secretion of IL-12 or IL-10, respectively. Physiologically, dendritic cells are responsible for the prevalence of Th2 response in the lungs, which are constantly affected by new antigens.[34,44] Once they are infected by mycobacteria, dendritic cells produce mainly IL-12 and TNF-α, and both of them are very important to control the immune response in TB.[42] Therefore, dendritic cells of very young children have reduced functional capacity in comparison to adults, what causes less stimulation to the cellular immune response and higher susceptibility to TB.[45]

The role played by the humoral immunity in the immune response in TB is controversial, although children and adults with TB usually have hypergammaglobulinemia.[34] Some studies have shown that specific antibodies do not provide adequate protection.[46] Other studies have demonstrated that B cells can improve protection against mycobacteria, regardless of the antibody production, that is, such cells could be antigen-presenting cells.[47] This protection could be confirmed by the finding that higher levels of antibodies are related to better prognosis[48]; however, Sant'Anna et al.[49] found higher levels of antibodies correlated with higher disease severity in Brazil. Nevertheless, children with TB are likely to present lower titers than adults. Such titers tend to increase proportionally to age and disease duration, reflecting the maturation of the immune system and the long-lasting exposure to mycobacteria.[50,51]

TNF-α plays an important role in the immune response to *Mycobacterium tuberculosis* and in the disease immunopathology.[8] It is a pro-inflammatory cytokine produced by macrophages activated as a response to several stimuli such as lipopolysaccharides and viral and bacterial infections.[52] TNF-α can also be expressed by activated T cells, B cells, NK cells (natural killer) and some tumoral cells. It can have two forms: soluble and transmembrane. Its mains actions are: antitumor and antiviral activities; mediation of shock and cachexia; and stimulation to apoptosis, mainly regarding inefficient macrophages, depriving mycobacteria from an environment for multiplication.[8] TNF, in mycobacterial infections in animal models, seems to increase the macrophages' ability to phagocyte and kill the aggressive agents, in addition to inducing the development of the granuloma, which restrains the dissemination of mycobacteria, producing an efficient immune response.[8,53,54] Therefore, mainly soluble TNF-α plays an important role in the coordination and restraining of mycobacterial infection, especially regarding the lungs, since the granuloma controls the growth and dissemination of mycobacteria, reducing the tissue damage.[8] TNF-α also seems to increase apoptosis of local neutrophils and decrease inflammatory tissue damage. Therefore, it is possible to infer that the use of antiTNF-α agents will result in accumulation of these cells, persistent inflammation and more severe damage.[55] Apoptosis of macrophages infected by mycobacteria, mediated by the action of TNF-α, is a characteristic of the normal response of the granuloma development in TB.[56,57] On the other hand, the excess of TNF-α seems to be associated with caseous necrosis. In clinical terms, it causes systemic inflammation, night sweats and cachexia, in accordance with its previous name, cachectin.[8] Therefore, the use of drugs that interfere with TNF-α could increase the incidence of infections, such as TB and deep mycoses, whose pathogenesis is highly influenced by the development of granulomas.[8,58,59]

Tuberculosis in children and immunosuppressed individuals

Epidemiologic data, medical history, physical examination and additional tests are used to diagnose latent TB and active disease. Children and adolescents represent a greater challenge, since they have more specific TB signs and symptoms, more frequent negative cultures for mycobacteria and higher risk of progression from infection to disease in comparison to adults.[33,34] TB in this age group is considered a sentinel event, reflecting a recent infection from a community source, since children are seldom primary sources of infection. If the child does not develop the disease, he/she might progress from latent infection to disease in the future.[33] Latent infection can persist for many years, posing a risk of progression to disease to approximately 10% of the general population.[8] In the pediatric age group, this rate can reach 43% in children younger than 1 year, 24% in those between 1 and 5 years old, and 15% in adolescents, when they do not receive treatment during the first 2 years after being infected.[35,60] One of the main factors associated with non-progression to disease seems to be the production of IFN-γ by host T cells, which is reduced in younger children.[61-64]

Certain conditions and diseases coincide with immunosuppression, and use of immunosuppressive drugs can lead to inefficient immune response and, as a consequence, make individuals prone to higher risk of opportunistic and non-opportunistic infections.[25-27,59,65-69] The development of TB in the immunosuppressed population involves several aspects: atypical forms that make diagnosis difficult, need for longer treatments, possibility of drug interaction between TB treatment and therapy of the underlying disease, adverse effects of antituberculous therapy associated with the underlying disease and, also, the possibility of transmission of infection to other immunosuppressed patients who are treated at the same health care facility. It is believed that one dose of prednisone higher than 15 mg/day for longer than 2-4 weeks is an increased risk for the development of TB.[69,70] The identification of infection carriers in this context can be difficult due to the fact that such patients are asymptomatic and because of the limitations of the diagnostic methods available.

The ideal assay to establish the diagnosis of latent TB should have high sensitivity and specificity, regardless of previous BCG vaccination and infection with other mycobacteria. The TST, which has been the method used as gold standard for almost one century to identify these patients, is an *in vivo* assay that assesses late hypersensitivity reaction, using a PPD as antigen. The PPD consists in a heterogeneous mix of proteins, what makes it difficult to differentiate between infection with *Mycobacterium tuberculosis* and other mycobacteria.[34,68] The TST has several limitations: patients need to go to the health care facility twice; false-positive results associated with previous BCG vaccination (reduced specificity), occurrence of booster effect (repeated TST can lead to anamnestic response, with recruitment of previously existing hypersensitivity) or, also, due to the previous contact with other mycobacteria; false-negative results (severe patients, including those with active TB, and immunosuppressed patients[6,12,55]); and errors in the performance or interpretation of results of the assay.[17,68,71-74] The TST could have a good predictive value in terms of the infection progression to active disease, but in populations with low incidence of TB it seems to have a predictive value lower than 50%.[69,75,76] In countries where the incidence of TB is high, BCG vaccination at birth will not have false-positive results in the interpretation of the TST after some years.[77,78] Since the TST does not have an internal positive control, it is very difficult to differentiate between true-negative results and false-negative results. The earlier the TST is performed in patients with immunosuppressive disease, the lower the risk of false-negative results due to anergy associated with the underlying disease. Therefore, there might be situations when the patients at risk are not diagnosed and treated, while in other situations they might be receiving unnecessary treatment due to diagnostic error. A TST positive result can suggest recent infection, even if asymptomatic, or past infection, susceptible to reactivation.[8,29,34,79] In patients at high risk of developing active TB in case of infection (HIV infection, recent contact with TB patient, immunosuppressed individuals), the cutoff point of the TST will be 5 mm for indication of chemoprophylaxis (ATS and Brazilian Ministry of Health).[69,80]

Biological agents that act on TNF-α (antiTNF-α agents) were initially recommended for the treatment of rheumatoid arthritis. Their use has become frequent also to treat other diseases that need immunosuppression. Several studies have approached the incidence of TB in rheumatic diseases and the role played by these drugs and other immunosuppressive agents in the reactivation of latent TB. Some studies have suggested that low doses of corticosteroids and cytotoxic agents used in rheumatoid arthritis do not increase the incidence of TB in this group of patients.[69,81,82] However, Yamada et al.[65] found increased risk of TB in Japanese individuals who were being treated for rheumatoid arthritis before the introduction of antiTNF-α agents. The same situation was reported in Sweden[83] and Spain,[84] but, in the United States, Wolfe et al.[85] showed that the incidence of TB did not increase with conventional immunosuppression, maybe due to the low prevalence of TB in that country. Information currently available suggests that this class of drugs increases the risk of TB and that most cases result from the reactivation of previous infection. The contribution of recent infections to such phenomenon is unknown.[8] It would be necessary that patients who will initiate therapy with biological agents, mainly the antiTNF-α agents, undergo latent or active TB investigation previously.[10,65,76] On the other hand, Keane & Bresnihan[12] did not find evidence in the literature on the association between TB and the use of other biologic agents commonly employed such as rituximab, abatacept and anakinra. In case the active disease is ruled out in patients with positive TST, therapy with isoniazid for 9 months is recommended as prophylaxis of latent infection progression to disease. In this case, the initiation of therapy with antiTNF-α agents should be postponed for at least 1 to 2 months.[8] It is believed that the reduction in the action of TNF-α leads to abnormal immune response even when there is a small number of bacilli, causing important clinicopathological consequences.[70]

Diagnostic methods based on the production of interferon-gamma

In vitro whole-blood assays have been recently developed with the purpose of assisting the diagnosis of TB. These assays are known as IGRAs (interferon-gamma release assays) because they assess the production of this cytokine by cells previously sensitized with specific agents. In May 2005, the FDA approved the assay QuantiFERON®-TB Gold (QFT-G) for diagnosis of latent infection or active disease

caused by *Mycobacterium tuberculosis* (65). The QFT-G uses the ELISA (enzyme linked immunosorbent assay) method to measure the production of IFN-γ by T cells of a whole-blood sample.[68] Another assay that has been commonly used, the ELISPOT (available in the market as T-SPOT.TB®, Oxford Immunotec, Abingdon, United Kingdom) uses the ELISPOT (enzyme linked immunospot assay) technique to assess the number of peripheral mononuclear cells that produce IFN-γ using magnifying lens or an ELISPOT reader.[18,68,86] The ELISPOT, which is commonly used in Europe, has been approved by the FDA in the United States in July 2008. Positive results of these assays must lead to investigation to determine whether there is TB disease before considering them as TB infection.[87]

The QFT-G and the ELISPOT are based on the production of IFN-γ by T lymphocytes previously sensitized after incubation with synthetic peptides simulating mycobacterial proteins such as the early secretory antigenic target 6 (ESAT-6) and the culture filtrate protein 10 (CFP-10). New antigens have been recently included in the assays with the purpose of increasing sensitivity. The TB7.7 antigen was included in the new QuantiFERON-TB Gold In-Tube (patient's blood is directly inserted into a vacuum tube with antigens ready for incubation),[15,68] and the Rv3879c antigen was included in the ELISpot^{plus}.[16] Such proteins, present in PPD and secreted by *Mycobacterium tuberculosis* and by pathogenic strains of *Mycobacterium bovis*, are not included in the BCG vaccine and in most non-tuberculous mycobacteria, except for *Mycobacterium kansasii*, *Mycobacterium szulgai* and *Mycobacterium marinum*.[88-90] Therefore, these assays must offer higher specificity than the TST for the diagnosis of infection with *Mycobacterium tuberculosis*. Lein et al.[91] showed that patients with lung disease caused by the *Mycobacterium avium* complex had negative assays.

Genes that codify these antigens are located in the so-called region of difference 1 of the genome of *Mycobacterium tuberculosis.* They are deleted from the genome of *Mycobacterium bovis* of BCG and are not present either in the genome of some non-tuberculous mycobacteria such as *Mycobacterium avium*.[68] Therefore, antigens used in the IGRAs are not present in BCG and in some non-tuberculous mycobacteria.[92] Budnick et al.[93] suggested that the QFT-G would indirectly identify the whole *Mycobacterium tuberculosis* complex: *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microcoti* and *Mycobacterium canetti*. Gey Van Pittius et al.[94] suggested that, although *Mycobacterium avium* and most non-tuberculous mycobacteria do not have genes able to codify the ESAT-6 and CFP-10 proteins, some genes present in some of these mycobacteria could produce proteins that would have cross-reaction with the genes associated with the region of difference 1 segment. However, Wang et al.[95] confirmed the accuracy of the IGRAs for the diagnosis of active TB even in endemic areas for non-tuberculous mycobacteria.

These assays seem not to be influenced by previous BCG vaccination[79,88] and they are less influenced by previous infection with non-tuberculous mycobacteria, which increases their diagnostic sensitivity and specificity when compared to the TST.[10,17,29,79,87,88,96] Other advantages of such assays are: only one visit to the health care facility (without second visit for reading, as it is the case for the TST), results in 24 hours, no performance or reading interpretation errors as it is the case for the TST, use of small amount of blood (which is good for the pediatric age group) and absence of booster effect, since it does not expose the patient to an antigen. Table 1 shows a comparison between the TST and the IGRAs. Richeldi et al.[18] commented that the fact that

Table 1 - Comparison between the TSTs and the IGRAs

	TST	IGRA
Visit to health care facility	twice	once
Time for results	72-96 hours	24 hours
False-positive results	Previous BCG, booster effect	No
False-negative results	Immunosuppressed individuals, severe TB	Possible, but hardly probable
Results interpretation	Susceptible to assessment errors	Computerized

BCG = Bacillus Calmette-Guérin vaccination; IGRA = interferon-gamma release assay; TST = tuberculin skin test; TB = tuberculosis.

the ESAT-6 and CFP-10 proteins are present in PPD can, at least theoretically, cause repeated TST assays to increase the T cells' response to such antigens. It would result in false-positive ELISPOT results, a method extremely sensitive even to a small number of T cells.[97] However, the same study[18] showed that, in practical terms, patients undergoing repeated TST did not have positive ELISPOT results. This assay would have high specificity even in patients with false-positive TST due to the booster effect, and it could be used in those patients being investigated and who underwent TST for several times. However, errors regarding collection, transportation, technique and interpretation reduce its accuracy. Blood must be incubated with the synthetic antigens at most 12 hours after collection. Other important limitations are the cost of the assay and the need of a laboratory adequate for the assay technique. This can limit its use in developing countries, exactly where TB is more often endemic. Other important limitation of the IGRAs, as well as the TST, is the lack of differentiation between TB infection and TB disease. The diagnosis of TB infection requires ruling out the disease based on clinical data and additional tests.[87]

The IGRAs can have undetermined results due to technical errors, because of the large amount of IFN-γ in the null control or, even, if the positive control with phytohemagglutinin (potent T-cell stimulator mitogen) determines the production of little or none IFN-γ.[9] This low mitogenic response can result in anergy or lack of ability of the immune system to prepare an efficient cell response. Therefore, the IGRAs can also be affected by immunosuppressive states. However, undetermined results can be found in immunocompetent individuals occasionally. The literature has shown that undetermined results were found in up to 11% of the patients tested with the QFT-G, and this finding must be associated with the small number of immunosuppressed patients included in these studies.[98-100] Ferrara et al.[79] found high rate of undetermined results (21.4%) in a population with 20% of the patients receiving immunosuppressive drugs. Ravn et al.,[101] confirming that study, found undetermined results with the QFT-G in nine patients. Of these, three had HIV infection, one was being treated with an immunosuppressive drug and two had severe TB. The undetermined results due to lack of mitogenic response suggest that they might be associated with immunosuppression. Therefore, physicians must be attentive to the possibility of anergy and must further investigate TB in patients with suspected latent infection or active disease.[9,29]

Few studies have approached the importance of the IGRAs in the context of immunosuppressed patients. Some studies have shown little reduction in the response of the IGRAs when compared to non-immunosuppressed population.[21,102] Other studies have shown that the prevalence of positive ELISPOT results was higher than that found using the TST, mainly in immunosuppressed patients.[16,102,103] However, Brock et al.[104] found low prevalence of positive QFT results in asymptomatic patients infected by the HIV. The undetermined results were more frequent in patients with CD4 count lower than 100 cells/mm^3.

Recent studies have shown that both assays have high sensitivity and specificity for the diagnosis of TB disease, but few of them have approached TB infection, which is the initial reason for the use of such assays.[21,79,96,102] Ferrara et al.[79] suggested that IGRAs specificity is higher than TST specificity for the diagnosis of tuberculosis infection. Pai et al.[105] showed that the IGRAs are more specific than the TST when populations previously vaccinated with BCG are assessed. In general, different studies involving adults have shown agreement of 60-80% when comparing the TST to the methods based on IFN-γ production. In terms of sensitivity, there are several studies that showed that the IGRAs are more sensitive than the TST.[15,102,103,106] Ponce de Leon et al.[15] recently showed that, in a population from an endemic area for TB, the ELISPOT was significantly more sensitive than the TST for diagnosis of latent TB in patients with rheumatoid arthritis, but the same result was not found in healthy patients. Such finding probably was associated with immunosuppression of the disease and/or medication used in the disease treatment. However, studying a group of 2,381 contacts of TB cases in Gambia, Jackson-Sillah et al.[107] showed that the ELISPOT had lower sensitivity than the TST, but the authors highlighted that this assay may be important for the diagnosis in the pediatric age group. In a recent meta-analysis, Pai et al.[108] concluded that the IGRAs (mainly the QFT using well-plates or tubes) have excellent specificity for the diagnosis of latent tuberculosis infection, whereas the TST has high specificity in the population that was not vaccinated with BCG, but low specificity in the previously vaccinated population. IGRAs and TST sensitivity remains controversial. It is difficult to be evaluated using meta-analysis because of the methodological differences between the studies included; however, the ELISPOT seems to be more sensitive than the QFT-G and TST. Both showed similar sensitivity; therefore, it is possible to conclude that this higher sensitivity rate of the ELISPOT can be useful in the evaluation of immunosuppressed patients.[68] It is worth mentioning that malnutrition, immunosuppression and severe TB can cause anergy and contribute to a decrease in the sensitivity of the IGRAs.[108]

With regard to the response to the IGRAs in the pediatric age group, there are few studies about it and several controversial aspects need to be clarified. As previously mentioned, the cellular immunity tends to mature as the individual grows older. Some studies have shown that the production of IFN-γ detected by the QFT-G can be affected by growth, but this does not occur with the ELISPOT, except in the first weeks of life. This assay can detect the production of IFN-γ even when there is a small number of

secretory cells. This could explain the higher frequency of undetermined QFT-G results when compared to the ELISPOT in the pediatric age group regarding adults.[14,29,109]

In South African children with active TB in an area with high incidence of AIDS, Liebeschuetz et al.[21] demonstrated that the ELISPOT was much more sensitive than the TST and that age younger than 3 years, coinfection with HIV and malnutrition reduced TST sensitivity, but did not affect ELISPOT sensitivity. In school outbreaks, Ewer et al.[110] showed that the ELISPOT had better correlation with exposure to *Mycobacterium tuberculosis* than the TST in a group of adolescents between 11 and 15 years old, and that previous BCG vaccination did not have an influence on the results of the assays. Higher sensitivity of the ELISPOT compared to the TST was also demonstrated in other studies.[95,111] ELISPOT sensitivity for the diagnosis of active disease was higher than QFT-G and TST sensitivity, whereas these assays had similar results. There are not reliable data for the assessment of IGRAs specificity for the diagnosis of active TB.[14,109,112]

Considering the latent infection, the lack of a gold standard for this diagnosis makes it difficult to compare new assays with the TST.[14] Regarding the QFT-G, there are controversial aspects; however, more recent data suggest that this assay can be less sensitive than the TST regarding the diagnosis of TB infection.[109] Several studies involving children have demonstrated that the ELISPOT has higher sensitivity than the TST in the diagnosis of latent infection.[18,19,110] Due to the fact that the ELISPOT correlates better with different patterns of exposure to TB than the TST, it is possible to conclude that the ELISPOT has better sensitivity than the TST. However, longitudinal studies are needed to confirm the actual higher sensitivity of the IGRAs. With regard to specificity, the IGRAs have proven to be more specific than the TST in the diagnosis of latent infection.[14,113,114]

Conclusion

The TST assesses an *in vivo* multimediated inflammatory response to multiple tuberculin antigens, whereas the IGRAs assess *ex vivo* production of IFN-γ by patients' lymphocytes stimulated by a small number of antigens of *Mycobacterium tuberculosis.* Although the IGRAs have been developed as a strategy to diagnose latent infection, some studies have shown that these assays can also be useful to diagnose active disease. In such case, the negative result would be sufficient to rule out the possibility of disease, that is, the IGRAs seem to have high negative predictive value. This would be particularly useful for children, immunosuppressed individuals and patients with negative cultures, for whom the diagnosis of active disease is more difficult to be established using the conventional assays.[13]

Although some aspects still remain controversial, in general, the IGRAs are accepted as being more sensitive and specific than the TST for the diagnosis of latent infection and active disease. However, there is need for longitudinal studies that make it possible to confirm these data in a reliable manner. Longitudinal studies are also crucial for the definition of future risk regarding the progression to active disease in patients with positive results, differently from what happens with the TST. Regarding the TST, the risks of disease and the benefits of treatment associated with different results of the assay have already been established.[68] Such facts reinforce the need for further studies on the IGRAs in different populations, mainly regarding those with high incidence of TB and including patients with conditions that lead to depression of the cellular immune response, in addition to specific studies involving the pediatric age group.

In terms of public health, the IGRAs could be, at least currently, a complement to the TST, assessing the cost-benefit ratio. Although these assays require only one visit and are not performed directly in the patient, they still are expensive assays and need specialized laboratories and professionals trained to perform them timely. Oxlade et al.[115] concluded that the best cost-benefit ratio of the QFT would be to test patients known to have positive TST results. At present, while the CDC (Centers for Disease Control and Prevention) recommends that the QFT-G replaces the TST in any situation, health institutions from England suggest that the IGRAs must be assessed along with the TST. Only when there is probability of finding false-negative TST results, as it is the case for immunosuppressed individuals and young children, these assays would be indicated for use in the initial phase of investigation.[13,14,68,87]

Negative TST and IGRAs results in immunosuppressed population do not rule out latent TB or active disease, at least currently. In practical terms, individuals with these negative assays, but with risk factors for progression from latent TB to active disease, could need chemoprophylaxis or disease treatment.[87]

The WHO, recognizing the importance of the IGRAs regarding clinical, epidemiological and basic research studies, co-sponsored a meeting of experts in Geneva, Switzerland, in March 2006. Seven major priority lines of research on the assays were defined during this meeting (Table 2).[13] In spite of the large number of recent studies published, many questions remain unanswered. The IGRAs ability to identify patients with latent infection at a higher risk of progression to active disease and, therefore, to give those patients a chance to benefit from preventive measures, as well as the behavior of such assays in longitudinal studies involving high risk populations, such as children and immunosuppressed individuals, provide a fertile ground for research, mainly in countries like Brazil, where TB is far from being eradicated.

Table 2 - Research priorities regarding the IGRAs suggested by the WHO

Development of new trials and biological considerations
Performance of assays in high risk populations and involving those little studied (including immunosuppressed patients and children)
Risk prediction models
Assay reproducibility
Assessment of T cells during tuberculosis treatment
Epidemiological applications
Implementation of the use of assays regarding different health systems and socioeconomic conditions

IGRA = interferon-gamma release assay; WHO = World Health Organization.

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Correspondence:
Flavio R. Sztajnbok
Rua Álvaro Ramos, 405/804, bloco 1
CEP 22280-110 - Rio de Janeiro, RJ - Brazil
Tel.: +55 (21) 2275.7372, +55 (21) 9994.7006
Fax: +55 (21) 2275.7372, +55 (21) 2264.2082
E-mail: flavios@skydome.net