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NOVELTIES IN DERMATOLOGY

Current Panorama in the Diagnosis of Cutaneous Tuberculosis

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Abstract. Pulmonary and cutaneous tuberculosis are caused by *Mycobacterium tuberculosis*. According to data from the World Health Organization, there are around 8 million new cases per year. The incidence of cutaneous tuberculosis has risen in parallel with that of pulmonary tuberculosis, and coinfection by *M tuberculosis* and human immunodeficiency virus is considered to be one of the main causes. Current diagnostic methods for pulmonary and extrapulmonary tuberculosis are far from perfect, leading to a delay in starting appropriate therapy. We present a review of these diagnostic methods and of their use in the cutaneous forms. In conclusion, histopathologic findings and isolation of *M tuberculosis* in cultures of biopsy material or by polymerase chain reaction are the most useful diagnostic tools in cutaneous tuberculosis.

Key words: cutaneous tuberculosis, diagnosis, mycobacteriosis.

PANORAMA ACTUAL EN EL DIAGNÓSTICO DE LA TUBERCULOSIS CUTÁNEA

Resumen. La tuberculosis pulmonar y cutánea está causada por *Mycobacterium tuberculosis*. Según datos de la Organización Mundial de la Salud (OMS) se presentan alrededor de 8 millones de casos nuevos al año. La incidencia de la tuberculosis cutánea se ha incrementado paralelamente con la de la tuberculosis pulmonar. La coinfección de *M. tuberculosis* y el virus de la inmunodeficiencia humana (VIH) se considera una de las principales causas. Los métodos diagnósticos utilizados en la actualidad para la identificación de tuberculosis tanto pulmonar como extrapulmonar dejan un amplio margen de error, retrasando el inicio de un tratamiento oportuno. Presentamos una revisión de dichos métodos y de su aplicación en las formas cutáneas. En conclusión, los hallazgos histopatológicos y el aislamiento de *M. tuberculosis* en cultivos de biopsias o por reacción en cadena de la polimerasa (PCR) son las herramientas diagnósticas más útiles para la tuberculosis cutánea.

Palabras clave: tuberculosis cutánea, diagnóstico, micobacteriosis.

Introduction

Tuberculosis (TB) is a health problem throughout the world. It has important consequences and is not limited to developing countries. According to the World Health Organization (WHO), there are approximately 8 million new cases per year and 1700 million people are thought to be infected. Pulmonary TB is the fifth most common cause of death in the world, with 3 million deaths annually. As many as 95% of cases of pulmonary TB are reported in developing countries.[1] Extrapulmonary forms, including cutaneous TB, represent 10% to 20% of the total number of cases. Cutaneous TB represents 1.5% of all forms of TB and is responsible for 0.1% to 1% of all skin disorders.[2,3] In the 20th century, the incidence of pulmonary TB was falling, although since the 1980s the number of cases has been rising, as has the incidence of cutaneous TB. The reasons for this phenomenon include AIDS, the emergence of resistant strains of *Mycobacterium tuberculosis*, and the increased use of immunosuppressive agents.[1,4-7]

The lack of a timely diagnosis has also played an important role in making this infection difficult to control. The development of new diagnostic tools is one of the components of the Global Plan to Stop TB 2006-2015 and of the New Stop TB Strategy of the WHO, which support the use of evidence-based medicine in the development of new policies in the diagnosis of TB. It is noteworthy that around $1 billion are spent annually on the diagnosis of TB.[1,7]

The available diagnostic methods range from very simple and cheap ones to extremely complex and expensive ones. The former include the traditional sputum smear, which,

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although the most widely used, has low sensitivity and variable specificity. The most sophisticated procedures, such as those based on molecular biology, are very specific yet expensive, and their sensitivity is variable; therefore, their use is somewhat limited in developing countries. The vast majority of the available diagnostic procedures prove most useful in pulmonary TB.[8] The present study aims to review these procedures and describe how they can be used to diagnose cutaneous TB.

Etiology and Pathogenesis

Both pulmonary and cutaneous TB are caused by *M tuberculosis* and, occasionally, by *Mycobacterium bovis*. The infection is usually acquired by inhalation of mycobacteria, although it can also be caused by ingestion or direct contact. Intact skin provides an effective barrier against invasion by the microorganism; however, any impairment in the integrity of the skin or mucous membrane makes it easier for the microorganism to enter and, therefore, for the process of infection to begin. Once tuberculosis bacteria have been inhaled, those contained in airborne particles measuring 1-5 µm can reach the alveoli and produce primary pulmonary infection, before spreading to extrapulmonary sites, including the skin. Cutaneous TB can also occur due to direct contact or proximity to lesions in other tissues such as lymph nodes, bone, the digestive tract, the pleura, and the lung itself.

The infection triggers a late-onset hyperreactive cellular immune response (responsible for the clinical and histologic manifestations), which takes between 2 and 10 weeks to develop completely and is confirmed by a positive tuberculin skin test result. Only 5% of infected individuals develop primary TB.[6] In 10%, the disease appears months or years later due to reactivation of latent bacteria, thus producing the type of TB that affects the highest number of people, postprimary pulmonary (reactivation) TB.[6] This is the contagious form. The infective potential of a patient with TB depends essentially on the amount of bacteria in secretions and on the frequency of cough. The possibility of becoming infected depends on the immune status of the exposed host and on the frequency of exposure. An individual with pulmonary TB spreads mycobacteria into the environment through respiratory secretions, although UV sunlight kills them rapidly. Under conditions of poor ventilation and scant sunlight, bacteria suspended in the air are viable for 3-5 days, thus enabling them to be inhaled by another person.[6] A knowledge of the transmission of pulmonary TB and its clinical manifestations is important for the dermatologist, since some cases of cutaneous TB present concurrently with pulmonary TB or in patients with a history of pulmonary TB. Cutaneous TB is seldom primary.

Cutaneous TB

Diagnosis of cutaneous TB is challenging: its manifestations are varied, typical dermatologic lesions are rare, and the bacterium is seldom identified by staining or culture. Skin involvement may arise due to infection of the skin, either in isolation or together with infection of other organs, that is, disseminated TB. The Table summarizes the main characteristics of each of the types of presentation of cutaneous TB. Lesions caused by direct contact with an external source usually appear as tuberculous chancre, TB verrucosa cutis (Figure 1), and, occasionally, lupus vulgaris. Skin infection originating from an endogenous site may appear as scrofuloderma (Figure 2), acute miliary TB, tuberculous gumma, lupus vulgaris, and orificial TB. Orificial TB is usually observed in patients with involvement of the lungs, intestine, or anogenital area, and the mouth is the most commonly affected site.[9,10] Lupus vulgaris is the most common clinical form of cutaneous TB in developed countries: up to 40% of patients present associated tuberculous lymphadenitis and 10%-20% of cases are associated with pulmonary or osseous TB, supposedly the foci of primary infection.[3,4,11-13] However, scrofuloderma is the type most commonly associated with active pulmonary TB.[4]

In addition to infectious lesions, there may be cutaneous eruptions resulting not from infection but rather from immune phenomena generated by distant infection. These lesions are known as tuberculids and there are 3 types: papulonecrotic lesions, lichen scrofulosorum, and erythema induratum.[9,10] Diagnosis of cutaneous TB becomes complicated when the possible differential diagnoses are taken into consideration. These include lesions due to atypical mycobacterioses or other infectious dermatoses (syphilis, sporotrichosis, chromomycosis, actinomycosis), and acne conglobata.[11] Clinical presentation does not usually enable an accurate diagnosis to be made, and, although the lesions arouse clinical suspicion, diagnosis must be confirmed by additional tests. The systemic manifestations that are characteristic of pulmonary TB, such as fever and weight loss, are unusual in the cutaneous forms, and their presence should point to systemic disease. Similarly, the presence of respiratory symptoms such as cough and hemoptysis suggest the presence of coexisting pulmonary disease. In such cases, diagnosis should focus on lung infection, since identification is easier and skin involvement could be diagnosed by inference. In patients infected with human immunodeficiency virus (HIV) and with other forms of immunosuppression, the most common skin lesions include the miliary form (associated or not with subcutaneous nodules), and the presence of pustules mimicking folliculitis.

Table 1. Transmission, Clinical and Histopathologic Characteristics, and Diagnosis of the Different Forms of Cutaneous Tuberculosis

	Tuberculous Chancre	*Tuberculosis Verrucosa Cutis*	*Lupus Vulgaris*	*Scrofuloderma*	*Acute Miliary Tuberculosis*	*Tuberculous Gumma*	*Orificial Tuberculosis*
Transmission	Direct contact on skin or mucosa. No specific immunity	Direct inoculation on skin or mucosa in a previously infected person. Moderate immunity	Direct extension. Hematogenous or lymphatic spread from a tuberculous focus. Reinfection	Involvement due to spread towards the skin from a focus of tuberculosis (lymph node, bone). Can be after BCG or PPD	Hematogenous spread from a primary pulmonary focus. Low immunity	Acute hematogenous spread from a primary focus during periods of bacteremia and low resistance	Self-inoculation from mucosa or skin adjacent to a natural orifice. Active internal tuberculous infection
Clinical characteristics	Initially a small papule or a lesion that is slow to heal. Painless ulcer with a granular or hemorrhagic base and that may be covered by necrotic tissue	Papule or papule-pustule with an inflammatory purple halo, presents hyperkeratosis: verrucous plaque. Asymptomatic	Friable café-au-lait–reddish papule or macule with a smooth or scaly surface that can progress to plaque. More common on the face (nose)	Firm subcutaneous nodules that ulcerate and develop sinuous tracts by draining caseous or purulent material	Affects the whole body, especially the trunk. Macular, papular, erythematous, or purpuric lesions. Occasionally vesicles, scabs, or central necrosis	Subcutaneous fluctuating abscesses that form fistulas and ulcers. Single or multiple on the trunk, extremities, or head	Affects the mucous membrane and periorificial sites. Yellowish or reddish nodule that ulcerates with desiccated edges and is observed in punch biopsy
Histopathology	Nonspecific inflammatory infiltrate; bacteria. Granulomatous inflammation with caseation, epithelioid cells, Langhans giant cells, and disappearance of bacteria	Acute inflammatory infiltrate in the epidermis, pseudoepitheliomatous hyperplasia, microabscesses in the upper dermis, granulomatous foci; bacteria occasionally present	Well-developed tubercles with scant caseation, absence of bacteria; nonspecific inflammatory infiltrate	Granulation tissue, caseous necrosis in the upper dermis; bacteria may be isolated	Abundant bacteria; necrosis; nonspecific inflammatory infiltrate surrounded by macrophages, sometimes forming microabscesses	Formation of abscesses, massive necrosis; AFB staining reveals a large number of *Mycobacterium tuberculosis*	Nonspecific inflammatory infiltrate and necrosis; some caseous tubercles in the deep dermis; bacteria are easily observed
Diagnosis	Histopathology, culture, conversion of PPD from (–) to (+), PCR	Histopathology, culture, response to antituberculous treatment	Histopathology, culture (50%-60%) PPD (+++) PCR	Clinical history of tuberculosis. Culture. PPD (+++)	Sputum smear test, histopathology, culture; presence of another focus of tuberculosis PPD (–)	Histopathology, culture	Clinical history of tuberculosis, histopathology, and culture. PPD (–), PCR

Modified and adapted from references 9-11.

Abbreviations: AFB, acid-fast bacteria; BCG, bacille Calmette-Guérin; PCR, polymerase chain reaction; PPD, purified protein derivative.

Diagnosis

Diagnosis of TB is based on 4 parameters: symptoms, histopathology testing, culture, and DNA sequencing by polymerase chain reaction (PCR).[14] An increasing number of new methods with suitable sensitivity and specificity that attempt to speed up diagnosis are being developed. Most focus on pulmonary TB, although they could also prove useful in extrapulmonary forms, such as cutaneous TB.[11,14,15]

Below, we review the most commonly used diagnostic procedures for TB in general and their specific application to cutaneous TB.

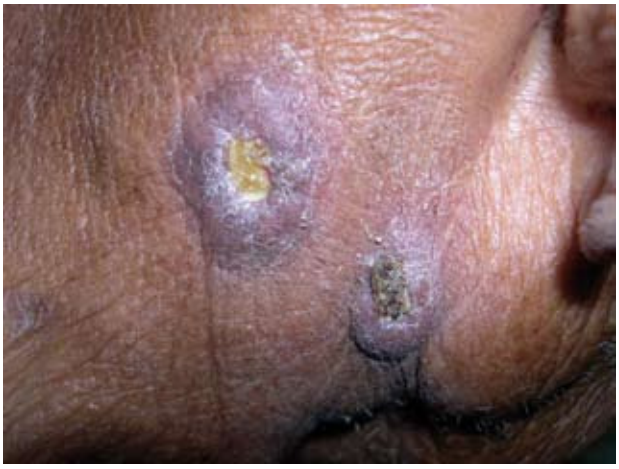

Figure 1. Tuberculosis verrucosa cutis.

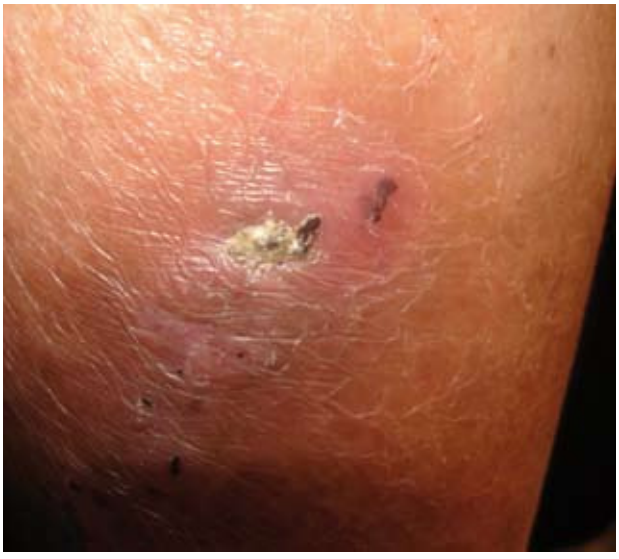

Figure 2. Cutaneous tuberculosis presenting as scrofuloderma.

Histopathology

Histopathology of a skin biopsy specimen shows that the different types of presentation may reveal similar findings, although characteristic data can point us to specific types of cutaneous TB.[9-11] In the case of tuberculous chancre, the acute form reveals a neutrophilic inflammation with nonspecific necrosis and, occasionally, the presence of bacteria; the chronic form shows centrally caseating granuloma, epithelioid cells, and giant Langerhans cells, although bacteria are usually absent. Early-onset TB verrucous cutis is characterized by acute inflammation in the epidermis, pseudoepitheliomatous hyperplasia, and microabscesses in the superficial dermis; granulomatous foci and bacteria can sometimes be observed. In scrofuloderma, bacteria can be isolated from the pus in lesions, and necrosis and caseation can be observed in the deep dermis. Bacteria are more easily observed in the orificial form, together with nonspecific changes such as necrosis and caseation in the deep dermis. The presence of well-developed tubercles with scant caseation, nonspecific inflammatory changes, and the absence of bacteria can be observed in lupus vulgaris. Tuberculous gumma can present massive formation of abscesses and necrosis; staining for *M tuberculosis* usually reveals large amounts of bacteria. Finally, acute miliary TB is characterized by abundant bacteria, necrosis, and nonspecific inflammatory infiltrates, with occasional microabscesses. Histopathology is useful, especially in tuberculous chancre, lupus vulgaris, tuberculous gumma, acute miliary TB, and orificial TB.[9-13]

Diagnosis by Tests: Staining and Culture

The mycobacterial wall is rich in complex lipids that enable the microorganism to be stained in the laboratory. The lipid wall of bacteria does not allow an acid-alcohol mixture to freely enter the bacterium. Hence the term acid-fast bacteria (AFB), although this does not necessarily mean that the mycobacteria are not vulnerable to these substances. Microscopic observation of AFB in staining of tissue or secretions is the first demonstration of the presence of mycobacteria, although this does not necessarily indicate the presence of *M tuberculosis*. It does, however, enable us to begin empiric therapy if there is sufficient clinical suspicion. AFB can also be observed with *Nocardia*, *Corynebacterium*, nontuberculous mycobacteria, and artifacts. Staining techniques include Ziehl-Neelsen, Kinyoun, and fluorochrome-based techniques with auramine-rhodamine. Ziehl-Neelsen staining is the most common. At least 10^4 bacteria per milliliter are needed to provide a positive diagnosis; therefore, their usefulness is somewhat limited in samples with a low bacterial load, a common occurrence in extrapulmonary forms such as

cutaneous TB. Sensitivity in pulmonary samples ranges from 40% to 80%. Therefore, we can conclude that an AFB-negative result in staining cannot rule out a diagnosis of TB.[16]

Culture is considered the gold standard for the diagnosis of active TB. Its sensitivity and specificity in pulmonary samples are 80% and 99%, respectively, although these percentages are significantly lower in skin samples. Culture of skin samples is necessary for diagnosis, especially in patients with AIDS or those taking immunosuppressive agents, since cutaneous manifestations and histopathologic lesions are usually atypical in these cases. Culture is only positive in 6% of cases of lupus vulgaris.[1] The most commonly used culture media for isolation of *M tuberculosis* are egg-based media (Löwenstein-Jensen [LJ]) and semisynthetic media with agar (Middlebrook 7H10 and 7H11).[6] Traditional solid culture media such as LJ are limited in that they take between 4 and 8 weeks to provide a result.[16] Liquid media speed up growth, and ingenious mechanisms have been designed to detect growth after periods as short as 3 to 7 days. The most widely used rapid-culture techniques are radiometric BACTEC (BACTEC 460) and nonradiometric BACTEC (BACTEC MGIT 960).[17] These techniques are based on the release of a marker (radioactive or fluorochrome) from a metabolite in the culture medium that is used by the mycobacteria. The release of the marker can be detected by a special device even before the mycobacterial colonies are visible. In addition to identification of mycobacteria, drug susceptibility tests can also be performed. A meta-analysis of 10 studies of the BACTEC MGIT 960 and BACTEC 460 systems revealed a sensitivity and specificity for detection of mycobacteria of 81.5% and 99.6% and 85.8% and 99.9%, respectively.[17] BACTEC 460 has the disadvantage that it requires radiolabeling, which renders it difficult and complicated to use. The combination of BACTEC MGIT 960 and BACTEC 460 with conventional solid LJ medium increases sensitivity to 87.7% and 89.7%, respectively.[16,17] Sensitivity of culture is generally greater than that of sputum smear, since very few bacteria are required for a positive result. Biopsy specimens can be cultured if they are preserved carefully in saline solution (not formol). Ideally, these should be taken before antituberculosis treatment has started.

Umapathy et al[18] performed a study in which cutaneous TB was confirmed using a bacteriologic approach (in LJ + pyruvate + 7H11 agar) or histologic approach in 88% of 193 samples. This is considered a high rate of positivity.

Nonradiometric biphasic culture medium (MB-Septi-Check) uses 20-mL bottles containing 7H12 broth. A device containing different solid media can be attached to the upper end of the bottle. Its advantages over BACTEC include greater sensitivity and growth in the solid phase to enable identification tests to be performed without the need for reseeding. Its disadvantages are that detection of growth is slower and does not enable in vitro sensitivity studies to be carried out.[6,16,17]

The increase in the number of infections by *Mycobacterium avium-intracellulare* and *M tuberculosis* in patients with AIDS has stimulated the development of techniques that enable these microorganisms to be detected in blood (blood culture), the most effective being lysis centrifugation and radiometric techniques.[6] The main advantage of lysis centrifugation is that it makes it possible to measure the number of bacteria per milliliter of blood and serially control the efficacy of treatment. Blood culture is indicated mainly in patients with AIDS and a CD4 lymphocyte count <50/mL and fever of unknown origin.

Amplification of Nucleic Acids (PCR)

Amplification of nucleic acids using PCR has revolutionized microbiology by facilitating direct detection and identification of infectious agents in clinical samples in a short time. PCR amplifies specific DNA sequences of the microorganism in vitro, and is a promising tool in the diagnosis of pulmonary TB and several forms of cutaneous TB, especially in those with a low bacterial load, such as lupus vulgaris and TB verrucosa cutis.[19,20] The technique can reveal the presence of mycobacterial DNA fragments in biological samples with a negative result by Ziehl-Neelsen staining or culture; this technique is very useful in bacteriologically negative disease and in patients with atypical symptoms associated with immunosuppression or with HIV infection.[20,21] PCR gives satisfactory results from as few as 100 bacteria per sample in a matter of hours.[16] However, this technique is not available in many countries, particularly developing countries.

Previous studies have shown PCR to be more useful in immunosuppressed individuals with positive bacterial counts and disseminated nodular or ulcerated lesions; therefore, PCR is not used in routine practice.[22] PCR provides a rapid diagnosis (the whole process takes around 5 days), much less than the conventional methods described above.[8,23] The sensitivity of PCR has proven to be better than direct microscopy and comparable with culture, especially in the case of lupus vulgaris.[7,8,20] It is also a useful tool for differentiating *M tuberculosis* from other species of mycobacteria.[24] The sensitivity of PCR is limited when the technique is used for negative AFB specimens or samples with a low bacterial load; sensitivity ranges from 50% to 72% in this type of sample.[7,21-23]

PCR can be performed on fresh biopsy samples or on paraffin-embedded tissue. With the latter, the possibility of false-negatives increases due to degradation of DNA during the embedding process. It has been suggested

that, if the tissue used for PCR is more than 5 years old, degradation leads to a significant reduction in amplification of DNA.[17,21,23,24]

Hsiao et al[14] observed that in forms of cutaneous TB with a low bacterial load and atypical symptoms and histopathology, PCR provides rapid and sensitive detection of *M tuberculosis* DNA in formalin-fixed samples and paraffin-embedded samples. In 2002, Senturk et al[15] reported the efficacy of PCR for *M tuberculosis* in paraffin-embedded tissue, concluding that embedding in this medium reduced sensitivity.

In another study, Salian et al[24] performed a trial based on paraffin-embedded tissue (pulmonary and extrapulmonary) to detect *M tuberculosis* using PCR. The test was able to detect as few as 9 microorganisms in 5-mm formalin-fixed paraffin-embedded sections. Of the 135 samples studied, *M tuberculosis* was detected in 11 that had been negative by AFB or culture. PCR gave a false-negative result in a sample that proved to be positive by culture, and 7 false-positive results that were positive by AFB were reported, although they had not been cultured or were culture-negative. These data led the authors to conclude that PCR is a useful tool for detecting the presence of *M tuberculosis* in formalin-fixed paraffin-embedded tissue.

A considerable number of regions/sequences of the mycobacterial genome (IS6110, IS986, 65 kDa, and 38 kDa) have been identified as target antigens of PCR. Most studies have used the IS6110 sequence; however, up to 40% of samples from southern India have been reported to lack 1 or more copies of this antigen. The 38-kDa protein antigen b is a phosphatase transport lipoprotein that is serodominant in humans and has been used in those pulmonary and extrapulmonary samples in which other regions or sequences have not been of help in confirming the diagnosis.[25-27]

The United States Food and Drug Administration (FDA) authorized the clinical use and commercialization of 3 different PCR tests: the Mycobacterium tuberculosis direct test (Gen-Probe MTD), the Enhanced MTD, and the Amplicor Mycobacterium tuberculosis test. They were all approved for the study of pulmonary TB. According to data from the FDA, the sensitivity of these tests for the diagnosis of pulmonary TB compared with culture is 95% in patients with an AFB-positive smear result, but only 50% in patients with an AFB-negative smear result. Specificity is greater than 95% in both, whether the result is AFB-positive or AFB-negative.[6,16] However, these tests were found to be less successful than expected and so will not replace sputum smear or culture. It is still unknown whether these tests can be applied to cutaneous specimens or whether or not they will prove useful.

Tuberculin Skin Test

This test uses the body's hypersensitivity to bacterial protein to reveal primary infection. It is used mainly to support a suspected diagnosis of TB or to detect latent tuberculous infection. It gives a positive result after infection by *M tuberculosis*, although this may also be the case after vaccination with bacille Calmette-Guérin (BCG) or infection by opportunistic environmental mycobacteria.[6,28] The tuberculin skin test is performed by injecting 5 U (0.1 mL) of purified protein derivative intradermally on the anterior aspect of the forearm. The result is considered positive when a visible and palpable induration greater than 10 mm in diameter appears at the inoculation site. This may be accompanied by edema, erythema, vesiculation, and, occasionally, regional lymphadenitis.[28] The transverse diameter of the induration in millimeters is read at 72 hours. In HIV-infected patients, a reading ≥ 5 mm is considered positive; thus, in individuals with no risk factors who have been vaccinated against BCG, an induration ≥ 15 mm must be considered positive. The reaction to tuberculin has been calculated to be negative in a high percentage of HIV-infected patients (30%-50%).[6]

A positive result in the tuberculin skin test or purified protein derivative test does not indicate an active infection, since the result will also be positive in cases of latent or previous infection.[6,28] The tuberculin skin test is indicated in cases where it could be of interest to confirm or rule out TB infection and there are no contraindications. However, interpretation of the results depends on factors such as the degree of immunosuppression and the technique used. False-negative results can be observed in severe systemic infections, including TB itself, and in immunosuppression.

Chromatography

The main use of this test is to identify species of mycobacteria once a positive culture is available. The mycobacterial cell wall is very rich in lipids, and mycolic acids are characteristic of the genus. These acids are relatively easily separated in methyl esters by silica gel thin-layer chromatography. Definitive identification is by gas chromatography. The usefulness of this technique in cutaneous TB is very limited.[6]

Serology Testing and Other Immunologic Techniques

Serology testing promised to be a very useful diagnostic technique when it was first used more than 100 years ago.

However, antigens were obtained using physical-chemical techniques and the results were discouraging. The technique developed over time and, in 1976,[29] the first enzyme-linked immunosorbent assay (ELISA) appeared. This assay uses highly purified antigens.[16,25,26]

Today, better results have been achieved with the analysis of a series of antigens, including the proteins Ag-60, 30 kDa, 38 kDa, 45/47-kDa complex, Kp90, the antigen DPEP or MPT32, the antigen Mtb81, and the novel ESAT-6.[29-34] The results of serology testing can vary according to the nature and purity of the antigen, the technique used (direct or indirect ELISA), the detection system, the TB stage, and associated conditions (eg, HIV infection). They can also vary depending on whether the patient harbors the bacteria or not, and whether the infection is extrapulmonary.[25,26] If individual antigens are used, sensitivity varies from 60% to 80% with a specificity of 84% to 100%. Combining antigens in the same test can increase sensitivity, although it decreases specificity.[35-37] Such combinations include *TbF6* (102 kDa), a fusion polyprotein composed of 4 antigens (38 kDa, Mtb8, Mtb11, and Mtb48), and *TbF10* (55 kDa), which is a fusion of 3 antigens (38 kDa with Mtb8 and Mtb11). Although user-friendly commercial kits are available, antibody detection has not formed part of routine diagnostic testing.

Recently, 2 new technological developments—QuantiFERON[28,37] and the enzyme-linked immunosorbent spot (EliSpot)[38,39]—have come onto the market. These were initially intended for the diagnosis of latent TB, although they are being promoted for the analysis of active cases. Both are based on the production of interferon (IFN) γ on stimulation of T cells with the specific *M tuberculosis* antigens ESAT-6 and CFP-10.[40-43] Response only occurs when the T cells have an immunologic memory generated by previous infection with *M tuberculosis*. QuantiFERON is currently approved by the FDA for the diagnosis of latent TB. Its sensitivity and specificity are 89% and 99%, respectively. Furthermore, EliSpot has a sensitivity of 98.8% and a specificity of 100%.[6,44] The advantage of these tests is that they require only 1 blood sample, and the result is available in 1 or 2 days. User-friendly kits of QuantiFERON are already commercially available. The response—production of IFN-γ—is read using ELISA. The procedures used in EliSpot are more laborious, although they are based on the same principle. Cells must be extracted for culture with antigens. The high cost is the main disadvantage of these 2 tests. Their main advantage is their excellent ability to detect disease in patients who have been vaccinated against BCG, a common problem. Whereas the qualitative result does not discriminate between latent or active infection, the quantitative result enables disease (active infection) to be detected.

Adenosine deaminase is a purine catabolic enzyme. It is widely distributed throughout the body, although its greatest activity is found in lymph tissue, mainly in T cells. It has proven most useful when determined in the effusion fluid of patients with pleural TB. Its sensitivity is high, although its specificity is limited. Its levels also increase in empyema, lymphoma, autoimmune diseases, and cancer.[45] This enzyme has also been used in peritonitis and pericarditis fluid and in cerebrospinal fluid extracted for diagnosis of TB. It plays no role in the diagnosis of cutaneous TB.

In order to improve specificity in the serologic diagnosis of TB, it is necessary to use *M tuberculosis*–specific antigens and to avoid possible bacterial contamination during the purification process. Some authors have suggested the combination of purified *M tuberculosis*–specific antigens to increase the sensitivity of serology-based diagnosis of TB.[44] To conclude, serology-based diagnosis of TB cannot be recommended for general use. Its applicability in cases of extrapulmonary TB remains unknown.

Conclusions

TB in all its forms continues to be a universal health problem and a challenge for the development of diagnostic tools that provide a specific, sensitive, rapid, and accessible result for the patient.

The new diagnostic methods used today focus on pulmonary TB, although it is unknown whether they can be applied in extrapulmonary forms, especially cutaneous TB. To determine this, tests must be applied in cases of cutaneous TB, whose low incidence renders such an approach difficult.

To date, histopathology testing and isolation of *M tuberculosis* in culture of skin samples or by PCR have been considered the best diagnostic tools for the detection and diagnosis of cutaneous TB.

The definitive criterion for cutaneous TB is isolation of the bacterium in culture or identification of mycobacterial DNA by PCR. Unfortunately, few institutions or laboratories can afford this procedure, particularly in developing countries.

Conflicts of Interest
The authors declare no conflicts of interest.

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