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REVIEW ARTICLE

# Risk of tuberculosis infection in anti-TNF-α biological therapy: From bench to bedside

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Anti-tumor necrosis factor-α (TNF-α) biological agents, including soluble TNF-α receptors and anti-TNF-α monoclonal antibodies, bring new hope for treating rheumatic diseases such as rheumatoid arthritis, but also increase the risk of infection, especially tuberculosis (TB) infection. Recent findings have shown that the physiological TNF-mediated signaling was somehow impaired by TNF antagonists, leading to the exacerbation of chronic infection associated with aberrant granuloma formation and maintenance. Although both receptor and antibody agents appear to pose an equally high risk in causing development of new TB infections, monoclonal anti-TNF-α antibody seems more inclined to reactivate latent TB infection. This review is focused on the underlying mechanisms that cause the TB risk in the anti-TNF-α therapy and also the strategies to deal with it, with the aim of reducing the TB incidence during anti-TNF-α biological therapies.


## Introduction

The development of anti-tumor necrosis factor-α (TNF-α) therapy brings new hope for treating rheumatic diseases in which TNF-α plays a crucial role, including rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PsA), and other inflammatory arthritis. Anti-TNF-α therapy not only relieves symptoms, but also reduces bone erosions to a minimal degree, which may lead to a better prognosis. However, TNF-α is also a key cytokine in host defense against intracellular infection, such as mycobacterium tuberculosis (MTb) infection.[1,2] In daily practice it is confirmed that anti-TNF-α therapy is also associated with increased susceptibility to infections, especially tuberculosis (TB).[3] It is also found that anti-TNF-α monoclonal antibodies appear to pose a higher risk in TB infection than

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TNF-α receptors.[4] In this review we focus on the underlying mechanisms that cause the differences in MTb risks among different kinds of anti-TNF-α agents, with the aim of reducing the MTb incidence to a minimal degree during anti-TNF-α biological therapies.

## TB infection

TB is caused by a bacterium called *Mycobacterium tuberculosis* (*M. tuberculosis*). The bacterium usually attacks the lungs, but it can also attack other parts of the body such as the kidney, spine, brain, intestines and so forth. If not treated properly, TB infection can be fatal. Not all patients infected with *M. tuberculosis* become symptomatic. Two TB-related conditions exist: active TB (ATB) disease and latent TB infection (LTBI).

It is noteworthy that LTBI refers to a TB infection status in which the patient is infected with *M. tuberculosis* but remains asymptomatic. The main adverse consequence of LTBI is that approximately 5—10% of these patients will eventually go on to develop ATB at a later stage of their life. The greatest risk of progression to ATB occurs in the first 2 years after infection.[5] However, the risk may be higher if the patient is receiving concurrent immunosuppressive therapy and it is even worse if a patient suffers a disease that disturbs the immune system or is complicated with malnutrition. Moreover, old age is also associated with TB infection, since the immune system weakens with age.[6,7]

## Factors increasing the risk of TB infection

There are many factors that increase the risk of TB infection. Among these factors, the immunosuppressive conditions top the list. The risk of developing TB is about 100-fold increased in the HIV-infected population.[8—10] Solid organ transplant recipients have an elevated risk of TB infection because of immunosuppressive therapy.[11,12] In an investigation in Spain, the incidence of TB in recipients was 26.6 times higher than that in the general population and the highest TB incidence was observed among lung transplant recipients, with a relative risk of 73.3.[12] In patients receiving anti-TNF-α treatment, the relative risk of TB infection varies from 1.5 to 17.[13,14] The risk of TB infection is higher with anti-TNF-α monoclonal antibody therapy than with soluble TNF-α receptor therapy.[4,15] In a study in the US, the risk of developing TB attributed to infliximab was more than twofold that of etanercept.[16] Tissue malignancy also has an influence on the risk for TB infection. In patients with hematological malignancies including leukemia and lymphoma, the relative risk of developing TB reaches 16.[17] Additionally, other conditions, such as diabetes mellitus, smokers, alcoholics, also have two- to threefold increased risks of developing TB.[18—20]

## Risks of TB infection by different TNF-α antagonists

With increasing biologic agents becoming available for clinical use, there are more and more concerns about the increased rates of infections secondary to the disturbance of physiological cytokine-mediated signalings by these agents.[21] It had been reported that an increased risk of TB infection was associated with biological therapies, including TNF-α antagonists (monoclonal anti-TNF-α antibody: adalimumab, infliximab, certolizumab pegol and soluble TNF-α receptor: etanercept),[3,22] anakinra (IL-1 receptor antagonist),[23] abatacept (CTLA-4 Ig),[24] rituximab (monoclonal anti-CD20 antibody),[25] efalizumab (monoclonal anti-CD11a antibody),[26] daclizumab (monoclonal antibody to the interleukin 2 receptor-CD25)[27]and so on, among which TNF-α antagonists seem extremely prominent for increasing the risks of TB infection.

In a retrospective cohort of 112,300 Canadian RA patients from 1998 to 2003, the rate of TB was investigated to determine whether different disease-modifying antirheumatic drugs (DMARDs) were associated with the risk of TB. The researcher found that the median time from the first prescription with infliximab or etanercept to the presentation of TB was 17 and 79 weeks, respectively.[14] Another research reviewed 90 RA patients treated with infliximab and 103 patients treated with etanercept, by analyzing the data of the Korean National Tuberculosis Association (KNTA) from 2001 to 2005; in the infliximab-treated RA group, two cases of TB developed, while there was no case of TB reported in the etanercept-treated RA group.[15]

In 2008, Wallis[28] reported 248 cases of infliximab-associated and 39 cases of etanercept-associated TB infections, which were recorded from January 1998 to March 2003 in the Adverse Events Reporting System (AERS) database of the Food and Drug Administration (FDA). According to the report, among 197,000 infliximab-treated and 113,000 etanercept-treated patients, the TB incidence rate was 54 per 100,000 and 28 per 100,000, respectively. Monte Carlo simulation revealed that the median time of TB onset after starting etanercept therapy is three to five times longer than infliximab (12—21weeks). A median rate of reactivation of LTBI by infliximab treatment was 12.1 times higher than the etanercept treatment ($p < 0.001$). In contrast, both TNF-α antagonists appeared to pose a high risk of progression of new TB infection.

A French prospective research[4] reported that the annual adjusted incidence rate of TB was 9.3 (range 0.0—9.4) per 100,000 for patients receiving etanercept, 187.5 (range 0.1—374.8) per 100,000 for infliximab, and 215.0 (range 0.0—521.7) per 100,000 for adalimumab, compared with 8.7 per 100,000 for the general French population. The standardized incidence ratio (SIR) was 1.8 (95% CI = 0.7—4.3; $p = 0.20$) for etanercept, 18.6 (95% CI = 13.4—25.8; $p < 0.0001$) for infliximab, and 29.3 (95% CI = 20.3—42.4; $p < 0.0001$) for adalimumab. British research[29] published in 2010 showed that the incidence rate of TB was highest for adalimumab (144 events/100,000 person-years), followed by infliximab (136/100,000 person-years) and then etanercept (39/100,000 person-years). Compared with etanercept, the adjusted incidence rate ratios (IRRs) and 95% CI for adalimumab and infliximab were 4.2 (1.4—12.4) and 3.1 (1.0—9.5), respectively. The median time from the initial TNF-α antagonist use to TB diagnosis was 13.4 months for cases exposed to etanercept, 5.5 months for infliximab and 18.5 months for adalimumab.

From the data above, it can be concluded that the incidence of TB induced by various TNF-α antagonists are different; monoclonal anti-TNF-α antibodies such as

infliximab or adalimumab poses a higher risk of TB incidence than soluble TNF-α receptor, etanercept. The median time of TB onset after receiving monoclonal antibody therapy was shorter than that of soluble receptor. Anti-TNF-α antibody seems more inclined to reactivate a latent TB infection, although both TNF-α antagonists appear to pose an equally high risk in causing the development of new TB infection.[30]

## Mechanisms of TB infection in different TNF-α antagonists

The mechanism that underlies increased TB infection in TNF-α antagonist therapies has aroused researchers' interests. TNF-α is a cytokine that plays a central role in establishing and maintaining the inflammatory response against infections.[1,2,31,32] TNF-α has soluble and transmembrane forms, both of which are biologically active and are produced by a wide variety of cells, including macrophages, natural killer cells, granulocytes, fibroblasts, and T cells. Both forms of TNF-α interact with two distinct types of receptors, TNF receptor 1 (TNFR1) and TNFR2. Either or both forms of TNF-α are found in practically all cells of the human body, except red blood cells. TNF-α blockade will result in the interruption of TNFR-mediated functions, which comprises cell activation and proliferation, production of cytokines and chemokines, and the formation and maintenance of granulomas.[33] In the host immune response, the mycobacteria arrives in the alveoli and is engulfed by alveolar macrophages. TNF-α is then released with an autocrine style, an action which further enhances the release of other cytokines and chemokines to attract and activate $CD4^+$, $CD8^+$ and γ/δ lymphocytes. These lymphocytes strengthen T-cell adhesion and antigen presentation, which results in the proliferation and recruitment of more T and B cells. These activated cells also release interferon-γ (IFN-γ), which accelerates antigen presentation and induces intracellular killing of bacilli and macrophage apoptosis. Finally, granuloma formation and infection occurs; however, if the patients have no symptoms, but have mycobacteria within the body, it is termed LTBI. Under such circumstances, MTb-specific central memory T cells ($CCR7^+CD27^+$) and effector memory cells ($CCR7^-CD27^-$) will permanently exist in the body. Thus, anti-TNF-α therapy disturbs the physiological TNF-α mediated immunoinflammatory responses and may cause TB reactivation or dissemination.[33–35]

Plessner et al[36] investigated the effects of anti-TNF-α antibody (MP6-XT22) and soluble TNFR fusion molecule (mTNFR2-Fc) in the murine TB model. Both MP6-XT22 and mTNFR2-Fc resulted in rapid morbidity in acute infection. During chronic infection, mTNFR2-Fc related infection could be controlled, whereas TB infection secondary to MP6-XT22 caused death within 1 month. Histological proof showed that the lung sections of mTNFR2-Fc-treated mice had lower bacterial burden than those of MP6-XT22-treated mice. Also, granuloma formation and maintenance were better in the mTNFR2-Fc group than the MP6-XT22 group, and this effect was more significant with the progression of time and increase in drug dose. During acute infection, flow cytometry revealed increased C3 deposition on $CD4^+$ T cells in the lungs of mice treated with MP6-XT22 compared with those of mTNFR2-Fc-treated mice; there were significantly fewer $CD4^+$ T cells, and not macrophages, in the lungs of MP6-XT22-treated mice compared with those of mTNFR2-Fc-treated mice, during primary infection but not in chronic infection. Drug fluorescent staining analysis showed a higher concentration of MP6-XT22 in the lungs. These findings were a good start to clarify the differences in the mechanism of inducing and exacerbating TB infection by different anti-TNF-α agents. In other research,[37] C57BL/6 mice with chronic TB infection were treated with the TNF-neutralizing antibody MP6-XT22 or normal rat IgG. The results showed that the lymphoid aggregates were well maintained in the infected lungs of rats in the IgG-treated group 9 days after treatment; in contrast, the dissolution of lymphoid nodules in the MP6-XT22-treated group became obvious and continued to be apparent at 21 days. Further immunohistochemical studies showed that the dissociation of B-cell-macrophage units in the infected lungs of the MP6-XT22 treated group was particularly conspicuous, with a marked decrease in the expression of cells with CD19, which is the early differentiation antigen of the B cell. Lin et al[38] undertook an investigation on a cynomolgus macaque model. Macaques were classified as having active or latent disease 6—8 months after TB infection. Then, macaques with acute infection were randomized to receive either adalimumab or no treatment, while macaques with latent infection were randomized to receive treatment with a TNF-neutralizing agent, either p55-TNFRI or adalimumab, or with saline. The results showed that neutralization of TNF in latently infected macaques caused reactivation in a majority of animals, as determined by gross pathologic examination and bacterial burden. A spectrum of dissemination was noted, including extrapulmonary disease. However, macaques that developed primary and reactivated TB after TNF neutralization had a similar granuloma structure and composition to that of control macaques with active disease. TNF neutralization was associated with increased levels of interleukin-12 and chemokine receptor expression, decreased levels of CCL4, and reduced mycobacteria-induced IFN-γ production in blood, but not in the affected mediastinal lymph nodes. Interestingly, the first sign of reactivation often occurred in thoracic lymph nodes. In an *in vitro* study,[39] Saliu et al found that infliximab and adalimumab significantly inhibited T cell activation and reduced the proportion of TB-responsive $CD4^+$ cells by 70% and 50%, respectively, while etanercept produced no significant effect. Infliximab and adalimumab obviously suppressed IFN-γ produced by TB-responsive $CD4^+$ cells. However, IL-10 production was equally suppressed by all three anti-TNF drugs.

Wallis[35] concluded that the differences in risks of TB infection among TNF antagonists are caused by their different structures and functions, including pharmacokinetics and dosing, soluble TNF binding kinetics and stoichiometry, the affinities of TNF antagonists for transmembrane TNF, cytotoxicity, apoptosis and T-cell activation, and cytokine expression.

## Diagnosis of latent TB infection before anti-TNF-α therapy

### Tuberculin skin test

LTBI is diagnosed in patients who are free from symptoms of ATB disease, but have a positive tuberculin skin test (TST).

TST is widely used to screen high-risk subjects all over the world. A number of countries have generated national guidelines that deal with LTBI before anti-TNF drugs therapy.[7,40,41]

In China, where TB prevalence is very high, the criterion of LTBI diagnosis may be less valuable and the guiding principle for LTBI treatment may not be as strict as in the US or European countries. Considering this, the risk of TB infection induced by biological drugs becomes more unoptimistic. Therefore, the Chinese Rheumatology Association suggested a TST induration diameter $\leq$ 10 mm as the threshold of applying biological agents in RA and AS.[42,43] The specific standards are as follows: (1) the induration diameter $\leq$ 10 mm, and no evidence of TB infection, biologics are available; (2) the induration diameter $>$ 10 mm and $\leq$ 15 mm, without evidence of TB infection, the use of biologics depends on the patient disease condition. If necessary, it is suggested to be applied simultaneously with anti-TB treatment; (3) the induration diameter $\geq$ 15 mm or $<$15 mm with blister or necrosis, biologics are not recommended unless the TB is controlled after anti-TB treatment. However, TST is easily influenced by many factors, such as Bacille Calmette Guérin (BCG), which is widely used in young Chinese populations.[42,43] Diagnosis of LTBI is necessary, but still challenging in daily practice (Fig. 1).

## Interferon-gamma release assay (IGRA)

IGRA is established as an alternative to the TST in TB infection diagnosis, especially when it comes to the diagnosis of LTBI, in which IGRA plays an important and unique role. The development of IGRA should be attributed to the progress of molecular biology and immunology.[44] In 1999, Behr et al[45] performed comparative hybridization experiments on a DNA microarray, to better understand the differences among *M. tuberculosis, Mycobacterium bovis,* and the various BCG daughter strains. Eleven regions (encompassing 91 open reading frames) of H37Rv deleted from BCG vaccines and *M. bovis* were confirmed by sequencing. Five additional regions representing 38 open reading frames were present in *M. bovis,* but absent from BCG strains. The differences between MTB and BCG were determined by these 16 regions denoted RD1—RD16. Of particular importance was that the region of deletion-1 (RD-1) was completely deleted from all BCG strains and most environmental mycobacteria. Therefore, detection of RD-1-encoded antigen-specific T-cells response revealed whether the suspected person was infected with MTB or not. Early secreted antigenic target 6-kDa protein (ESAT-6) and culture filtrate protein 10 (CFP-10), which were two of the RD-1-encoded proteins, can theoretically evoke robust $CD4^+$ and $CD8^+$ T-cell responses. When the body was re-stimulated by a mycobacteria-specific antigen, memory cells which had been immunized primarily would be re-activated rapidly and release IFN-γ. By detecting IFN-γ secretion, it was possible to find out if the body had been infected by TB without interference by previous BCG vaccination or atypical mycobacterial infection. It can be inferred from the abovementioned mechanisms that IGRA is particularly important in the diagnosis of LTBI, since it is asymptomatic. Unfortunately, the current diagnostic kits utilizing production of IFN-γ in response to TB antigens, are unable to distinguish between LTBI and ATB disease. Efforts had been made to distinguish between LTBI and ATB disease, by assessing multiple cytokines such as TNF-α, IL-12 and IL-17, which is helpful to confirm the diagnosis of ATB disease in a TB-endemic population.[46]

There are two different sets of IGRA test, including T-SPOT.TB and Quanti FERON-TB Gold (or Quanti FERON-TB Gold in Tube). The T-SPOT.TB test is based on the high sensitive enzyme-linked immunospot assay that enumerates the number of T-cells releasing IFN-γ in the peripheral blood, which responds to the stimulation by MTb-specific antigens. The method of Quanti FERON-TB is based on the whole-blood ELISA test of IFN-γ released from T-cells, in response to the stimulation by MTb-specific antigens. Because of different local commercial permissions, head-to-head comparisons between these two assays for LTBI are rare and most of them were performed in Japan. A study speculated that T-SPOT.TB might reduce the frequency of indeterminate results of Quanti FERON-TB Gold.[47] However, another study noted that LTBI in younger patients may be better diagnosed by Quanti FERON-TB Gold or Quanti FERON-TB Gold in Tube, while in middle-aged and elderly patients, T-SPOT.TB may be more suitable to exclude LTBI, although the validity of two methods in the exclusion of LTBI are similar and not fully enough to diagnose LTBI separately.[48]

## Comparison and interaction between TST and IGRA

It is newly accepted that the specificity of IGRA is greatly improved compared with TST (97% for Quanti FERON-TB Gold and 92% for T-SPOT.TB vs. only 66% for TST).[49,50] It had been widely accepted that T-SPOT.TB is superior on the predictability of TB infection in immunosuppressed patients, and it was not influenced by immunosuppression status, such as HIV infection.[51] The new data showed that T-SPOT.TB may be more sensitive than the TST in patients receiving anti-TNF-α therapy.[52]

Considering that the TST has a low specificity in TB diagnosis and its result is always affected by many other factors, IGRA is regarded as an alternative to TST in the diagnosis of ATB disease and LTBI in the US.[53] However, in Britain, the guideline recommends the combination of both methods.[54] Currently, there is a tendency that IGRA may be used as a substitute for TST in the diagnosis of LTBI before starting TNF-α antagonists, especially in countries where TB prevalence is intermediate and the BCG vaccination is mandatory at birth, such as in Korea.[55] In China, T-SPOT.TB was approved by the State Food and Drug Administration in September 2010. A consensus report of a Chinese infliximab and etanercept study group also suggested that T-SPOT.TB should be advised in TST-positive patients before the administration of TNF-α antagonists.[42,43] However, the phenomenon of poor agreement between TST and IGRA was found in many studies of different populations and multi-centered, large-scaled and randomized comparative studies between the TST test and IGRA are still lacking. A strategy of simultaneous testing to optimize diagnostic sensitivity is suggested in the clinical use of biological drugs.

A recently published study[56] aimed to figure out the impact of TST on subsequent IGRA results. TST-mediated

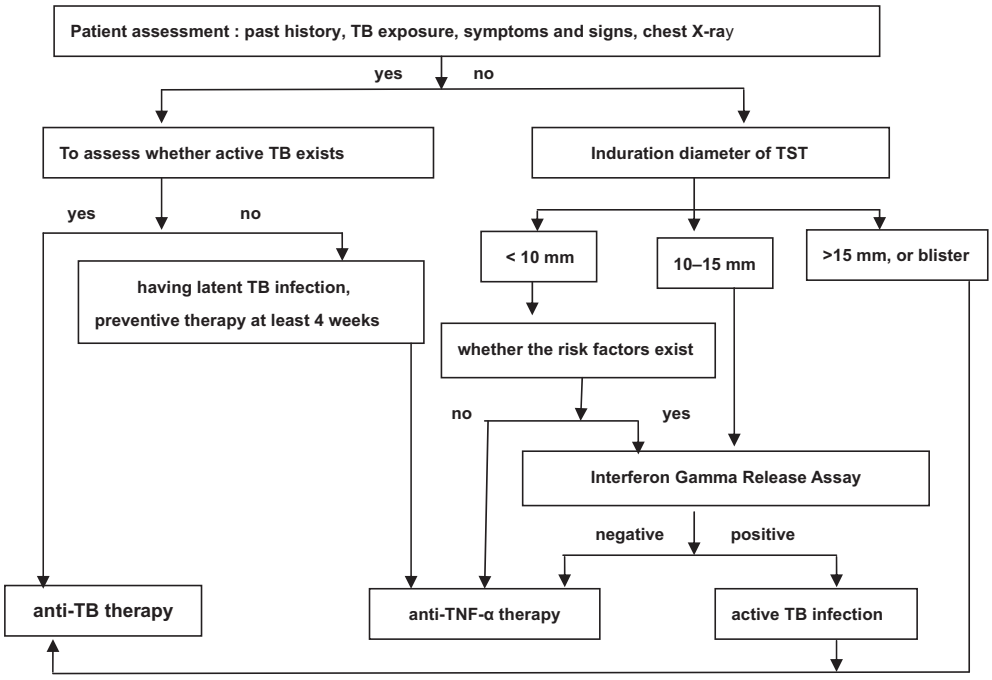


**Figure 1.** Tuberculosis screening diagram in rheumatoid arthritis (RA) and ankylosing spondylitis (AS) patients for anti-TNF-$\alpha$ therapy in our clinic.

boosting of IGRA responses were evaluated in 26 South African volunteers. IGRAs were performed pre-TST, and were repeated four times on Days 3, 7, 28, and 84 post-TST administration. The results showed that IGRAs performed post-TST were obviously elevated since Day 3. The reporter advised that when using a two-step screening strategy, it is better to perform an IGRA within 3 days after performing the TST.

## Screening new antigens for diagnosis of TB

Finding new TB antigens specific for MTb is ongoing. Heparin-binding hemagglutinin (HBHA) became a candidate. A study showed that LTBI individuals mount a strong T-cell response to HBHA, whereas patients with ATB disease do not, suggesting that HBHA is a good marker for the immunodiagnosis of LTBI, and that HBHA-specific Th1 responses may contribute to protective immunity against ATB.[57] An updated report published in Nature also demonstrates an underappreciated role of type I IFN-$\alpha\beta$ signaling in the pathogenesis of TB, which has implications for the development of a vaccine and therapy.[58]

## Decline in the incidence of LTBI after strengthening TB control

With increasing concerns in the safety of anti-TNF-$\alpha$ agents, especially in patients with possible LTBI, there appeared a decline in the incidence of reactivation of LTBI in the general population. Several retrospective and observational studies suggested that treatment of LTBI before or during anti-TNF-$\alpha$ therapy prevented reactivation effectively.[40,41] In a single-center retrospective study,[59] before starting etanercept use, 78/84 patients were fully or partially treated for LTBI with a mean of 2.5 months (0—12 months); no cases of ATB or newly occurred TB were found, although they were thought to be at high risk for ATB because 80% were born in TB-endemic countries. Similar findings were reported by Carmona et al, who analyzed the Spanish-based BIOBADASER and EMECAR databases. After adoption of formal guidelines to treat LTBI before initiating TNF-a antagonists, 324/384 patients (84%) with a positive purified protein derivative (PPD) received anti-LTBI therapy, and there was a 78% ($p = 0.008$) reduction in TB risk for RA patients treated with infliximab; the TB risk fell to the background rate of RA patients not receiving anti-TNF-a therapy.[41] In Japan, of 5000 cases of RA patients treated with infliximab, 11 TB cases developed in the first 2000 patients, however, after intensified TB screening and prophylactic treatment, only three TB cases developed in the last 3000 patients who received stricter preventive anti-TB treatment (22.3% in the last 3000 patients vs. 14.0% in the first 2000 patients taking prophylactic treatment). [60]

However, treatment of LTBI before anti-TNF-$\alpha$ therapy is not universally effective. A retrospective study[61] in Greece reported that in 613 patients who received anti-TNF-$\alpha$ therapy from July 2000 to June 2004, 11 cases of ATB developed. Of the 11 cases, eight cases received infliximab and three received adalimumab; none received etanercept. Ten cases of ATB developed in the PPD-positive cohort and one case of ATB in the PPD-negative cohort, however, seven of the 11 cases of ATB occurred in patients who had completed or were undergoing LTBI therapy. The failure to prevent developing ATB in this Greek cohort may be attributable to inadequate compliance with recommendations, inadequate length of therapy, higher threshold for initiation of LTBI therapy (10-mm induration on PPD), or the use of infliximab instead of etanercept in a population with a significant background incidence of TB infection.

ATB or reactivation of LTBI associated with anti-TNF-α agents should be paid more attention to, for the safety of biological treatment in rheumatic diseases. Although there lie many puzzles in the mechanism of TB infection under these circumstances, it is convincing that with stricter screening for TB infection, especially LTBI, also combined with aggressive preventive anti-TB treatment, TB infection associated with anti-TNF-α agents will be under control. Based on the available data, we recommended IGRA to be a routine complementary test with TST before anti-TNF-α treatment.

## Conflicts of interest

All contributing authors declare no conflicts of interest.

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