High Incidence of Tuberculosis Infection in Rheumatic Diseases and Impact for Chemoprophylactic Prevention of Tuberculosis Activation during Biologics Therapy

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We conducted a long-term follow-up study in patients with rheumatic diseases who were candidates for biologics treatment to evaluate the effects of biologic agents on the risk of tuberculosis infection and the effect of prophylactic treatment on tuberculosis activation. One hundred one patients with rheumatic diseases who were candidates for biologics treatment were recruited, and 57 healthy subjects were recruited as controls. Tuberculin skin test (TST) and the T-SPOT.TB test were performed for all subjects at baseline. Follow-up testing by the T-SPOT.TB assay was performed every 6 months in patients with rheumatic diseases and at 2 years of recruitment in the healthy controls. In patients with rheumatic diseases and healthy controls, the TST-positive (induration, ≥10 mm) rates were 37.6% (38/101) and 34.0% (18/53), respectively (P > 0.05), while the T-SPOT.TB-positive rates were 46.5% (47/101) and 21.1 (12/57), respectively (P = 0.0019). Fifty-two patients were followed up at month 6 with a T-SPOT.TB-positive rate of 40.4%, and 49 were followed up for ≥12 months with a T-SPOT.TB-positive rate of 36.7%, with no significant difference in the positive rate at different time points including baseline (P > 0.05). Long-term follow-up revealed that conversion to T-SPOT.TB positivity occurred only in the biologics treatment group, with a positive conversion rate of 11.2% (4/38). Most importantly, no latent tuberculosis developed into active tuberculosis during follow-up with T-SPOT.TB screening and preemptive treatment with isoniazid. Biologics treatment appears to increase the risk of tuberculosis infection. However, tuberculosis activation could be prevented by preemptive isoniazid treatment in patients with latent tuberculosis infection while receiving biologics therapy.

Biologic agents, including tumor necrosis factor (TNF) antagonist and interleukin-1 (IL-1) receptor antagonist, have become invaluable treatments against chronic inflammatory diseases, such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and psoriasis (1–5). Studies have shown that following TNF antagonist therapy, the relative risk for activation of latent tuberculosis (TB) is increased up to 25 times, depending on the clinical setting and the TNF antagonist used (6–10). Activation of latent tuberculosis following IL-1 receptor antagonist therapy has also been reported (11, 12). Therefore, latent tuberculosis infection (LTBI) screening and preemptive antituberculosis treatment are recommended prior to biologics treatment in patients with latent tuberculosis infection.

The diagnosis of LTBI is traditionally based on tuberculin skin test (TST) positivity in the absence of active tuberculosis. However, TST has a low sensitivity in patients with rheumatoid diseases (13) and has a low specificity in patients with prior Mycobacterium bovis bacillus Calmette-Guérin (BCG) vaccination (14, 15). The new gamma interferon release assays (IGRAs) have been introduced to compensate for the drawback of TST in detecting LTBI (16, 17), but evidence supporting the use of IGRAs to identify new tuberculosis infection and prevent reactivation of tuberculosis in patients with chronic rheumatic conditions is lacking (8).

We conducted a long-term follow-up study in patients with rheumatic diseases who were candidates for biologics treatment. TST and IGRAs were performed to detect LTBI and new tuberculosis infection. The rate of conversion to IGRA positivity, the effects of different biologic agents on the risk of tuberculosis infection, and the prophylaxis were evaluated.

MATERIALS AND METHODS

Patients and study population. One hundred one patients with rheumatic diseases who were candidates for biologics treatment, including etanercept, anakinra, and infliximab, were recruited in the Shanghai Guang Hua Hospital and Huashan Hospital from April 2008 to September 2009. Rheumatic diseases included rheumatoid arthritis (RA), ankylosing spondylitis (AS), and psoriatic arthritis (PsA), which were diagnosed according to the American College of Rheumatology (formerly the American Rheumatism Association) criteria for RA (18) or the European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy (19). All subjects were screened at enrollment by IGRA and TST using purified protein derivative (PPD). Patients with active tuberculosis were excluded. Meanwhile, 57 healthy subjects who had no history of exposure to known active tuberculosis were recruited as controls. Among them, all were tested by IGRA and 53 received the TST.
After recruitment, patients were evaluated and treated with biologics, such as soluble TNF receptor fusion protein (etanercept), chimeric hu-
man-mouse anti-tumor necrosis factor alpha (TNF-α; infliximab), or in-
terleukin-1 receptor antagonist (anakinra), and/or conventional immu-
nosuppressive agents, such as disease-modifying antirheumatic drugs
(DMARD), corticosteroid, or methotrexate. All the patients were then
followed up regularly, and IGRA was performed every 6 months. The
healthy controls were all followed up, and IGRA was repeated after 2 years
of recruitment. If necessary, chest X ray was employed to exclude active
tuberculosis. The criteria used to exclude active tuberculosis included no
cough with or without sputum production or hemoptysis; no fever, night
sweats, or weight loss; normal chest X ray; and no signs of extrapulmonary
tuberculosis.

For patients with a high risk of tuberculosis reactivation, prophylactic
treatment with isoniazid (INH) was advised, and with the consent of the
patients, oral INH at 300 mg daily for 6 months was prescribed. INH
prophylaxis was administered concurrently with infliximab or etanercept
for patients with positive baseline IGRA results but not for candidates for
anakinra because tuberculosis reactivation had not been reported as a
common complication during anakinra treatment. Meanwhile, none of
the T-SPOT.TB-positive healthy controls received INH prophylactic
treatment. The study was approved with written consent by the Ethics
Committee of Huashan Hospital, Fudan University, and written informed consent
was obtained from all the participants.

### TABLE 1 Baseline characteristics of patients at recruitment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with rheumatic diseases</th>
<th>Biologics treated (n = 65)</th>
<th>Other antirheumatics treated (n = 36)</th>
<th>Healthy controls (n = 57)</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range) age (yr)</td>
<td>47 (18–70)</td>
<td>47 (18–70)</td>
<td>47 (20–63)</td>
<td>44 (21–70)</td>
<td>0.3761</td>
</tr>
<tr>
<td>No. of males/no. of females</td>
<td>29/72</td>
<td>21/44</td>
<td>8/28</td>
<td>23/34</td>
<td>0.3607</td>
</tr>
<tr>
<td>No. (%) of subjects with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCG vaccination history</td>
<td>79 (78.2)</td>
<td>53 (81.5)</td>
<td>26 (72.2)</td>
<td>51 (89.5)</td>
<td>0.3189</td>
</tr>
<tr>
<td>TB history</td>
<td>5 (5.0)</td>
<td>1 (1.5)</td>
<td>4 (11.1)</td>
<td>0</td>
<td>0.0531</td>
</tr>
<tr>
<td>Abnormal chest X ray</td>
<td>11 (10.9)</td>
<td>6* (9.2)</td>
<td>5* (13.9)</td>
<td>0</td>
<td>0.5152</td>
</tr>
<tr>
<td>No. (%) of subjects with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TST positive</td>
<td>38 (37.6)</td>
<td>20 (31.2)</td>
<td>18 (50.0)</td>
<td>18 (34.0&lt;sup&gt;b&lt;/sup&gt;)</td>
<td>0.0855</td>
</tr>
<tr>
<td>T-SPOT.TB positive</td>
<td>47 (46.5)</td>
<td>27 (41.5)</td>
<td>20 (55.6)</td>
<td>12 (21.1)</td>
<td>0.2136</td>
</tr>
<tr>
<td>No. (%) of subjects with the following diagnosis:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>82 (81.2)</td>
<td>52</td>
<td>30</td>
<td></td>
<td>0.3534</td>
</tr>
<tr>
<td>Others&lt;sup&gt;e&lt;/sup&gt;</td>
<td>19 (18.8)</td>
<td>13</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range) disease duration (mo)</td>
<td>60 (1–360)</td>
<td>60 (1–360)</td>
<td>66 (1–240)</td>
<td></td>
<td>0.5272</td>
</tr>
<tr>
<td>No. (%) of subjects taking the following biologic:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etanercept</td>
<td>28 (43.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anakinra</td>
<td>25 (38.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Infliximab</td>
<td>12 (18.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%) of subjects with the following duration of</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>biologics treatment (yr):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>37 (56.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>28 (43.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No. (%) of subjects receiving other antirheumatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMARD</td>
<td>20 (19.8)</td>
<td>0</td>
<td>20 (55.5)</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>13 (12.9)</td>
<td>5 (7.69)</td>
<td>8 (22.3)</td>
<td></td>
<td>0.0597</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>81 (80.2)</td>
<td>47 (72.3)</td>
<td>34 (94.4)</td>
<td></td>
<td>0.0084</td>
</tr>
<tr>
<td>No. (%) of subjects receiving preventive anti-TB TB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>therapy</td>
<td>8 (7.9)</td>
<td>8 (12.3)</td>
<td>0</td>
<td></td>
<td>0.0479</td>
</tr>
<tr>
<td>No. (%) of subjects withdrawn from biologics treat-</td>
<td>11 (10.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ment due to positive T-SPOT.TB result</td>
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<td></td>
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</tbody>
</table>

<sup>a</sup> Comparison between biologics–treated and other antirheumatic-treated patients.
<sup>b</sup> The X ray indicated previous pulmonary tuberculosis.
<sup>c</sup> The X ray indicated previous pulmonary tuberculosis (n = 4) and increased lung markings (n = 1).
<sup>d</sup> Fifty-three subjects were tested.
<sup>e</sup> Others included AS and PsA.
pected by manual counting. The laboratory technicians were blinded to the subject identifiers.

TST. TST was carried out, using the Mantoux technique on the volar surface of a forearm, with five tuberculin units (TU) of tuberculin PPD RT23 (Statens Seruminstitut, Copenhagen, Denmark), and the results were read by one individual. Tests were read at 48 to 72 h, and the results were measured with a ruler as induration diameters along and across the arm. The result was classified as negative if the TST induration was <10 mm in diameter and as positive if the reaction was ≥10 mm in diameter or there was blistering.

Data analysis. The data were compared by using the nonparametric Mann-Whitney test and chi-square test or Fisher’s exact test. Significance was inferred for $P$ values of <0.05.

RESULTS

Latent tuberculosis infection rate in patients with rheumatic diseases in a high-prevalence region of tuberculosis. The baseline characteristics of the patients are shown in Table 1. Among the 101 recruited patients, the median age was 47, the age range was 18 to 70 years, and 29 were male. In comparison, the median age of the 57 healthy controls was 44 years, and the age range was 21 to 70 years; 23 of the healthy controls were male.

Most patients were diagnosed with RA ($n = 82$); 16 were diagnosed with AS and 3 were diagnosed with PsA. Sixty-five patients eventually received biologics treatment, including etanercept (soluble TNF receptor fusion protein, $n = 28$), anakinra (interleukin-1 receptor antagonist, $n = 25$), and infliximab (chimeric human-mouse anti-TNF-α, $n = 12$). The remaining 36 patients who received routine antirheumatic treatment were recruited as nonbiologic-treated controls.

In patients with rheumatic diseases and healthy controls, the TST-positive rates were 37.6% (38/101; 95% confidence interval [CI], 28.8% to 47.4%) and 34.0% (18/53; 95% CI, 22.6% to 47.5%), respectively ($P > 0.05$), while the T-SPOT.TB-positive rates were 46.5% (47/101; 95% CI, 37.1% to 56.2%) and 21.1% (12/57; 95% CI, 12.3% to 33.4%), respectively ($P = 0.0019$; Fig. 1). Among the 38 TST-positive patients, 11 were T-SPOT.TB positive and 27 were T-SPOT.TB negative. The T-SPOT.TB-positive rate (46.5%) was higher than the TST-positive rate (37.6%) in patients with rheumatic diseases, but the difference was not significant ($P > 0.05$). Eleven (10.9%) withdrew from biologics treatment due to positive T-SPOT.TB results. Moreover, at baseline, 8 (7.9%) received preemptive antituberculosis treatment with isoniazid for 6 months.

Sensitivity of IGRA for detection of LTBI during prolonged immunosuppressive treatment. The patients were followed up for 6 to 24 months, with a median duration of 6 months, and the T-SPOT.TB assay was performed at each follow-up. Fifty-two patients were followed up at month 6 with a T-SPOT.TB-positive rate of 40.4%, and 49 were followed up for ≥12 months with a positive rate of 36.7% (Fig. 2). It seemed that the T-SPOT.TB-positive rate tended to be lower during long-term follow-up. However, there was no significant difference in the positive rate at different time points, including baseline ($P > 0.05$).

To understand the influence of biologics treatment on tuberculosis infection, we divided the patients into 2 groups: biologics treatment ($n = 65$) and other antirheumatic treatment without biologics ($n = 36$). The baseline characteristics of the two groups are shown in Table 1. Long-term follow-up showed no significant difference in the positive rate at different time points, including baseline ($P > 0.05$), even between the biologics treatment and other antirheumatic treatment without biologics groups, and the T-SPOT.TB-positive rates did not change significantly at each time point ($P > 0.05$ for all comparisons) (Fig. 2).

Risk of tuberculosis infection during biologics treatment. When we compared the T-SPOT.TB results between follow-up and baseline, it was found that positive conversion occurred only in the biologics treatment group, with a positive conversion rate of 11.2% (4/38; Fig. 3), while no positive conversion was found in the non-biologics treatment control group (0/16) or healthy controls (0/45). On the other hand, among all patients with negative T-SPOT.TB results at baseline, no active tuberculosis developed in either the biologics treatment group or the control group with other antirheumatic treatment (Fig. 3).

Effects of preemptive antituberculosis treatment on activation of latent tuberculosis in T-SPOT.TB-positive patients. In our study, 4 patients converted to T-SPOT.TB positivity during follow-up. All 4 of these patients were treated with biologics, such as etanercept or anakinra, and in all 4 patients, conversion to T-SPOT.TB positivity occurred after 6 months of biologics treatment. Two of the 4 patients received preemptive anti-tuberculosis therapy with INH at 300 mg daily at month 6 of biologics treat-
ment for 6 months, and none developed active tuberculosis at the end of follow-up (Table 2). Fortunately, no active tuberculosis developed during long-term follow-up in patients with positive baseline T-SPOT.TB results.

**DISCUSSION**

It has been demonstrated that IGRAs are superior to TST in identifying LTBI in immunosuppressed and BCG-vaccinated populations (14–17, 20). In this study, we found that the T-SPOT.TB-positive rate was slightly higher than the TST-positive rate (46.5% versus 37.6%) in patients with rheumatic diseases before biologics treatment, which may indicate higher sensitivity in immunosuppressed patients. Furthermore, the LTBI rate in patients with rheumatic diseases (46.5%) was significantly higher than that in healthy people (21.1%) but lower than that in HIV-infected individuals (67.6%) in China (16, 17). This is an important finding which seems to suggest that rheumatic disease is a risk factor for tuberculosis infection. However, future studies are needed to confirm the findings of this study.

For the first time, we employed T-SPOT.TB to monitor the effect of immunosuppressive biologics treatment on new tuberculosis infection in China. Long-term follow-up found that the T-SPOT.TB-positive rates at different time points of biologics treatment remained similar, which suggests that the sensitivity of IGRA was not influenced by prolonged immunosuppressive treatment. In our cohort, there were 4 patients (10.5%) whose T-SPOT.TB result converted to positive during follow-up, while the rate was reported to be 32.6% (28/86) when patients were tested by TST in areas with intermediate tuberculosis burdens (21). Two reasons may contribute to this difference. First, TST might overestimate the new tuberculosis infection rate in high-prevalence areas of tuberculosis due to its limited specificity. Second, 36 candidates eventually did not receive biologics treatment, including 20 with a baseline positive result by T-SPOT.TB.

It has been reported that the risk of tuberculosis is higher with anti-TNF antibody therapy (infliximab) than with soluble tumor necrosis factor receptor therapy (etanercept) (22, 23). The conversion to positive T-SPOT.TB results during anakinra treatment was not reported previously. Surprisingly, 2 patients treated with anakinra became T-SPOT.TB positive, suggesting new tuberculosis infection. Fortunately, no active tuberculosis developed during our long-term follow-up.

The increased risk of reactivation of tuberculosis among patients with rheumatic diseases becomes the major concern during treatment with infliximab and other immunosuppressive agents (24, 25). Three measures were taken in our cohort to prevent activation of latent tuberculosis. One was baseline screening for LTBI and a recommendation to abandon biologics treatment in T-SPOT.TB-positive patients at baseline. Therefore, 20 patients with a positive baseline T-SPOT.TB result did not receive biologics treatment in our study. Another was to stop biologics treatment in patients who converted to T-SPOT.TB positivity during long-term follow-up.

**TABLE 2 Clinical characteristics of 4 patients with conversion to T-SPOT.TB positivity**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Follow-up duration (mo)</th>
<th>Treatment regimen</th>
<th>Biologics treatment duration (mo)</th>
<th>Steriod treatment history</th>
<th>TST result at: Baseline</th>
<th>End of follow-up</th>
<th>Preemptive INH antituberculosis treatment (duration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH017</td>
<td>Female</td>
<td>24</td>
<td>18</td>
<td>Etanercept + methotrexate + penicillamine</td>
<td>24</td>
<td>Yes</td>
<td>Negative</td>
<td>Not tested</td>
<td>Initiated at mo 6 of biologics treatment (6 mo)</td>
</tr>
<tr>
<td>GH021</td>
<td>Female</td>
<td>63</td>
<td>12</td>
<td>Anakinra + methotrexate</td>
<td>6</td>
<td>No</td>
<td>Positive</td>
<td>Positive</td>
<td>No</td>
</tr>
<tr>
<td>GH076</td>
<td>Male</td>
<td>48</td>
<td>12</td>
<td>Anakinra + methotrexate</td>
<td>6</td>
<td>No</td>
<td>Negative</td>
<td>Not tested</td>
<td>Initiated at mo 6 of biologics treatment (6 mo)</td>
</tr>
<tr>
<td>GH202</td>
<td>Female</td>
<td>63</td>
<td>12</td>
<td>Etanercept + methotrexate</td>
<td>24</td>
<td>No</td>
<td>Negative</td>
<td>negative</td>
<td>No</td>
</tr>
</tbody>
</table>

*All patients had a diagnosis of RA. For all patients, the time to conversion to T-SPOT.TB positivity was 6 months after biologics treatment, and no active tuberculosis developed in any of the patients.
follow-up. Lastly, preemptive antituberculosis treatment was used for patients who both had a positive T-SPOT.TB result at baseline and converted to T-SPOT.TB positivity during follow-up. Preemptive antituberculosis treatment was recommended for at-risk populations with LTBI, including TNF antagonist-treated patients (26). In our study, 8 patients received preemptive antituberculosis therapy at baseline, and 2 patients who were preemptively treated converted to T-SPOT.TB positivity during biologics treatment, but active tuberculosis did not develop in any of the patients.

Conclusions. The rate of LTBI is significantly higher in patients with rheumatic diseases than healthy controls on the basis of detection by T-SPOT.TB. The biologics etanercept and anakinra appear to have similar capacities to increase the risk of tuberculo-
sis infection. Activation of latent tuberculosis could be prevented by preemptive INH treatment in certain patient populations with positive T-SPOT.TB results at baseline or during biologics treatment.

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We do not have any conflict of interest to declare.

D.H. and W.Z. contributed to the design of the study, data collection, analysis, and interpretation. F.B., Z.S., T.J., J.S., Q.Z., and T.Y. contributed to data collection and interpretation. L.S., H.Z., and W.Z. contributed to data interpretation and drafted the manuscript. X.W. and Y.Z. contributed to the design of the study and data analysis and critically revised the manuscript. Y.G. and Y.F. contributed to data collection and analysis. All authors read and approved the final manuscript.

REFERENCES
1. Braun J, Brandt J, Listing J, Zink A, Alten R, Golder W, Gromnic-


4. Mancarella L, Bobbio-Pallavicini F, Cuccarelli F, Falappone PC, Fer-


11. Mertens M, Singh JA. 2009. Anakinra for rheumatoid arthritis: a system-
atic review. J. Rheumatol. 36:1118–1125.

12. Settas LD, Tsimiriakas G, Vosvotekas G, Triantafyllidou E, Nicolaides P. 2007. Activation of latent tuberculosis could be prevented by preemptive INH treatment in certain patient populations with LTBI, including TNF antagonist-treated pa-

matoid arthritis: study in a population with a high prevalence of tubercu-


atic review. J. Rheumatol. 36:1118–1125.


