A B S T R A C T

Introduction: It is universally agreed that screening for latent tuberculosis infection prior to biologic therapy is necessary, especially in endemic areas such as Hong Kong. There are still, however, controversies regarding how best to accomplish this task. The tuberculin skin test has been the routine screening tool for latent tuberculosis infection in Hong Kong for the past decade although accuracy is far from perfect, especially in patients who have been vaccinated with Bacillus Calmette–Guérin, who are immunocompromised, or who have atypical mycobacterium infection. The new interferon-gamma release assays have been shown to improve specificity and probably sensitivity. This study aimed to evaluate agreement between the interferon-gamma release assay and the tuberculin skin test in the diagnosis of latent tuberculosis infection in patients with arthritic diseases scheduled to receive biologic agents.

Methods: We reviewed 38 patients with rheumatoid arthritis, psoriatic arthritis, or spondyloarthritis at a local hospital in Hong Kong from August 2013 to April 2014. They were all considered candidates for biologic agents. The patients underwent both the interferon-gamma release assay (ASACIR.TB; A.TB) and the tuberculin skin test simultaneously. Concurrent medications were documented. Patients who tested positive for either test (ie A.TB+ or TST+) were prescribed treatment for latent tuberculosis if they were to be given biologic agents. All patients were followed up regularly for 1 year and the development of active tuberculosis infection was evaluated.

Results: Based on an induration of 10 mm in diameter as the cut-off value, 13 (34.2%) of 38 patients had a positive tuberculin skin test. Of the 38 patients, 11 (28.9%) also had a positive interferon-gamma release assay. The agreement between interferon-gamma release assay and tuberculin skin test was 73.7% (kappa=0.39). Six patients were TST+/A.TB−. When positive tuberculin skin test was defined as an induration of 5-mm diameter, the agreement between the two tests improved with a kappa value of 0.47. In that case, half of the patients had a positive tuberculin skin test; among them, nine were TST+/A.TB−. Only one was TST−/A.TB+. Subgroup analysis showed that the agreement between both tests improved further (kappa=0.69) in patients not taking a concurrent systemic steroid. For patients prescribed systemic steroid, the agreement was only slight with a kappa value of 0.066. Finally, none of the 38 patients, of whom 32 had an exposure to biologic agents, developed active tuberculosis during the 1-year follow-up period.

Conclusion: In a tuberculosis-endemic population, although 10-mm diameter induration is the usual cut-off for a positive tuberculin skin test, the level of agreement between the interferon-gamma release assay and tuberculin skin test improved from fair to moderate when the cut-off was lowered to 5 mm. A dual testing strategy of tuberculin skin test and interferon-gamma release assays appeared to be effective and should be pursued especially in patients who are on systemic steroid therapy. Nonetheless, the issue of potential overtreatment is yet to be evaluated.

New knowledge added by this study
- In Hong Kong, a tuberculosis-endemic area, the level of agreement between tuberculin skin test (TST) and interferon-gamma release assay (IGRA) for detecting latent tuberculosis infection was only fair in arthritis patients scheduled to receive biologic therapy.
- Although 10 mm is the cut-off for positive TST according to the local guideline, the level of agreement between the two tests improved when a 5-mm cut-off was used.

Implications for clinical practice or policy
- Dual testing strategy with TST and IGRA appeared to be effective and should be employed, especially in patients who are prescribed systemic steroid therapy.
Introduction

The advent of biologic agents has revolutionised the treatment of patients with rheumatoid arthritis (RA), psoriatic arthritis (PSA), and spondyloarthritis (SPA). The outcome is now greatly improved. This, however, comes at the price of a clear heightened risk of active tuberculosis (TB) as a progression of latent TB infection (LTBI). Therefore, it is universally agreed that screening for LTBI prior to biologic therapy is necessary, especially in endemic areas such as Hong Kong. Unfortunately, there remains controversy regarding how best to accomplish this task.

The tuberculin skin test (TST) has been the routine screening tool in Hong Kong for the past decade. It's accuracy, however, is far from perfect, especially in patients who have been vaccinated with Bacillus Calmette–Guérin (BCG), are immunocompromised, or have been infected with atypical mycobacterium. Recently, interferon-gamma release assays (IGRAs) that measure interferon-gamma secretion in response to Mycobacterium tuberculosis—specific antigens have become available to detect LTBI. They have been shown to offer improved specificity and probably sensitivity. Other shortcomings of the TST, such as the need for return visits and reader variability, are also overcome. One of the IGRAs, the ASACIR.TB (A.TB; Haikou VTI Biological Institute, Hainan, China), has shown encouraging results in a large-scale clinical trial conducted in China and might be more appropriate in Chinese populations.

On the other hand, IGRAs are not flawless. The rate of indeterminate results has been reported to be as high as 40%. The immunocompromised state of arthritic patients will also induce a depressed response to a T-cell reaction leading to an inaccurate IGRA result. There are recent data to argue that an IGRA alone is insufficient to identify all patients at risk. Furthermore, various studies have suggested very different concordance figures between the IGRA and the TST, likely as a result of heterogeneity (eg differing background TB prevalence, variable immunosuppressive therapies, or underlying BCG status).

This study aimed to evaluate the agreement between the IGRA and the TST in the diagnosis of LTBI in patients with arthritic diseases scheduled to receive biologic agents in Hong Kong.

Methods

Patients

We reviewed 38 patients with RA, PSA, or SPA at a local hospital in Hong Kong from August 2013 to April 2014. They were diagnosed according to the 2010 classification criteria for RA of the American College of Rheumatology/European League Against Rheumatism, the Classification Criteria for Psoriatic Arthritis, and the Assessment of SpondyloArthritis international Society classification criteria, respectively. Patients were included if they were considered candidates for biologic agents. Concurrent medications were documented. Candidates were excluded if they had active TB infection, a history of incomplete TB treatment, or no measured induration. Patients underwent both the IGRA and the TST simultaneously. Those who tested positive for either test and who were due to be prescribed biologic agents were given latent TB treatment with isoniazid or rifampicin for 9 months. All patients were followed up regularly for 1 year and the development of active TB infection was evaluated.
evaluated. This study conforms to the provisions of the Declaration of Helsinki and the guidelines of the local ethical committee. Informed consent was considered not necessary due to the retrospective nature of the study.

**Tuberculin skin test**

The TST was performed by rheumatologists. A 0.1 mL of 2-TU PPD (tuberculin units of purified protein derivative) was injected intradermally into the volar aspect of the forearm. The indurations were measured in millimetres after 48 hours of inoculation by rheumatologists who were blinded to the IGRA results. According to the local guideline, induration of ≥10 mm was considered a positive result of LTBI.3

**Interferon-gamma release assay**

We performed the A.TB IGRA test (Haikou VTI Biological Institute) in all study patients. This assay employs Haikou VTI’s patented technology (US patent number 7754219) that enables intracellular delivery of the full-length protein CFP-10 and the antigen ESAT-6 to stimulate antigen-specific T-cells through the major histocompatibility complex class 1 pathway.12,13 The assay was performed according to the user manual. In brief, negative control phosphate buffered saline (N), positive control concanavalin A (P), and the TB stimulators CFP-10 and ESAT-6 (T) were mixed with fresh heparinised whole blood and incubated for approximately 24 hours at 37.8°C. The plasma was collected and stored at 48˚C for up to 2 weeks. The interferon-gamma level in the plasma was then determined by enzyme-linked immunosorbent assay. If N was <0.5 IU/mL and (T-N)/(P-N) ≥0.6, or if N was ≥0.5 IU/mL and (T-N)/(P-N) ≥0.85, the test was considered to be positive (A.TB+), otherwise the result was negative (A.TB−).

**Statistical analysis**

Descriptive statistics were presented as frequencies and means ± standard deviations as appropriate. The concordance between TST and IGRA was evaluated by the Cohen’s weighted k statistic. A kappa value of >0.6 represents substantial agreement, 0.41 to 0.60 moderate agreement, 0.21 to 0.40 fair agreement, and <0.21 slight agreement. The concordance was sub-analysed in patients with and without prednisolone.

**Results**

The demographic and clinical characteristics of 38 patients are summarised in Table 1. All patients were residents of Hong Kong. Half of the patients were prescribed systemic steroid therapy that comprised prednisolone at a dose of 2.5 mg daily to 15 mg daily. All except three patients with SPA were on various conventional disease-modifying antirheumatic drugs.

The results of the concomitant TST and IGRA are shown in Table 2. Of the 38 patients, based on an induration of 10-mm diameter as the cut-off value, 13 (34.2%) had a positive TST, 11 (28.9%) had a positive IGRA. The agreement between A.TB IGRA test and TST was 73.7%. Six patients were TST+/A.TB− and four were TST−/A.TB+. Subgroup analysis showed that four of the six divergent TST+/A.TB− results were in patients on systemic steroid, and only three patients with systemic steroid were A.TB+ versus eight patients without. When positive TST was defined as an induration of 5-mm diameter, half of the patients had a positive TST, among them nine were TST+/A.TB−. Only one was TST−/A.TB+. In patients prescribed a systemic steroid, with 5-mm induration as positivity, TST missed no patients who had positive IGRA.

Analysis of the agreement between the two tests, assessed by kappa statistic, showed only fair strength in our study, with a kappa value of 0.39 (Table 3). When a 5-mm induration was taken as a positive TST, however, the agreement between the two tests improved to moderate with a kappa value of 0.47. Subgroup analysis revealed that the agreement between both tests improved further (kappa=0.57) in patients not taking a concurrent systemic steroid. For patients taking a systemic steroid, the agreement was only slight (kappa=0.066). Again, the agreement of the TST and IGRA was substantial (kappa=0.69) in patients not on systemic steroid therapy when a 5-mm induration was regarded as positive.

At the end of the study, 32 of the initial 38 patients had received biologic agents. None of them
developed active TB during the 1-year follow-up period.

Discussion

In clinical practice there is no gold standard test for diagnosing LTBI. Both IGRA and TST have strengths and weaknesses. In a meta-analysis performed on an unselected population, the specificity of IGRA was 99% in a non-BCG-vaccinated population and 96% in a BCG-vaccinated population. The specificity of the TST was 97% in a non-BCG-vaccinated population but dropped to 59% in a BCG-vaccinated population. In addition to BCG history, comparison between the two tests must take into account the underlying disease, the immunosuppression status, and the background TB burden of the population being screened.

In this study, we found only a fair agreement (kappa=0.39) between the results of TST and A.TB IGRA in arthritis patients from a TB-endemic area. Some studies have also reported a discrepancy between the two tests in countries with intermediate TB burden. There was also discordance between the two tests in LTBI diagnosis among individuals infected with the human immunodeficiency virus. The patients on prednisolone in our study had a lower rate of positivity for both tests. It seems intuitive to assume that an immunosuppressed state will induce a depressed response to a T-cell reaction. In the literature, a systematic review showed that both positive IGRA and positive TST results were significantly influenced by immunosuppressive therapy.

The Hong Kong guideline for the TST cut-off value for LTBI diagnosis before anti-tumour necrosis factor treatment is an induration of >10 mm. In the current study, we showed that the TST cut-off value that achieved better agreement between IGRA and TST results was 5 mm. If we cannot rely on IGRA to diagnose LTBI, it may be more appropriate to lower that TST cut-off to 5 mm. We also showed that our approach to LTBI screening with both TST and IGRA was successful in preventing the development of active TB in patients who would receive biologic therapy. This dual testing strategy might be especially applicable to patients on systemic steroid, as they are at higher risk of developing active TB and the two test results are more discordant. The consequent improved sensitivity will invariably lower the

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<th>TABLE 2. Tuberculin skin test (TST) and A.TB* test results</th>
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<td>All patients, with 10-mm induration as cut-off (n=38)</td>
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<td>TST+/A.TB+</td>
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<td>Patients on prednisolone (n=19)</td>
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<td>All patients, with 5-mm induration as cut-off (n=38)</td>
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* A.TB denotes ASACIR.TB interferon-gamma release assay

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<th>TABLE 3. Agreement between the two tests</th>
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specificity and cause a potential overtreatment. In these high-risk settings, however, it is reasonable to favour sensitivity in screening for LTBI.

Only five patients could give a definite history of BCG vaccination. For other patients, such information was uncertain. While this might reflect the local clinical situation, it is one of the limitations of the present study. Despite the mechanistic similarity, because of the different interpretation methods employed for the test results and the lack of comparative trials of the performance of A.TB IGRA and other IGRAs, the conclusions drawn from the current study may not be applicable to patients who are given a different IGRA. Further studies using individual IGRAs may be warranted to address the same question.

**Conclusion**

In arthritis patients in a TB-endemic population, the level of agreement between TST and A.TB IGRA for detecting LTBI was only fair. Although 10 mm is the usual cut-off for TST, the level of agreement between the two tests improved from fair to moderate when a 5-mm cut-off was used. A dual testing strategy with TST and IGRA appeared to be effective and should be pursued, especially in patients who are prescribed a systemic steroid. The issue of potential overtreatment is yet to be evaluated.

**Declaration**

All authors have disclosed no conflicts of interest.

**References**