EXTENDED REPORT
Screening for latent tuberculosis infection: performance of tuberculin skin test and interferon-γ release assays under real-life conditions

S Kleinert,1 H-P Tony,1 K Krueger,2 J Detert,3 F Mielke,4 K Rockwitz,5 R Schwenke,6 G R Burmester,3 R Diel,7 M Feuchtenberger,1 C Kneitz8

ABSTRACT
Objectives To characterise optimal screening strategies for latent tuberculosis infection (LTBI) prior to the initiation of anti-tumour necrosis factor therapy.
Methods Patients in 62 German rheumatology centres were evaluated for LTBI. Each patient was screened with a tubercul skin test (TST) and one form of an interferon-γ release assay (IGRA), either TSPOT.TB (TSPOT) or Quantiferon TB Gold (QFT).
Results A total of 1529 patients with rheumato logical disease were tested with a TST, 844 with TSPOT and 685 with QFT. TST was positive in 11.3% (n = 173). The prevalence of LTBI was 8.0% when defined as a positive TST and no previous Bacille Calmette-Guérin (BCG) vaccination and 7.9% when based on a positive IGRA. Combining both estimates increased the prevalence of LTBI to 11.1%. Clinical risk factors for LTBI were found in 122 patients (34 with a history of prior TB, 81 close contacts and 27 with suggestive chest X-ray lesions). A compound risk factor (CRF) was defined as the presence of at least one of these three risk factors. Statistical analyses were conducted to examine the association between CRF and LTBI test outcomes. In multivariate analysis, TST was influenced by CRF (OR 6.2; CI 4.08 to 9.44, p < 0.001) and BCG vaccination status (OR 2.9; CI 2.00 to 4.35, p < 0.001). QFT and TSPOT were only influenced by CRF (QFT, OR 2.6; CI 1.15 to 5.98, p = 0.021; TSPOT: OR 6.7; CI 4.83 to 15.82, p < 0.001). CRFs and the agreement of TST and IGRA test results varied by rheumatological disease.
Conclusion LTBI test results in an individual patient need to be considered in the context of prior BCG vaccination and clinical risk factors. In patient populations with low rates of TB incidence and BCG vaccination, the use of both TST and IGRA may maximise sensitivity in detecting LTBI but may also reduce specificity.

INTRODUCTION
The introduction of tumour necrosis factor (TNF) antagonists was a milestone towards better disease control in rheumatoid arthritis (RA). However, it was soon evident that TNF inhibitor therapy was associated with an increased risk of reactivation of latent tuberculosis infection (LTBI).1 National guidelines regarding screening for LTBI were established based on patients’ history, clinical examination, chest X-ray and tuberculin skin test (TST) results. The effectiveness of recommendations based on TST testing was proved by a Spanish cohort of patients treated with anti-TNF agents.2 Cases of TB reactivation that occurred in this cohort after establishment of recommendations were mainly due to non-adherence to recommendations.3
Interferon-γ release assays (IGRAs) have shown superior results to TST in screening for TB exposure.4 Some national guidelines have recently favoured IGRA testing over TST for detection of LTBI.5–7 The US Centers for Disease Control suggest using IGRA and TST if risk for progression to active tuberculosis is increased.8 Nevertheless, there are still open questions concerning the performance of different test systems in immunocompromised patients, such as patients with rheumatic diseases on immunosuppressive therapy,9 particularly with respect to the performance of IGRAs under real-life settings in daily practice. We therefore conducted a multicentre study to compare the utility of IGRA and TST in LTBI screening in a large cohort of patients with rheumatic diseases receiving immunosuppressive therapy.

METHODS
Study design and patients
This was a prospective study in which patients eligible for anti-TNF treatment were successively screened for LTBI at 62 participating centres located throughout Germany according to standard of care and national recommendations. Ethical approval for data management was obtained from the Ethics Committee of the Charité – Universitätsmedizin Berlin and approval for secure data management procedures was obtained from the data protection officer.

LTBI screening
Patients were screened for LTBI according to national guidelines. The patient’s history of prior TB and close contact with TB patients was noted and Bacille Calmette-Guérin (BCG) vaccination status was documented. All patients underwent physical examination. Chest X-rays were obtained from 1409 patients. Interpretation regarding pulmonary lesions suggestive of latent or prior TB was based on the judgement of the local radiologist.

All patients received a TST and one type of IGRA, either TSPOT.TB (TSPOT) or Quantiferon TB Gold (QFT), depending on what was available in the corresponding laboratory. In accordance
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with contemporary guidelines for immunosuppressed patients, a TST with a diameter of ≥5 mm skin induration was considered positive. The cut-off for TSPOT positivity was ≥6 spots. At the time of testing, IGRA’s were mainly based on the two peptide antigens ESAT-6 and CFP-10.

Statistical analyses
Clinical risk factors for LTBI were defined as a history of prior TB (prior TB), close contact to a patient with TB (contact) or a chest x-ray suggestive of LTBI (CXR). A compound risk factor (CRF) was defined as the presence of at least one of these three risk factors.

Statistical analyses were performed using SPSS V.17.02 for Windows (SPSS, Chicago, Illinois, USA). Univariable regression analyses were used to calculate the influence of single risk factors; multivariable analyses used the variables CRF and BCG vaccination status to estimate the influence on test results. The results were reported as OR with CI. Cohen’s k was calculated for test agreement (higher values indicate greater agreement).

Lymphocyte count and prednisone dose at the time of testing were documented in a subset of patients, and regression analyses were used to calculate the influence of these factors on OR for a negative or indeterminate test result.

RESULTS
Patient characteristics
A total of 1609 patients eligible for anti-TNF treatment were successively screened for LTBI. Eighty patients were excluded from the main analysis due to indeterminate IGRA results, leaving a total of 1529 patients. All of the patients had rheumatic diseases, including rheumatoid arthritis (RA; n=852, mean DAS28 4.35±1.88, median disease duration 7.4 years), ankylosing spondylitis (AS; n=294, median disease duration 7.5 years), psoriatic arthritis (PsA; n=215, median disease duration 6.9 years), undifferentiated spondyloarthropathy (SpA; n=92, median disease duration 3.6 years) and various other rheumatologic disorders (n=76, median disease duration 1.3 years). More than half of the patients (61.5%) were women. Of the 1529 patients, 204 (13.5%) had been vaccinated with BCG. Patient characteristics and risk factors by TST and IGRA status are shown in figure 1.

Estimation of LTBI prevalence
Positive TSTs were recorded in 11.3% (n=173) of patients (figure 1). The prevalence of LTBI was 8.0% (n=123) as estimated by a positive TST in patients without previous BCG vaccination or 7.9% (n=120) based solely on a positive IGRA (8.3% in the TSPOT group and 7.3% in the QFT group). The estimated prevalence of LTBI increased to 11.1% if LTBI was defined as meeting either of these criteria. Only 4.3% of patients (n=66) were positive for both TST and IGRA.

Influence of risk factors on test results
Clinical risk factors for LTBI were found in 122 patients (34 with a history of prior TB, 81 close contacts and 27 with a chest x-ray suggestive of LTBI). A CRF was defined as the presence of at least one of these risk factors. Risk factors were more common in patients with positive LTBI screening results (table 1).

Statistical analyses were used to examine the impact of risk factors on screening results. For TST and TSPOT, all single risk factors influenced test results while, for QFT, only the risk factor prior TB had an influence on test results (see table S1 in online supplement).

We further analysed the influence of CRF and BCG vaccination status on the LTBI screening results. In multivariate analyses, patients with CRF had a 6.2-fold increased risk for positive TST in the total population (table 2). CRF was associated with a 2.6-fold increased risk for a positive QFT and an 8.7-fold increased

Table 1 Age, BCG vaccination status and clinical risk factors by LTBI screening results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TST (n=1529)</th>
<th>QFT (n=685)</th>
<th>TSPOT (n=844)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean years (±SD)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Positive (n=173)</td>
<td>52.0±12.1</td>
<td>51.6±14.1</td>
<td>56.4±12.7</td>
</tr>
<tr>
<td>Negative (n=1356)</td>
<td>58.4±12.7</td>
<td>51.0±13.9</td>
<td>59.5±12.9</td>
</tr>
<tr>
<td>BCG,† n (%)</td>
<td>50 (28.9)</td>
<td>51 (10.0)</td>
<td>127 (20.0)</td>
</tr>
<tr>
<td>Positive (n=50)</td>
<td>18 (3.2)</td>
<td>12 (2.0)</td>
<td>30 (4.7)</td>
</tr>
<tr>
<td>Negative (n=635)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact,‡ n (%)</td>
<td>29 (16.8)</td>
<td>3 (6.0)</td>
<td>11 (15.7)</td>
</tr>
<tr>
<td>Positive (n=70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=774)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF,§ n (%)</td>
<td>15 (8.7)</td>
<td>3 (6.0)</td>
<td>7 (11.1)</td>
</tr>
<tr>
<td>Positive (n=27.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=74)</td>
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</tr>
</tbody>
</table>

Data represent patient numbers (%) positive for vaccination or stated risk factor unless otherwise indicated.

CRF was defined as the presence of at least one of the following three clinical risk factors: history of prior TB, close contact and chest x-ray suggestive of LTBI (CXR).

‡Chest x-ray was performed in 88.2–97.1% of patients in each group.

§BCG, Bacille Calmette-Guérin; CRF, compound risk factor; LTBI, latent tuberculosis infection; QFT, Quantiferon TB Gold; TSPOT, TSPOT.TB; TST, tuberculin skin test.

Figure 1 LTBI screening results in 1529 German rheumatology patients preparing to start treatment with tumour necrosis factor (TNF) inhibitors. TST+, tuberculin skin test positive; IGRA+, interferon-γ release assay positive. BCG +/–/uk: BCG vaccinated/not vaccinated/vaccination status unknown. Data represent patient numbers (%).
risk for a positive TSPOT. Univariate analyses revealed very similar results (data not shown). Test results were independent of the time interval between TST and IGRA (p=0.21; data not shown).

Influence of risk factors in disease subgroups
Multivariate regression analyses of patients with RA (n=852) showed that CRF influenced TST (OR 8.44, CI 4.71 to 15.14, p<0.001) and TSPOT (OR 10.69, CI 5.01 to 22.81, p<0.001) but not QFT (OR 0.69, CI 0.08 to 4.66, p=0.625). In patients with PsA (n=215), CRF had no significant influence on TSPOT (OR 3.54, CI 0.56 to 22.33, p=0.179) or TST (OR 1.98, CI 0.61 to 6.38, p=0.253) and could not be calculated for QFT due to the absence of patients with CRF and positive QFT. In patients with AS or SpA (n=485), CRF influenced TST (OR 7.88, CI 3.44 to 18.52, p<0.001), but not QFT (OR 7.29, CI 2.87 to 18.52, p<0.001) and TSPOT (OR 7.83, CI 2.76 to 17.89, p<0.001). Complete disease subgroup data are shown in table S2 in the online supplement. Univariate results were very similar.

Agreement between TST and IGRA
The patients’ characteristics in the concordant and discordant subgroups are shown in table 3. Concordance of TST and IGRA was 89.5% but Cohen’s κ was 0.40 (p=0.001), indicating that the agreement was only fair to moderate, although significant. Concordance was 87.6% (κ=0.34, p<0.001) between TST and QFT and 91.1% (κ=0.44, p<0.001) between TST and TSPOT. Agreement was significant in the total population and all disease subgroups except those with PsA (see table S3 in online supplement). The subgroup of TST-positive patients with a skin induration ≥15 mm (n=71) included 27 that were not BCG vaccinated and were IGRA-negative.

Influence of BCG vaccination
Of the 204 patients who were BCG vaccinated, 50 were TST-positive and 14 were IGRA-positive (table 1). BCG status influenced TST but not QFT and TSPOT (table 2). Concordance was lower in the BCG vaccinated group (79.9%, p=0.27, p<0.001) than in the non-vaccinated group (n=1503; 91.0%, p=0.45, p<0.001).
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dates are comparable to those from a German study in which an
LTBI prevalence of 7.2% (defined by QFT testing) was reported
among healthcare workers.60

Owing to the lack of a gold standard for LTBI testing, we
calculated the association between positive tests results and
known LTBI clinical risk factors (prior TB disease, contact his-
tory and chest x-ray lesions suggestive of latent or prior TB) to
help assess the performance of these testing systems. All the risk
factors influenced the test results for TST and TSPOT whereas,
for QFT, only prior TB exerted an influence. CRF influenced
the results for all three of the tests but had less influence on
QFT than on the other test systems. It should be noted that our
study was not designed to compare the two IGRA (QFT and
TSPOT) directly, so the correlation of clinical risk factors with
test results provided the only means of estimating their relative
utility. By this standard, TSPOT appears to perform better than
QFT due to its greater correlation with known LTBI risk factors.
Nevertheless, we cannot exclude the possibility that a poorer
 correlation with clinical risk factors is due to a higher specificity
rather than a lower sensitivity. A better understanding of the
relative merit of QFT versus TSPOT will require head-to-head
tests under real-world conditions.

Important differences in the influence of risk factors on
screening results and concordance of TST and IGRA tests were
observed among disease subgroups. In the RA subgroup, QFT
was not influenced by CRF. In the PsA subgroup, CRF did not
influence any test and concordance of TST and IGRA was not
significant whereas, in the SpA group, the influence of CRF on
all tests was high. These discrepancies could indicate unknown
confounders due to immunological disease characteristics or to
the intensity and duration of previous immunosuppressive ther-
apieties. Our findings suggest that optimal LTBI screening strate-
gies for patients with PsA may be particularly elusive.

A subgroup analysis of 507 patients with appropriate data did
not reveal a statistically significant influence of prednisone use
or lymphopenia on test results. Data on previous therapies and
concomitant disease-modifying antirheumatic drugs were not
recorded completely and therefore were not analysed.

Our study has some limitations that should be considered
when evaluating the findings. The lack of a gold standard for
LTBI diagnosis means that we cannot directly determine the
sensitivity or specificity of the LTBI tests, nor were we able
to obtain subsequent clinical information on the development of
TB in these patients. In addition, the multicentre nature of our
study, which involved the use of many different clinicians admin-
istering tests and laboratories assessing results, may add some
unknown confounders but definitely resembles real-life condi-
tions. Finally, these results were obtained in a country with a
low incidence of TB (approximately four active TB cases per 100
000 according to 2010 World Health Organization figures) and
low BCG vaccination rate (13.3% in our study). Accordingly, the
conclusions may not necessarily apply to countries with higher
rates of either TB or vaccination.

The results from our study and others lend some support to
the suggestion that IGRA may be preferable to TST when
diagnosing LTBI in populations with a low incidence of TB and
a high vaccination rate.6 A single-centre study of 142 Swiss
patients with autoimmune diseases, 83% of whom had been
vaccinated with BCG, reported a 12% positive rate for QFT and
32% for TST; the tests showed low concordance (64%) with
poor agreement (κ =0.17), and risk factors exerted a much stron-
ger influence on QFT than on TST.11 It is likely that the high
vaccination rate reduced the specificity of TST in this popula-
tion. Our study and others, including a pooled analysis of data
from multicentre phase III studies of rheumatology patients
(n=2282),12 have shown higher concordances between TST and
IGRA (87–89.5%), but κ values indicate only fair to moderate
agreement (ranging from 0.22 in the pooled phase III studies
with 38.5% BCG vaccinated to 0.40 in our study with 13.3%
BCG vaccinated). Furthermore, our data indicate that the con-
cordance of TST and IGRA is lower in the BCG vaccinated
group. As expected, IGRA test results were not influenced by
prior BCG vaccination, suggesting a higher specificity for diag-
osing LTBI in these patients. The higher specifity in BCG
vaccinated patients and the ease in doing a blood test without
requiring the patient to return to the clinician’s office, as for skin
tests, has contributed to the success of IGRA.

Although IGRA may have some advantages over TST in cer-
tain settings (eg, populations with high vaccination rates), our
study also raises important concerns about this testing method
for patients with rheumatological diseases evaluated prior to biolog-
ical therapy. Although LTBI testing based on TST has been pro-
ved to be effective in patients with rheumatological diseases prior to
anti-TNF treatment,2 in our study there was only a partial over-
lap in the populations judged to have LTBI based on TST and
IGRA. Special concern needs to be raised regarding the subgroup
of 71 TST-positive patients with a skin induration ≥15 mm, usu-
ally considered a sign of true infection. Of this group, 27 (38%) were
IGRA-negative and had not been vaccinated with BCG. This
finding suggests that the real-world effectiveness of IGRA in
diagnosing LTBI and therefore preventing TB reactivation in
patients prior to biological therapy requires further study. TST
could have the additional benefit of detecting latent infection
with atypical mycobacteria whereas IGRA does not. However,
the impact of anti-TNF treatment on these infections needs
further elucidation.

We conclude that, in patient populations with low rates of
TB incidence and BCG vaccination, employing both TST and
IGRA may maximise sensitivity in detecting LTBI but may also
reduce specificity. From the clinician’s perspective, it is impor-
tant to bear in mind that false positive or false negative tests can
occur with either IGRA or TST. Accordingly, test results in each
individual patient should be considered in the context of BCG
vaccination status and probable LTBI risk as defined by clinical
risk factors.

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recommendations to prevent reactivation of latent tuberculosis infection in patients