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Editorial

Tuberculosis infection diagnosis: Current prospects

Diagnóstico de la infección tuberculosa: perspectivas actuales

Tuberculosis (TB), one of the oldest and most important infectious diseases, continues to be the leading cause of worldwide mortality produced by a microorganism. According to the World Health Organization (WHO), there were 10.8 million new TB cases and 1.25 million deaths from this disease in 2023.[1]

One critical aspect that aggravates TB control is the presence of a vast population with tuberculosis infection (TBI). This is defined as the state in which a person infected with the *Mycobacterium tuberculosis* complex, presents no signs, symptoms, radiological, microbiological or anatomopathological evidence of disease, and furthermore has no capacity for contagion.[2] It is estimated that approximately a quarter of the world's population is infected with this microorganism, and therefore is a huge reservoir of potential tuberculosis cases,[3] because 5–15% of those infected will develop TB at some point in their life,[4] especially in the presence of risk factors such as patients with HIV infection, silicosis, terminal renal failure, immunosuppressive treatments, as well as, epidemiological situations such as contact with TB patients, persons deprived of liberty, or migrants from high prevalence countries.[5]

TB has traditionally been described in dichotomous terms, with individuals being classified as either "infected" (classically referred to as latent infection) or "TB disease" (active TB). However, it is now known that infection with this pathogen is a more complex process, which should be considered as a continuous spectrum of stages, ranging from initial exposure to the bacillus to final manifestation of disease, but not limited to the strict classification of latency and activity.[6] The immune system attempts to control infection by the bacillus through various responses, and predominantly through the action of macrophages and T cells, which seek to limit bacterial multiplication. In many cases, this immune response can contain the infection, and prevent its progression to TB disease, but does not completely eliminate the bacillus,[7] which although phagocytosed by macrophages, manages to survive within these cells, in the form of granulomas. These granulomas are immune isolation structures wherein the bacillus can persist for years, without causing significant damage to the body. Thus, a dynamic balance is maintained between the pathogen and the host immune system. In this period, patients show no symptoms of the disease, are not contagious, and are traditionally referred to as "infected without active disease". As mentioned earlier, progression from infection to disease occurs in a small percentage of cases, not as a single event, but as a continuum caused by various factors that decompensate the immune response. Hence, some individuals may experience a multiplication of bacilli without presenting symptoms, but have the ability to transmit the pathogen to others, in the so-called *subclinical tuberculosis*.[6] This intermediate process can last months or years and patients may remain asymptomatic throughout life or sometimes develop symptoms in the long term. This new, more flexible and complex approach has important implications for diagnosis and treatment by highlighting the need for not only diagnosing the disease, but detecting TB cases, especially, those in the earlier or intermediate stages, thereby enabling early intervention and preventing progression to symptomatic disease. Therefore, the proper identification of TBI individuals is a priority in TB control strategies.[8]

The TBI diagnostic methods are based on the detection of the specific immune response against the *M. tuberculosis* complex, either through skin tests, such as the classic tuberculin skin test (TST) or by interferon gamma release assays (IGRA).[9]

TST is the oldest traditional method used to detect TBI. This test detects the delayed-type hypersensitivity immune response mediated by T lymphocyte against the tubercle bacillus. Although Robert Koch had presented it as the cure for tuberculosis in 1890, it was later observed that it had a great diagnostic capacity to differentiate between infected and uninfected individuals. This test is based on intradermal injection into the flexor surface of the forearm (Mantoux technique) of a purified protein derived (PPD) of *M. tuberculosis* and measurement of the skin reaction (induration) in millimetres after 48–72 h. A debated aspect is the cut-off point for considering the test as positive since there are multiple individual and epidemiological factors that need to be taken into account. There is general agreement that a transverse diameter $\geq$5 mm of induration is positive for TBI and therefore would warrant preventive treatment, depending on the context in which it can be applied. Generally speaking, the main advantages of TST are that it is an economical, simple, and easily available test, with extensive accumulated evidence of its effectiveness, especially in children. However, TST has a lack of specificity and can give false positive results in individuals vaccinated with BCG or exposed to non-tuberculous/environmental mycobacteria. Moreover, sensitivity may be limited by false negative results in immunocompromised individuals or in young children without fully developed immunity. And lastly, TST presents a certain variability in result interpretation,

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since this depends on the observer's experience, which may vary according to different clinical contexts.[9]

The beginning of the 21st century witnessed the incorporation of IGRA techniques, which are *in vitro* tests that measure the release of gamma-interferon (IFN-γ) in the blood by circulating T cells, in response to several specific antigens of the *M. tuberculosis* complex (excluding BCG strains), such as the protein 6-kD *M. tuberculosis* early-secreted antigenic target (ESAT-6) and the 10-kD culture filtrate protein (CFP-10). Prior contact with the tuberculous bacillus results in T lymphocyte response through release of a large number and variety of cytokines that can be detected. At present, there are several commercially available techniques that are based on the detection of IFN-γ in whole blood via ELISA or chemiluminescence, one such being QuantiFERON-TB Gold Plus (QFT-Plus), which is the most widely used assay, but there are also others such as Wantai TB-IGRA, Standard E TB-Feron, QIAreach QFT-TB, LIAISON QFT-Plus, VIDAS TB-IGRA, T-Track TB, AdvanSure TB-IGRA and ichroma IGRA-TB. Moreover, there are IGRA methods based on ELISPOT quantification of the number of IFN-γ-producing T cells, in a mononucleated cell concentrate such as T-SPOT.TB. Unlike TST, IGRA are not affected by BCG vaccination, since they are based on specific antigens of *M. tuberculosis*, and furthermore, they may suffer interference only from a few non-tuberculous or environmental mycobacterial species (*Mycobacterium kansasii*, *Mycobacterium marinum*, *Mycobacterium szulgai*, and *Mycobacterium flavescens*), which in turn improves the specificity of the test and has facilitated reduction in the number of unnecessary preventive treatments.[10,11] On the other hand, these are objective reading tests with more reproducible results and which do not require a second follow-up visit as in TST.[11] A relevant aspect of these tests is the incorporation of positive (with universal stimulants such as phytohemagglutinin) and negative controls. Therefore, the IGRA results are interpreted as positive, negative or indeterminate. The indeterminate results are rare and may arise as a result of incorrect sample processing, reduced blood lymphocyte count or their reduced activity due to HIV infection, immunosuppressive treatment, cancer, autoimmune or chronic diseases such as diabetes mellitus, chronic kidney failure and liver disease, as well as extreme stages of life and some genetic factors.[2] On the other hand, IGRA are significantly more expensive than TST and require well equipped laboratories and personnel with specific training, thus limiting their implementation in certain low-resource settings. We also need to bear in mind that results may be affected by immunological alterations, even though they present less false negatives than TST.[12]

Just like in TST, IGRA do not distinguish between TBI and TB disease, and are poor predictors of progression from infection to disease, since a large number of individuals with positive TST or IGRA do not progress to TB disease. This is one of the most relevant and difficult aspects in every-day clinical practice diagnosis seen especially in patients with subclinical TB, who may pass unnoticed precisely due to the absence of symptoms, despite the fact that they can transmit the disease. Consequently, patients diagnosed with TBI, but with TB disease (even in the subclinical phase) would not only fail to get cured, but may generate a drug-resistant TB.[13] Therefore, several improvements or technical innovations have been carried out, such as the incorporation of new peptides of ESAT-6 and CFP-10 antigens in QTF-Plus, to generate responses from T-CD4+ and T-CD8+ lymphocytes, wherein the latter could improve detection of recently infected cases and consequently lead to a greater theoretical probability of progression to disease, despite there being no clear evidence to that effect. Recent developments in the identification of new antigens such as Rv2028c, Rv2029c and Rv0475, likewise attempt to pave the way to improve IGRA value as highlighted by Dong et al.[14] These could overcome some of the current limitations, especially by combining multiple antigens that could mitigate variability in individual responses, thereby improving their diagnostic accuracy.

Besides these improvements in antigen selection, rapid microfluidic technologies in miniaturized devices, as well as flow cytometry, are also being developed. The objective is to offer same-day results and try to increase diagnostic accuracy.[15] These are expensive methods for the time being, that require certain types of infrastructure, and where scientific evidence is still quite limited.

Other diagnostic innovation aspects include the incorporation of new immunological biomarkers, as well as, molecular and transcriptomic tests in TBI patients who are at high risk of progression to disease. These would be targeted to patients with incipient TB, in accordance with the present continuous spectrum interpretation of the disease. The incorporation of these biomarkers into daily TBI treatment practice would greatly improve efficiency through better patient selection for targeted treatment.[16,17]

On another note and with a view to a simpler and more universal implementation in low-resource areas, skin tests with specific tuberculous antigens have been developed in the last decade to improve the specificity of TST. These include Diaskintest, C-TST and Cy-TB (formerly C-TB test), which are based on recombinant ESAT-6 and CFP-10 proteins. Cy-TB has just received a positive opinion from the European Medicines Agency (EMA) and recommends approval of this product (SIILTIBCY). In general, despite the varying quality of the studies carried out, results seem to be good, and in some cases even comparable to the IGRA.[18]

The incorporation of emerging technologies such as artificial intelligence, e.g. machine learning to analyse complex patterns in IGRA results and other techniques, could contribute to increasing their predictive capacity. Some studies indicate that TBI patients have biomarker profiles similar to that of patients with disease, indicating that such profiles may correspond to TBI patients in incipient TB stage that are at greater risk of progression to disease.[19,20]

To summarise, IGRA represent a significant progress in tuberculosis infection diagnosis when compared to the classic tuberculin skin test. However, usefulness is limited, since they do not facilitate differentiation between infection and disease, and furthermore are a poor predictor of the risk of progression to TB disease. Although significant progress has been made in the development of new technologies with high diagnostic potential, there is still a need for more scientific evidence for widespread implementation in clinical practice.

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