Investigation of latent tuberculosis infection in patients with psoriasis who are candidate for receiving immunobiological drugs

Investigação de infecção tuberculosa latente em pacientes com psoríase candidatos ao uso de drogas imunobiológicas

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Abstract: The use of tumor necrosis factor inhibitors for the treatment of patients with psoriasis has been related to a higher incidence of tuberculosis, specially the disseminated and extrapulmonary forms. Despite their efficacy, these drugs increase the risk of reactivating latent tuberculosis infection, thus requiring diagnosis of the condition before their administration. Investigation of latent tuberculosis infection with tuberculin skin test is ineffective due to its low specificity and the dubious results that it generates in patients with psoriasis. Assays based on the detection of synthesis of gamma interferon in vitro by peripheral monoclonal cells, stimulated by specific antigens (ESAT-6 and CFP-10), seem to offer better accuracy when compared to the Mantoux test in identifying latent tuberculosis infection. This diagnosis tool has demonstrated higher specificity, since it has no correlation with indirect forms of exposure to M. tuberculosis such as BCG vaccination or with infections by other mycobacteria.

Keywords: Psoriasis; Tuberculosis; Tumor necrosis factor-alpha

Resumo: O uso dos inibidores do fator de necrose tumoral no tratamento de pacientes com psoríase vem sendo relacionado a uma maior incidência de tuberculose, particularmente, nas suas formas extrapulmonar e disseminada. Apesar de sua indiscutível eficácia, essas drogas elevam o risco da reativação de infecção tuberculosa latente (ITBL), tornando obrigatório o diagnóstico da referida condição antes da sua administração. A investigação da infecção tuberculosa latente pelo teste cutâneo da tuberculina é falha, dada sua baixa especificidade, além de apresentar resultados duvidosos em pacientes com psoríase. Ensaios baseados na detecção da produção de interferon-gama in vitro por células monoclonais periféricas, estimuladas por antígenos específicos (Esat-6 e CFP-10), parecem oferecer maior acurácia quando comparados ao teste de Mantoux na identificação de infecção tuberculosa latente. Essa ferramenta diagnóstica tem oferecido maior especificidade, já que não apresenta correlação com medidas indiretas de exposição ao M. tuberculosis, como a vacinação por BCG, e com infecções por outras mycobactérias.

Palavras-chave: Fator de necrose tumoral alfa; Tuberculose; Psoríase

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INTRODUCTION

We will discuss some important points in this article before we report the factors that make the investigation of latent tuberculosis mandatory prior to exposure to biological medicines.

Psoriasis and comorbidities

Impaired quality of life consequent to psoriasis severity associated with identification of comorbidities related to the chronicity of the disease has led to a review of the proposed therapeutic regimens for these patients and encouraged the use of treatments based on the pathogenesis of the inflammation. Severe psoriasis is associated with depression, alcohol abuse and heavy smoking, which contributes to progression of the disease and subsequent association with other ones. The incidence of depression in psoriasis patients is around 24%, with improvement of the lesions being usually accompanied by relief of this condition. Based on the similarity of the immunological factors responsible for the formation of atheromatous plaques and those involved in the installation and progression of chronic inflammatory diseases such as psoriasis, it was possible to establish a relationship with the incidence of cardiovascular diseases. Corroborating these findings, patients with severe psoriasis present a high incidence of psoriatic arthritis, cardiovascular disease, hypertension, obesity, diabetes and increased risk of acute myocardial infarction. Gisondi et al. (2006) studied a group of patients with psoriasis and found greater prevalence of metabolic syndrome in psoriasis patients when compared to a control group. Cohen et al. (2007) studied 340 patients with psoriasis and 6,643 controls and identified an association of the disease with acute myocardial infarction, diabetes, hypertension, obesity and dyslipidemia, specially in men between 35 and 50 years old, suggesting the presence of metabolic syndrome in these patients. An evaluation of 16,851 patients with psoriasis found increased levels of total cholesterol and triglycerides associated with decreased serum levels of HDL when compared with controls.

Supporting these findings, more than 20 gene loci interfering with susceptibility to psoriasis and related to metabolic syndrome, type II diabetes, familial hyperlipidemia and cardiovascular disease were detected.

To associate these findings, Gottlieb, Chao and Dann (2008) proposed a model of interrelations between psoriasis and other diseases of a chronic inflammatory nature (Figure 1).

Studies on the pathogenesis of psoriasis, similarly to what had been identified in rheumatoid arthritis and Crohn’s disease, given the immune-mediated inflammatory characteristic of these diseases, showed that it is characterized by high levels of TNF and interleukin 1 in the lesion site. TNF can stimulate the proliferation of T cells, but can also promote their apoptosis and consequently stop immune response. TNF can also increase chemotaxis of T cells to the lesion site by regulating adhesion molecules in endothelial cells.

In autoimmune processes, TNF can sequestrate auto-reactive T cell precursors in the thymus or even make circulating T cells anergic. This finding suggests that TNF may exert both an immunosuppressive and immunostimulatory role, depending on the genetic makeup of the individual, disease duration or level of circulating TNF.

In psoriasis, TNF is synthesized in macropha-
ges, keratinocytes and intraepidermal Langerhans cells and is distributed throughout the epidermis, specially along the blood vessels of the upper dermis. TNF receptors have diversified distribution. In lesioned skin, TNFR1 predominates in keratinocytes, intraepidermal Langerhans cells and blood vessel walls, whereas TNFR2 is predominant in dermal blood vessels and perivascular infiltrating cells.  

From this observation, it was hypothesized that drugs that can block the action of TNF and its receptors, by reducing the inflammatory process, could be useful in treating diseases such as psoriasis, rheumatoid arthritis and Crohn’s disease.  

**Biological therapies**

Biological therapies are therapeutic approaches that use complex proteins which can interrupt signaling for triggering and maintaining the inflammatory response in psoriasis. These proteins are synthesized by recombinant DNA technology in various levels of purification. The decision to use these therapies has been driven by their efficacy, short response time, severity of the disease, associated comorbidities, intolerance or poor response to other treatments.

The Food and Drugs Administration has approved TNF-α inhibitors (adalimumab, etanercept and infliximab) and modulators of T lymphocyte activation (alefacept and efalizumab) for treatment of patients with psoriasis. Their mechanisms of action are detailed in figure 2. Although anti-TNF are considered first-line drugs for certain cases - given their cost-benefit, their use has been limited due to the risk of reactivating latent tuberculosis infection (LTBI) in patients previously treated.

The initial warning was given by a study of 70 patients who were treated for rheumatoid arthritis with infliximab and had their tuberculosis reactivated. Out of these patients, 56% had the extrapulmonary form of the disease and 24% had the disseminated form.  

Estimates by the World Health Organization (WHO) show that there were 9.27 million new cases of tuberculosis (139 cases per 100,000 population) in 2007. A group of 22 countries is responsible for 80% of the tuberculosis burden in the world, with Brazil ranking 18th on the list. WHO recommends treatment success of 85% of the tuberculosis cases, and these results are not yet achieved in Brazil.

At the beginning of the century, Europe registered a significant increase in new tuberculosis cases, secondary to the administration of anti-TNF drugs. Among all patients exposed to infliximab between

![Figure 2: Mechanism of action of the biological agents approved by the FDA](Adapted Source: Nestle F et al. 2009.)
January 2000 and June 2003, 56 individuals developed tuberculosis, which corresponds to an incidence of 8-24 cases per 10,000 population versus an incidence in the general population of 1.1/10,000. In 2001, screening prior to administration of anti-TNF drugs was implemented in France, England and the United States in order to stop or slow the increase in new cases of latent tuberculosis associated with this therapy. The purified protein derivative (PPD) test, chest X-ray and detailed anamnesis are the most recommended procedures for screening prior to biological therapy, especially in countries like Brazil, which have a high incidence of tuberculosis. Figure 3 shows a flowchart of the screening scheme.

**Anti-TNF action and tuberculosis triggering**

Immunocompetence is a determining factor in an individual’s ability to control infection with *M. tuberculosis* through activation of antigen-specific T cells, with recruitment of inflammatory cells, cytokine release and activation of macrophages in the site of infection. At the end of this process, the resulting few viable bacilli along with giant cells, necrotic material, macrophages and lymphocytes will constitute the tuberculous granuloma. TNF, an important pleiotropic cytokine, acts in the maintenance of the granuloma formed, keeping the infection in its latent state and promoting apoptosis of the infected cells. In the pulmonary alveolus, macrophages - after phagocytosing the bacilli - migrate together with granulocytes and monocytes to the interstitium under the action of TNF and other pro-inflammatory cytokines. The antigen-presenting cells, also recruited by TNF, reach the lymph nodes and increase the response of specific T cells. These effector lymphocytes produce interferon gamma (INF-γ) and TNF, triggering the enzymatic synthesis of macrophages with full antimicrobial activity and induction of adhesion molecules, which are essential for the T cells and macrophages to bind to the granuloma. Although it is still not well established, it is believed that the TNF is responsible for maintaining the macrophage viable and, consequently, the granuloma intact (Figure 4).

Studies involving mice unable to produce TNF show a significant reduction in the granulomatous response to *M. tuberculosis*, leading the animals to die from tuberculosis. TNF participation in the pathogenesis of psoriasis and stabilization of the granuloma in tuberculosis supports the hypothesis that anti-TNF therapy promotes reactivation of LTBI.

Between 1998 and 2002, the Adverse Event Reporting System of the FDA found 716 granulomatous infections associated with anti-TNF therapies, including tuberculosis, histoplasmosis, aspergillosis, listeriosis and cryptococcosis. Patients who developed tuberculosis during the anti-TNF therapy presented inability to form a granuloma, prominent interstitial fibrosis and lymph node inflammation. In the same time period, Wallis et al. identified 323 cases of granulomatous infection: 138 tuberculosis, 40 histoplasmosis, 18 listeriosis and other 127 infections in patients who had undergone this treatment.

Despite the association of tuberculosis with the use of anti-TNF, its incidence has shown a different
behavior between the three drugs. Patients treated with etanercept seem to present a lower incidence of tuberculosis compared to those treated with infliximab and adalimumab, probably due to significant differences in the kinetics and mechanism of action of the latter two drugs, soluble receptors and monoclonal antibody, which are described below: 11

- The avidity with which monoclonal antibodies bind to TNF is higher compared to the soluble receptor;
- TNF suppression is greater and longer during treatment with infliximab and adalimumab, which may produce functional ablation of the activity of macrophages, resulting in increased susceptibility to infections, which, on the other hand, may improve the efficacy of the therapeutic response;
- Infliximab and adalimumab induce cell lysis and apoptosis of monocytes and activated T cells, which are key components in the formation of the granuloma;
- Infliximab and adalimumab, but not etanercept, inhibit INF-γ production, which is crucial to the host’s defense against bacteria.

Tuberculosis triggering due to the introduction of anti-TNF drugs has been commonly observed in the extrapulmonary or disseminated forms, as well as pulmonary involvement, which occurs less frequently, further hindering early diagnosis through conventional methods.

Relevance of accurate diagnosis of LTBI in patients with psoriasis

Tuberculosis is considered a serious public health problem in Brazil due to its high rates of incidence, prevalence and mortality. Bierrenbach et al. 11 studied the incidence of tuberculosis in 2004, based on data from the National Information System for Notifiable Diseases (SINAN), and noted an incidence of 41 per 100,000 population, with notification of 74,540 new cases.

Early and accurate diagnosis is essential to control the disease. The combination of clinical examination, sputum smear microscopy and culture is recognized as the gold standard for diagnosis of LTBI. Since it may take *M. tuberculosis* about eight weeks to grow in culture or it may not develop properly, which happens in 10% to 20% of the cases, diagnosis is usually based on clinical and radiographic findings, causing latent tuberculosis to be underdiagnosed.

An individual is considered to have LTBI when presenting a positive PPD test, negative bacteriological examination and no clinical or radiographic evidence of active tuberculosis 12 and the following risk situations: 11,13 a) equivocal history of tuberculosis treated in the past, b) HIV infection, c) immunosuppression, d) household contact with tuberculosis, or e) risk factors for reactivation of infection in case of a positive PPD test or radiographic changes consistent with sequelae of tuberculosis (calcified nodular lesions, apical fibrosis or pleural scarring).

It is estimated that one third of the world’s population have LTBI. This population is at risk of developing the active disease, thus triggering an epidemic worldwide. The measurement of reaction to PPD is used to classify individuals according to their
probability of being infected with *M. Tuberculosis*. Criteria for interpreting the results, using cut-off point, have been established with the aim of categorizing the measurements according to this probability.

The result, registered in millimeters, is interpreted as follows: 1) 0-4 mm - no reaction: individual not infected with *M. tuberculosis* or with reduced hypersensitivity; b) 5 to 9 mm - weak reaction: individual vaccinated with BCG or infected with *M. tuberculosis* or other mycobacterial; c) 10 mm or more - strong reaction: individual infected with *M. tuberculosis*, sick or not, and individuals vaccinated with BCG in the last two years.

Tuberculin skin test may give false negative results in the following situations: immunosuppressive diseases (sarcoidosis, acquired immunodeficiency syndrome, cancer and lymphoproliferative diseases), systemic corticosteroid therapy or use of immunosuppressive drugs, pregnancy, children younger than two years old and individuals older than 65. Thus, an induration of 5 mm or more is indicative of infection with *M. tuberculosis* in patients with acquired immunodeficiency syndrome and in those treated with immunosuppressive drugs (such as anti-TNF). In addition, individuals immunized with BCG within less than two years must have their results carefully interpreted, since an average measurement of 10 mm or more is expected. 15

**Psoriasis patient’s behavior in relation to the tuberculin skin test**

To obtain a reliable result with the tuberculin skin test, it is necessary that the individual assessed be immunocompetent, since the result must come from a delayed hypersensitivity reaction mediated by T cells. Diseases such as rheumatoid arthritis and Crohn’s disease have immunological characteristics similar to psoriasis. They have a defect in the differentiation of T cells, impairing their memory mechanism and consequently their ability to respond when recruited. 16

The understanding of psoriasis as a disease of chronic activation of the immune system, mediated primarily by T lymphocytes, suggests a significant deficit in the excellence of this response. The decrease in proliferation of T lymphocytes in the presence of preparations of bacterial antigens has already been demonstrated in psoriasis. 17

Patients with severe psoriasis, when compared with healthy individuals of the same population, present a low incidence of positive tuberculin skin test (29%) versus 51% positive tuberculin skin test in the comparison group. When the memory function of T cells was tested, by means of a study in vitro via measuring the production of INF-γ, a decrease in this response was also observed, suggesting a characteristic similar to that presented by patients with rheumatoid arthritis. 18

The anti- *M. tuberculosis* reaction in these individuals is probably affected, especially if the housing area is endemic for infection with this bacillus. Therefore, one can expect that there is a higher incidence of false negative results for the PPD test in these patients compared with the population not affected by the disease, further distancing this test from being recognized as the gold standard in diagnosing latent tuberculosis. 19

Reactivity of the skin of psoriasis patients is altered, and local reactions may be overestimated (Koebner phenomenon), leading a skin test to show results that are not accurate, which characterizes the low reliability of the PPD test in these individuals. Added to this fact, the extensive involvement of the skin in more severe cases makes it very difficult or even impossible to perform the PPD test, since a sound area in the forearm is needed for inoculation of the reagent.

A depression in reactivity to PPD has also been reported. A number of patients with guttate psoriasis were compared to controls, and it was found that the former present compromised innate immunity; therefore, even in an area of healthy skin, antigen inoculation (bacterial preparation) can result in an inappropriate immune response. 20, 21

Lindholm et al (1978) compared patients treated at the dermatology clinic and patients from the general medical practice clinic and reported higher probability of positive results (weak or strong reaction) to PPD in the first group. Among these patients, those with a diagnosis of psoriasis had more pronounced responses, indicating the occurrence of false-positive results.

Even in the non-lesioned skin of patients with psoriasis, there is an increase in the number of dendritic cells and activated inflammatory cells, as well as higher concentration of TNF, which can compromise the reliability of a test whose result depends on immune-mediated inflammatory response. 23, 25 Plasma cells - dendritic cell precursors - are more abundant in the non-lesioned skin of patients with psoriasis, and they are probably the main sources of IFN-α, the key cytokine in the inflammatory response of psoriasis after PPD inoculation.

Dermal dendritic cells stimulate the synthesis of cytokines (IFN-γ, IL-2) in Th1 cells and may cause spontaneous proliferation of T lymphocytes, thus perpetuating the TNF stimulation response. 21

These pieces of evidence suggest that the tuberculin skin test is not a gold standard for diagnosing latent tuberculosis, especially in patients with mode-
rate and severe forms of the disease. This raises the need for researching new methods for investigation of LTBI.

**New tools for the diagnosis of LTBI**

The development of new tests for the diagnosis of LTBI was only possible after the regions of the genome of *M. tuberculosis* were identified, resulting in the creation of more specific tests, without the inconvenience of cross-reactivity with BCG vaccination and other environmental mycobacteria.25

The genes encoded by the region of difference 1 (RD1), present in *Mycobacterium bovis* (*M. bovis*), are primarily eliminated in the production of BCG. This region includes the genes Rv3871a to Rv3879c, which encode specific proteins: the early-secreted antigenic target (ESAT-6, with 6 kDa) and the culture filtrate protein (CFP-10, with 10 kDa). These proteins are specific antigens produced by this bacillus, since it cannot be produced by the BCG vaccine or other environmental mycobacteria.

Based upon this evidence, two assays using the ESAT-6 and CFP-10 as stimulating antigens were developed: QuantiRON-TB-Gold® (QFT) and T-SPOT.TB®:

- QuantiRON-TB-Gold® (QFT) is an assay that, in whole blood, quantifies the presence of INF-γ through the ELISA (enzyme-linked immunosorbent assay) method.26
- T-SPOT.TB® (Oxford Immunotec, Marlborough, USA) uses an ELISPOT (enzyme-linked immunospot assay) assay to identify the presence of peripheral blood mononuclear cells producing INF-γ in response to the stimulus produced by the ESAT-6 and CFP-10. The test, which has already been approved in Europe, is recommended by the National Institute for Health and Clinical Excellence as an ideal screening test for detecting LTBI in England and Wales.27

In addition to the two commercial kits described, other assays have been developed in research labs, but they are not yet commercially available.28

The specificity of these tests is similar (98% to 99%), whereas sensitivity is higher for the T-SPOT.TB when compared to the QFT (90%). This difference is primarily pronounced in immunosuppressed patients and children, whose frequency of indeterminate results has been high.25

Although the tests performed in vitro to detect LTBI are based on measuring the production of INF-γ, their operational characteristics are different, as follows:

- The incubation period can vary from a few hours (T-SPOT.TB and QFT) to a few days (commercially unavailable tests);
- The substrate to be investigated can be whole blood (QFT) or peripheral blood mononuclear cells (T-SPOT.TB);
- The antigen used can be the PPD (early versions of the QFT) or the RD-1 antigens (T-SPOT.TB and QFT);
- The technique used can be ELISA or ELISPOT.

Despite the good correlation between the two tests, the results in clinical practice may be discordant. A study aiming to compare the performance of these tests showed that the number of indeterminate results was higher for the QFT when compared to the T-SPOT.TB test, and also that the indeterminacies in both tests were associated with immunosuppression. Therefore, we can conclude that the choice of the diagnostic tool to be used must be based on the population studied, on the objective and resources available.29

Evidence suggests that assays based on detection of INF-γ have a better performance than the tuberculin skin test, since they have higher specificity, better correlation with indirect measures of exposure to *M. Tuberculosis* and lower cross-reactivity with BCG vaccination and environmental mycobacteria infection.30,31,32

The operational fundamentals of the T-SPOT.TB test in the diagnosis of LTBI are not yet completely standardized, despite the many studies conducted with this objective.33 In an attempt to solve this problem, two approaches are proposed:

- The results of the T-SPOT.TB are compared with the results of the PPD, with subsequent calculation of the concordance level. This option is useful, since it compares the performance of a new test with an already established one; 26
- The results of the T-SPOT.TB test are compared to a validity instrument that comprises both clinical and epidemiological characteristics related to LTBI.34

The T-SPOT.TB test was initially validated and compared with the PPD test in patients with positive culture for tuberculosis infection and controls without the disease. The achieved sensitivity of 96% was significantly higher than that of the PPD test (69%). The T-SPOT.TB test, unlike the PPD test, is not likely to give false-negative results in the presence of active tuberculosis infection and presents high sensitivity in HIV-positive patients infected with *M. tuberculosi*.35

A study compared the sensitivity and specificity of the T-SPOT.TB with the PPD test in the diagnosis of LTBI in healthy individuals and immunosuppressed patients. In this meta-analysis, the T-SPOT.TB test had 87% sensitivity (95%CI 78% - 95%) and 92% specificity (95%CI 88% - 95%): percentages higher than the
parameters obtained for the PPD test (71% sensitivity, 95%CI 65% -0.74% and 66% specificity, 95%CI 0.46% - 0.86%).

When the five studies that evaluated the performance of the tests in immunosuppressed patients were examined separately, two showed a slight response reduction of more specific tests in immunosuppressed patients compared to immunocompetent patients, and three studies that evaluated the results of the T-SPOT.TB test compared with the PPD test identified a significantly higher prevalence of positive results in the first test.

The cost/benefit of using the T-SPOT.TB test has frequently been debated. Diel et al. conducted a study in Germany to evaluate the cost/benefit of screening LTBI and compared results of the PPD and T-SPOT.TB tests in household contacts of patients with tuberculosis. The authors demonstrated that the PPD had a good cost/benefit for contacts that had been established up to 20 years earlier, followed by the T-SPOT test when PPD was positive. However, when the average was calculated, the strategy that showed the lowest cost was the T-SPOT only. For this calculation, the unnecessary use of isoniazid (in cases of false-positive PPD, which could have been confirmed by the T-SPOT.TB) and progression to tuberculosis in groups where LTBI had not been diagnosed by the PPD were considered. When performed in countries with high incidence of tuberculosis, the cost/benefit relation was only interesting to confirm a PPD positive result in patients at high risk of tuberculosis infection.

CONCLUSION

The severity and presentation characteristics of psoriasis have a strong impact on the quality of life of affected individuals and their families, a fact which is further complicated by the chronic nature of the disease and its aggravation, which is associated with a high incidence of comorbidities. The use of anti-TNF drugs to control severe cases or cases that are unresponsive to other treatments is recognized in the literature worldwide and recommended by the Brazilian Consensus on Psoriasis (2009). On the other hand, there is the imminent risk of triggering tuberculosis in patients using anti-TNF drugs, a fact that has limited their use by dermatologists, even in those cases in which careful evaluation shows the need of their use.

Legitimate identification of cases of LTBI prior to the administration of anti-TNF therapy is a preliminary recommendation. Still, the resources available for the diagnosis of LTBI are faulty, making it difficult to exclude this condition. In view of what has been discussed, a new diagnostic tool whose value is recognized in the literature is presented with the proposal to offer greater reliability to the investigation of LTBI in patients with psoriasis: the T-SPOT.TB test.
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