# Usefulness of Enzyme-Linked Immunospot Assay (Elispot) Compared to Tuberculin Skin Testing for Latent Tuberculosis Screening in Rheumatic Patients Scheduled for Anti-Tumor Necrosis Factor Treatment

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***ABSTRACT.*** ***Objective.*** Treatment with tumor necrosis factor (TNF) antagonists in patients with rheumatic diseases has been associated with increased rates of tuberculosis due to reactivation of latent *Mycobacterium tuberculosis* (*MTb*) infection (LTBI). Diagnosis of LTBI is based mainly on the tuberculin skin test (TST), which has certain limitations.

***Methods.*** We compared the TST with an enzyme-linked immunospot interferon-γ (IFN-γ) release assay (Elispot; T SPOT® TB) for the diagnosis of LTBI in 70 patients with various rheumatic diseases starting treatment with anti-TNF agents. All patients underwent a standard initial evaluation for LTBI including clinical examination, chest radiograph, and standard TST. Freshly isolated peripheral blood mononuclear cells were stimulated *ex vivo* with *MTb*-specific antigens (ESAT-6 and CFP10), and IFN-γ-producing cells were counted (Elispot assay).

***Results.*** Twenty-seven patients (38.6%) were TST+ and 16 were Elispot+ (22.8%). The overall level of agreement between the 2 tests was 72.8%, being much higher in patients who were TST– (39/43, 90.6%) than in those who were TST+ (12/27, 44.4%). Discordant results were observed in 19 patients (27.1%). Among TST– patients (n = 43), 4 were Elispot+ (9.3%); we also identified 15 Elispot– patients among 27 TST+ patients (55.6%). Multivariate analysis showed that a history of bacillus Calmette-Guérin (BCG) vaccination was associated with TST+/Elispot– discordant results (p = 0.01), whereas steroid use was linked to TST–/Elispot+ discordant results (p = 0.04).

***Conclusion.*** Elispot assay is a useful test for diagnosis of LTBI in rheumatic patients scheduled for anti-TNF therapy and identification of patients with false-positive TST results due to previous BCG vaccination. (First Release Mar 15 2008; J Rheumatol 2008;35:1271–6)

*Key Indexing Terms:*
TUMOR NECROSIS FACTOR INHIBITORS RHEUMATOID ARTHRITIS TUBERCULOSIS
SPONDYLOARTHROPATHIES TUBERCULIN TEST DIAGNOSIS

Tumor necrosis factor (TNF) antagonists represent an established treatment modality for an expanding number of inflammatory rheumatic diseases including rheumatoid arthritis (RA) and spondyloarthropathies[1]. Their use has been linked to increased risk of tuberculosis (TB), mainly from reactivation of latent *Mycobacterium tuberculosis (MTb)* infection (LTBI)[2,3]. Variable inhibition of TNF, a pleiotropic cytokine with a critical role in host immune defenses against *MTb* by the anti-TNF agents, is the presumed mechanism for TB reactivation[4].

Following initial reports of increased rates of TB in patients receiving anti-TNF agents[5-8], universal screening procedures for *MTb* infection were implemented[9,10]. These measures have dramatically decreased TB rates, especially in areas highly endemic for *MTb*[11]. Available screening methods include clinical history and physical examination, the tuberculin skin test (TST), and chest radiographs[9]. The TST, which contains a mixture of *MTb* antigens, remains the standard method for diagnosis of LTBI, but its administration can frequently lead to false-positive and negative results[12,13]. False-positive TST is due mainly to previous bacillus Calmette-Guérin (BCG) vaccination or sensitization to environmental nontuberculous mycobacteria, while a false-negative TST can be the result of immune suppression caused by immunosuppressive therapies or coexisting serious illnesses (HIV infection, cancer, chronic renal failure, etc.)[13]. Subjective administration or reading of the test as well as

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differences in TST preparations could lead to additional variability in test results.

To overcome these limitations, new *ex vivo* diagnostic assays that quantify interferon-γ (IFN-γ) release by peripheral blood mononuclear cells (PBMC) either by enzyme-linked immunosorbent assay (QuantiFeron-TB Gold/QTG test; Cellestis Inc., Valencia, CA, USA) or immunospot assay (Elispot) after stimulation with *MTb*-specific antigens have been developed[13]. These tests have demonstrated a higher sensitivity (76%–88%) and specificity (92%–97%) compared to TST (71% and 66%, respectively)[13].

There has been inadequate experience with the performance of these assays in patients with rheumatic diseases[14-17], especially in direct comparison to the standard TST. We compared the performance characteristics of a commercial Elispot assay to TST for the diagnosis of LTBI in a larger cohort of rheumatic patients scheduled for anti-TNF treatment.

## MATERIALS AND METHODS

*Patients.* Consecutive patients with various rheumatic diseases seen at the Outpatient Rheumatology Clinic of Hippokration General Hospital, Athens, who were considered candidates for treatment with anti-TNF agents according to published guidelines[1], were included in the study. Patients with concomitant or recent (< 1 year) treatment with antituberculosis agents, including isoniazid (INH) treatment for LTBI, were excluded. All patients signed an informed consent prior to their participation; the study was approved by the institutional review board.

A detailed history regarding previous TB, TB exposure, or BCG vaccination was taken from each patient and a complete physical examination, including documentation of BCG vaccination scars, was also performed. Patients were categorized to those with a definite, unknown, or negative history of BCG vaccination.

Current use of immunosuppressive medications including steroid or disease modifying antirheumatic drugs (DMARD) was also recorded. A chest radiograph was done in each patient and evaluated for signs of active or previous TB infection. Patients with suspicious findings for active TB were evaluated by a pulmonary physician specialized in the evaluation and care of patients with TB and underwent further appropriate testing (including sputum cultures for *MTb*, chest computed tomography, etc.). Patients with either a positive TST (≥ 5 mm) or Elispot assay and no signs of active TB were started on INH prophylaxis (300 mg/day orally for 1 month) prior to the initiation of anti-TNF therapy.

*Tuberculin skin test.* TST was performed by intradermal injection (Mantoux method) of 0.1 ml (2 units) of purified protein derivative (PPD RT 23, Statens Serum Institute, Copenhagen, Denmark) according to the American Thoracic Society/CDC guidelines[12]. The diameter of cutaneous induration was measured 48–72 h later with a ruler, by a trained physician. A TST was considered positive when the diameter of transverse induration was ≥ 5 mm.

*Elispot assay.* The Elispot assay (T SPOT® TB, Oxford Immunotec, Oxford, UK) was performed as described[18], on the same day of TST application. Briefly, peripheral blood was drawn from each patient in heparinized syringes (prior to TST application). PBMC were isolated over Ficoll-Histopaque 1077-1 (Sigma, St. Louis, MO, USA) density gradient centrifugation. Freshly isolated PBMC were resuspended in serum-free medium (AIM-V, Gibco BRL, Grand Island, NY, USA) and seeded in wells ($2.5 \times 10^5$ cells/well) containing either no antigen (negative control), phytohemagglutinin (PHA; as a positive control), or 2 different pools of recombinant *MTb*-specific antigens including the early secretory antigenic target-6 (ESAT-6) and the culture filtrate protein 10 (CFP10). Plates were incubated overnight at 37°C in a humidified 5% $CO_2$ incubator, and the number of spots indicative of IFN-γ-producing PBMC were measured in each well using a handheld magnified lens.

Specific predefined criteria for Elispot assay positivity were used, as described[18]. A test was considered positive when the number of spots in the *MTb* wells (one or both) minus the number of spots in the control well was ≥ 6 (if the number of spots in the control well was ≤ 5) or if the number of spots in 1 or 2 wells containing the *MTb* antigens was more than twice the number of spots in the control well (if the number of spots in the control well was > 6). No patient tested demonstrated more than 5 spots in the control wells, while all patients gave positive responses (> 20 spots/well) to PHA (data not shown).

*Statistical analysis.* Statistical analysis was performed using the Fisher exact test for comparison of proportions between groups. The concordance between TST and the Elispot test results was assessed by the kappa (κ) test. Multivariate analysis was performed in order to identify variables associated with discordant test results (TST+/Elispot– or TST–/Elispot+). Variables studied included age, sex, history of BCG vaccination, history of TB exposure, positive TST in the past, presence of comorbid conditions, and current steroid or DMARD use. SPSS version 10.0 was used for analysis (SPSS Inc., Chicago, IL, USA), while statistical significance was set at the 0.05 level.

## RESULTS

*Patient characteristics.* The characteristics of the rheumatic patients in the study are summarized in Table 1. Seventy consecutive patients with various rheumatic diseases including RA (n = 32), ankylosing spondylitis (n = 18), psoriatic arthritis (n = 12), Crohn-associated spondyloarthropathy (SpA; n = 2), and undifferentiated SpA (n = 8) were included. There were 37 women (53%) and the mean age of patients was 50.9 years. More than half the patients were receiving DMARD

*Table 1.* Patient characteristics.

| Characteristic | |
|---|---|
| n | 70 |
| Sex, M/F | 33/37 |
| Age, yrs ± SD | 50.9 ± 16.9 |
| Underlying rheumatic disease (%) | |
| Rheumatoid arthritis | 32 (45.7) |
| Ankylosing spondylitis | 18 (25.7) |
| Psoriatic arthritis | 12 (17.1) |
| Crohn's-associated spondyloarthropathy | 2 (2.9) |
| Undifferentiated spondyloarthropathy | 6 (8.6) |
| Immunosuppressive use (DMARD and/or steroids) | 43 (61.4) |
| DMARD use | 39 (55.7) |
| Steroid use | 29 (41.4) |
| Daily steroid dose, mg ± SD | 6.8 ± 5.2 |
| Duration of steroid treatment, months ± SD | 26.4 ± 29.1 |
| Comorbid conditions (%) | 15 (21.4) |
| Chronic liver disease (HBV/HCV related) | 9 |
| Diabetes mellitus | 3 |
| COPD | 2 |
| Amyloidosis | 1 |
| Status of BCG vaccination (%) | |
| No vaccination | 16 (22.9) |
| Definite history of vaccination | 28 (40) |
| Unknown | 26 (37.1) |
| History of TB exposure or chest radiograph findings consistent with previous TB | 8 (11) |

DMARD: disease modifying antirheumatic drug, HBV/C: hepatitis B/C virus, COPD: chronic obstructive pulmonary disease, BCG; bacillus Calmette-Guérin, TB: tuberculosis.

(39/70, 56%) and about 40% were taking steroids at low doses (mean daily dose 6.8 mg prednisolone) at the time of inclusion in the study. In steroid-treated patients, the mean treatment duration was 26 months (median 15 mo).

Fifteen patients (21.4%) had comorbid conditions including chronic liver diseases (n = 9), diabetes mellitus (n = 3), chronic obstructive pulmonary disease (n = 2), and amyloidosis (n = 1). Eight patients (11%) had either a history of past TB exposure or chest radiograph findings consistent with previous, healed TB.

A definite history of BCG vaccination was documented in 28 patients (40%), while 16 patients (29%) had never received the BCG vaccine. Nevertheless, a clear history of BCG vaccination could not be elicited in more than one-third of the patients (37.1%).

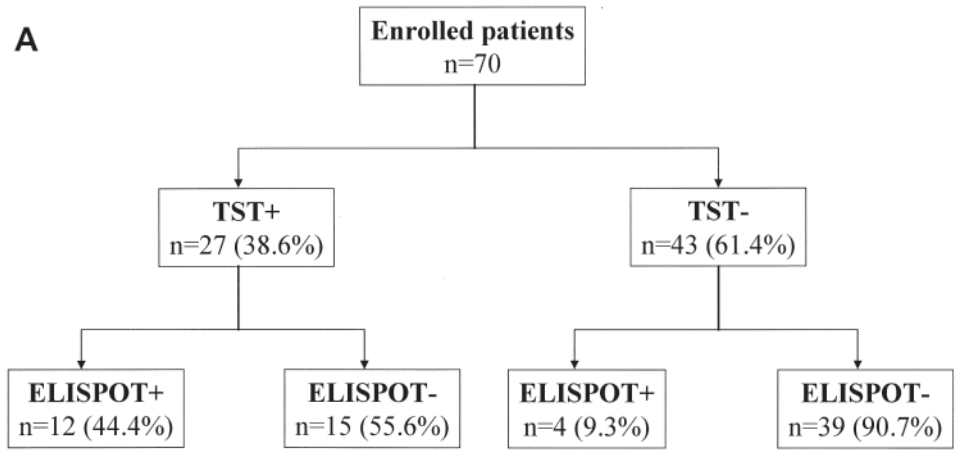
*TST and Elispot results.* Among 70 patients enrolled in the study, 27 (38.6%) displayed a positive TST (≥ 5 mm induration) and 16 (22.8%) a positive Elispot test. Among the TST+ patients (n = 27), 12 (44.4%) had a positive and 15 (55.6%) a negative Elispot assay; whereas among the 43 TST– patients, 39 (90.7%) were Elispot– and 4 (9.3%) Elispot+ (Figure 1). Overall, 39 patients (55.7%) had both tests negative, while 12 (17.1%) had both tests positive. Thus, the level of agreement between the 2 tests was 72.8% with a κ value = 0.38 (Table 2).

The rate of Elispot positivity was higher among TST+ patients (44.4%) compared to TST– (90.6%) patients. The distribution of positive Elispot assays in different patient subgroups stratified according to their TST diameter is shown in Figure 2.

Among the 8 patients with history of possible TB exposure or chest radiograph findings consistent with previous TB, 6 were both TST+ and Elispot+, while 2 were negative for both tests.

Among the 16 patients with a positive Elispot assay, 6 (37.5%) displayed reactivity against both *MTb* antigens (ESAT-6 and CFP10), while 5 had responses against ESAT-6 and 5 against CFP10 only.

*Discrepancy of TST and Elispot test results.* Nineteen patients (27.1%) had discrepant results between the TST and Elispot tests. Four patients displayed Elispot+/TST– results, while 15 patients had Elispot–/TST+ tests (Figure 1). The characteristics of the 4 patients with Elispot+/TST– tests are summarized in Table 3. One patient was a healthcare professional (ambulance driver) with a positive TST in the past (Patient 1), while another (Patient 2), who received initially 2 doses of infliximab without INH prophylaxis, showed a positive TST on repeat testing 1 month later. In the other 2 patients, one also had hepatitis B virus-related cirrhosis and the other was undergoing chronic steroid and methotrexate therapy. None of these patients had chest radiograph findings consistent with previous TB. In the subgroup of patients (n = 27) who had received immunosuppression (DMARD and/or steroids), the




B

| ELISPOT | TST + | TST - | |
|---|---|---|---|
| + | 12 | 4 | 16 |
| - | 15 | 39 | 54 |
| | 27 | 43 | 70 |

Figure 1. A. Distribution of Elispot results among tuberculin skin testing (TST)-positive and TST-negative patients. B. The same results in 2 × 2 format.

*Table 2.* Effect of BCG vaccination on Elispot and tuberculin skin test (TST) agreement.

| | Total Population, n = 70 | BCG-Vaccinated n = 28 | Unvaccinated, n = 16 | Unknown BCG Status, n = 26 |
|---|---|---|---|---|
| Concordant results | | | | |
| TST+/Elispot+ | 12 | 4 | 2 | 6 |
| TST–/Elispot– | 39 | 13 | 10 | 16 |
| Discordant results | | | | |
| TST–/Elispot+ | 4 | 1 | 0 | 3 |
| TST+/Elispot– | 15 | 10 | 4 | 1 |
| Agreement (%) | | | | |
| Overall | 72.9 | 60.7 | 75 | 84.6 |
| TST+ | 44.4 | 28.6 | 33.3 | 85.7 |
| TST– | 90.6 | 92.9 | 100 | 84.2 |

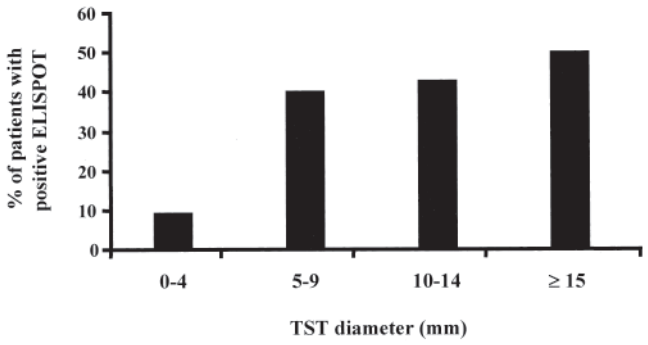



Figure 2. The proportion of Elispot-positive tests among patient subgroups defined according to their TST diameter (0–4 mm = 9.3%, 5–9 mm = 40%, 10–14 mm = 43%, ≥ 15 mm = 50%).

number of patients with discrepant Elispot+/TST– results was higher (3/43, 7%) compared to non-immunosuppressed patients (1/27, 3.7%), although the difference was not statistically significant (Table 4).

Among the 15 Elispot–/TST+ discordant patients, 10 (66.7%) had been vaccinated in the past with BCG (Table 2). The number of vaccinated individuals among the other 3 groups (i.e., Elispot–/TST–, Elispot+/TST+, and Elispot+/TST– patients) was much lower (33%, 33%, and 25%, respectively). None of these Elispot–/TST+ patients had been exposed to TB or had chest radiograph findings suggestive of previous TB.

*Table 4.* Role of immunosuppression in Elispot and tuberculin skin test (TST) results.

| | Immunosuppression (DMARD and/or steroids), n = 43 (%) | No Immunosuppressison, n = 27 (%) |
|---|---|---|
| Concordant results | | |
| TST+/Elispot+ | 9 (20.9) | 3 (11.1) |
| TST–/Elispot– | 23 (53.5) | 16 (59.3) |
| Discordant results | | |
| TST–/Elispot+ | 3 (7) | 1 (3.7) |
| TST+/Elispot– | 8 (18.6) | 7 (25.9) |

The TST diameter in these patients was smaller (11.9 ± 4.4 mm) compared to Elispot+/TST+ (16.4 ± 12.1 mm) patients, but the difference was not statistically significant (p = 0.41). A lower number of discordant Elispot–/TST+ results was observed in immunosuppressed (8/43, 18.6%) compared to non-immunosuppressed patients (7/27, 25.9%; p = nonsignificant; Table 4).

In order to account for various factors that could be responsible for discordant Elispot/TST results (such as history of TB exposure, sex, age, history of BCG vaccination, underlying rheumatic disease, etc.), multivariate analysis was performed that showed that a history of previous BCG vaccination was associated with a discordant Elispot–/TST+ result (p = 0.01) and steroid use with a discordant Elispot+/TST- test result (p = 0.041).

*Table 3.* Characteristics of patients with Elispot+/TST– tests.

| Patient | Age, yrs | Rheumatic Disease | Disease Duration, yrs | TST, mm | Previous TST | Possible TB Exposure | Steroid Use | DMARD Use | Comorbid Conditions |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 27 M | uSpA | 4 | 0 | 10 mm (5 yrs ago) | Healthcare professional | Yes | SSZ | None |
| 2* | 47 M | AS | 10 | 4 | ND | Unknown | No | No | None |
| 3 | 59 F | RA | 2 | 0 | ND | Unknown | Yes | MTX | None |
| 4 | 70 M | RA | 8 | 4 | Negative | Unknown | Yes | No | Cirrhosis |

* Repeat TST testing 1 month after initiation of anti-TNF therapy with infliximab was positive, and the patient was started on isoniazid prophylaxis. uSpA: undifferentiated spondyloarthropathy, SSZ: sulfasalazine, MTX: methotrexate, ND: not done.

*Patient followup.* None of the 70 patients included in the study had evidence of active TB during initial evaluation. Among these patients, 51 were started on anti-TNF treatment, after appropriate prophylaxis with INH for those who had either positive TST and/or positive Elispot assays. The rest of the patients (n = 19) either started INH prophylaxis and are awaiting anti-TNF treatment (10/19) or declined immediate treatment and were placed on a different therapeutic regimen (9/19).

Among the 51 patients treated with anti-TNF agents, 26 (50.9%) received adalimumab, 14 infliximab (27.4%), and 11 (21.6%) etanercept. None of these patients has developed active TB during the followup period (mean followup 7.1 mo, range 0–20 mo). Two patients who were either TST+ or Elispot+ did not initially receive INH prophylaxis. The first patient (TST–/Elispot+) is described in Table 3 (Patient 2), while the second, a 35-year-old man with longstanding ankylosing spondylitis who was TST+/Elispot–, declined INH prophylaxis. To date, he has received 9 months of adalimumab therapy with no evidence of active TB.

## DISCUSSION

This is the largest study to date directly comparing the results of an IFN-γ release assay to the standard TST for TB screening in patients with rheumatic diseases. Our results show that in rheumatic patients scheduled for anti-TNF treatment, the Elispot assay is a highly promising tool for *MTb* infection screening compared to the standard TST, as it is unaffected by previous BCG vaccination or steroid use.

A modest rate of agreement between the 2 assays, the TST and Elispot (72.9%, $\kappa = 0.38$), was observed. This concordance is close to the rate recently reported in healthy populations with varying risk for LTBI (78.9%)[13]. The overall agreement between the 2 tests was much higher among TST– (90.6%) than TST+ patients (44.4%). This was due to the increased number of discordant TST+/Elispot– results among the TST+ population (15/27, 55.6%). Multivariate analysis revealed that only BCG vaccination was statistically associated with this discordant result. A recent review has shown that BCG vaccination after the first year of life (as in our population) can lead to positive TST (≥ 10 mm) in roughly 20% of the cases[19]. Thus, these discordant results most likely represent "false-positives" due to previous BCG vaccination. Another possible explanation for this finding could be previous sensitization to environmental nontuberculous mycobacterial antigens present in the TST and not the Elispot assay. The true prevalence of such sensitization in our population is difficult to ascertain.

The discovery of a high proportion of patients with Elispot–/TST+ results is challenging, as it indicates that these patients could be unnecessarily exposed to INH treatment. This is particularly important because INH-related liver toxicity may be even higher in rheumatic patients who, especially with RA, are being treated with other potentially hepatotoxic medications such as methotrexate, nonsteroidal antiinflammatory drugs, leflunomide, sulfasalazine, etc[20].

Approximately 10% of our TST– rheumatic patients (4/43) were Elispot+. Current steroid use was the only factor statistically associated with this result. Thus, although the percentage of TST–/Elispot+ patients was relatively low, their identification could lead to early LTBI detection and TB prevention in this vulnerable population.

The main inherent limitation of our study is the inability to directly calculate and compare the true sensitivity and specificity of the TST and Elispot assays for LTBI detection. This is due to the absence of a "gold standard" method for the diagnosis of LTBI, as indicated in recent reviews on this topic[3,13]. Nevertheless, similar comparative analyses have been reported in other populations[13].

As noted, there are limited data on the performance of IFN-γ release assays in rheumatic patients. In a recent study, Sellam *et al*[16] examined the performance of different *in vitro* assays with TST in 68 BCG-vaccinated French rheumatic patients starting anti-TNF treatment. In that study, an in-house Elispot assay with a much lower positivity threshold was used (CFP10: 2.5 spots/$2.5 \times 10^5$ cells; ESAT-6: 1.25 spots/$2.5 \times 10^5$ cells, compared to 6 spots/$2.5 \times 10^5$ cells for both antigens in our study). The use of an in-house assay may pose a problem for generalized use of this method. In addition, a direct comparison to TST using both antigens was performed only in a minority of patients (n = 7 with "definite" LTBI). Nevertheless, similarly to our study, better "sensitivity" and "specificity" of the Elispot assays compared to TST for diagnosis of LTBI were found.

There are no reports in the literature directly comparing the QuantiFeron TB Gold (QTG) test with the TST in rheumatic patients. Efthimiou, *et al* described 2 patients with radiographic findings suggestive of old TB and negative TST who were positive by the QTG test[17]. Takahashi, *et al* used this test in a limited number of patients with RA (n = 14) treated with infliximab[15]. Among these, 4 were QTG-positive, but only 2 had the test prior to infliximab treatment. In a recent study of a large cohort of patients with RA in the UK (n = 101) who were screened with the QTG test, 7 were found to be positive[14] but no direct comparison to the TST was reported.

The possibility that some of the negative Elispot results in our study were falsely negative cannot be definitely ruled out. This is particularly true for the 4 out of 15 patients with discordant TST+/Elispot– results and no clear history of BCG vaccination, as well as for patients with double negative results. The effect of immunosuppression on Elispot results has not been adequately studied in rheumatic patients, and it is the subject of current investigation by our group (data not shown) and others. Moreover, only studies in rheumatic patients with a definite history of TB exposure can give more information regarding the true "sensitivity" of the Elispot test for diagnosis of LTBI, as suggested recently[21].

Similarly, it is difficult to ascertain the rate of "false-posi-

tive" Elispot results in our patient population. That would require either a study of a large number of rheumatic patients with low risk for TB exposure or longterm followup of TST–/Elispot+ patients without INH treatment (thus measuring the rate of TB development). The latter type of study, however, raises ethical issues that need to be carefully considered.

The results of our study show that the Elispot assay is a useful test for diagnosis of LTBI in rheumatic patients scheduled for anti-TNF treatment. The test could be particularly helpful in BCG-vaccinated patients with a false-positive TST who could be otherwise exposed to INH treatment in addition to other potentially hepatotoxic medications. However, prospective longterm studies examining the rate of TB reactivation under anti-TNF treatment in such patients without INH prophylaxis are needed before avoidance of prophylactic treatment can be recommended. The detection of approximately 10% of TST– as Elispot+ patients is equally important, since it identified patients with high risk for TB reactivation that could be missed with the traditional screening methods.

At this point, based on the available data, replacement of the TST by the Elispot assay cannot be definitely recommended. More data examining the test's cost, feasibility, and reproducibility as well as the outcome of anti-TNF-treated rheumatic patients with discordant TST/Elispot results are needed before evidence-based recommendations can be made.

## REFERENCES


1. Furst DE, Breedveld FC, Kalden JR, et al. Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2006. Ann Rheum Dis 2006;65 Suppl 3:iii2-15.
2. Keane J. TNF-blocking agents and tuberculosis: new drugs illuminate an old topic. Rheumatology Oxford 2005;44:714-20.
3. Gardam MA, Keystone EC, Menzies R, et al. Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. Lancet Infect Dis 2003;3:148-55.
4. Furst DE, Wallis R, Broder M, Beenhouwer DO. Tumor necrosis factor antagonists: different kinetics and/or mechanisms of action may explain differences in the risk for developing granulomatous infection. Semin Arthritis Rheum 2006;36:159-67.
5. Wolfe F, Michaud K, Anderson J, Urbansky K. Tuberculosis infection in patients with rheumatoid arthritis and the effect of infliximab therapy. Arthritis Rheum 2004;50:372-9.
6. Keane J, Gershon S, Wise RP, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001;345:1098-104.
7. Gomez-Reino JJ, Carmona L, Valverde VR, Mola EM, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. Arthritis Rheum 2003;48:2122-7.
8. Dixon WG, Watson K, Lunt M, Hyrich KL, Silman AJ, Symmons DP. Rates of serious infection, including site-specific and bacterial intracellular infection, in rheumatoid arthritis patients receiving anti-tumor necrosis factor therapy: results from the British Society for Rheumatology Biologics Register. Arthritis Rheum 2006;54:2368-76.
9. Winthrop KL, Siegel JN, Jereb J, Taylor Z, Iademarco MF. Tuberculosis associated with therapy against tumor necrosis factor alpha. Arthritis Rheum 2005;52:2968-74.
10. BTS recommendations for assessing risk and for managing Mycobacterium tuberculosis infection and disease in patients due to start anti-TNF-alpha treatment. Thorax 2005;60:800-5.
11. Carmona L, Gomez-Reino JJ, Rodriguez-Valverde V, et al. Effectiveness of recommendations to prevent reactivation of latent tuberculosis infection in patients treated with tumor necrosis factor antagonists. Arthritis Rheum 2005;52:1766-72.
12. American Thoracic Society. Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med 2000;161:S221-47.
13. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med 2007;146:340-54.
14. Pratt A, Nicholl K, Kay L. Use of the QuantiFERON TB Gold test as part of a screening programme in patients with RA under consideration for treatment with anti-TNF-alpha agents: the Newcastle (UK) experience. Rheumatology Oxford 2007; 46:1035-6.
15. Takahashi H, Shigehara K, Yamamoto M, et al. Interferon gamma assay for detecting latent tuberculosis infection in rheumatoid arthritis patients during infliximab administration. Rheumatol Int 2007;27:1143-8.
16. Sellam J, Hamdi H, Roy C, et al. Comparison of in vitro-specific blood tests with tuberculin skin test for diagnosis of latent tuberculosis before anti-TNF therapy. Ann Rheum Dis 2007;66:1610-5.
17. Efthimiou P, Sood S. QuantiFERON TB Gold Test: the new standard for screening of latent tuberculosis in patients with rheumatoid arthritis? [letter]. Ann Rheum Dis 2007;66:276.
18. Richeldi L, Ewer K, Losi M, et al. T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. Am J Respir Crit Care Med 2004;170:288-95.
19. Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? Int J Tuberc Lung Dis 2006; 10:1192-204.
20. Vanhoof J, Landewe S, Van Wijngaerden E, Geusens P. High incidence of hepatotoxicity of isoniazid treatment for tuberculosis chemoprophylaxis in patients with rheumatoid arthritis treated with methotrexate or sulfasalazine and anti-tumour necrosis factor inhibitors. Ann Rheum Dis 2003;62:1241-2.
21. Kunst H, Khan KS. New tests for the diagnosis of latent tuberculosis infection. Ann Intern Med 2007;147:672-3.