Interferon γ -release assays for detecting latent tuberculosis infection in patients scheduled for anti-TNF α therapy

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Abstract

Screening for, and treatment of, latent tuberculosis infection (LTBI) before anti-tumour necrosis factor α therapy has been shown to decrease the incidence of active tuberculosis by more than 80% and is recommended before initiation of treatment. In the absence of a 'gold standard' test for LTBI, current screening involves taking a clinical history of risk factors, chest radiograph and tuberculin skin test. Alternative cellular immune-based screening tests have been developed to detect *Mycobacterium tuberculosis* infection. This paper summarises the current position and advances in the use of newer screening strategies for LTBI.

Background

Tumour necrosis factor α (TNF α) is a proinflammatory cytokine with an important role in the pathogenesis of a variety of inflammatory conditions including rheumatoid arthritis, psoriasis and inflammatory bowel disease (IBD).¹ The anti-TNF α agents infliximab and adalimumab are currently licensed for use in Crohn's disease, and the former additionally for ulcerative colitis. They are high-affinity, neutralising monoclonal antibodies directed against TNF α . While efficacious in IBD,² these agents also block TNF α -mediated biological processes which protect humans from, or ameliorate the effect of, a number of other conditions. Foremost among these is the now well-documented risk of serious infection in patients treated with anti-TNF α , in particular the rapid development of active tuberculosis (TB).³

About one-third of the world's population is thought to be infected with *Mycobacterium tuberculosis*. This estimate is based on positive tuberculin skin

test (TST) results, such as the Mantoux or Heaf tests, in healthy, asymptomatic people. The TST uses a purified protein derivative of *M. tuberculosis* as the test antigen; and detects T-cell-mediated immunity through the development of an area of induration in the skin, usually after 48–72 h. What happens in the skin may not reflect immunity elsewhere; and so this is not a particularly precise measure of the risk of an individual developing active, symptomatic disease. Five to ten per cent of TST positive individuals will progress from this clinically asymptomatic state (here termed latent TB infection (LTBI)) to active disease.⁵

It is not really understood why some people are healthy for their entire lives and others develop clinical disease. However, the use of anti-TNF α agents increases the risk of LTBI progressing to active TB by approximately fivefold.⁶ This is often rapid and aggressive, occurring at a median of 12 weeks from anti-TNF α initiation in the earliest reports.⁴ It is likely to be a direct effect of the anti-TNF α agents themselves.⁷ Interestingly, there is a suggestion that infliximab and adalimumab are more likely to promote active TB than etanercept; and that disease occurs earlier during anti-TNF α therapy with the first two agents.^{4 8 9} However, overall, all such drugs should be assumed to greatly increase the risk of active TB over background rates.

The United Kingdom is generally a country with a low incidence of TB. In 2008 there were approximately 8700 new cases of active disease reported. While the majority of these are in non-UK born individuals resident in metropolitan areas, it

is not possible to accurately predict at an individual level who has LTBI, and therefore is at risk of developing active TB.¹⁰ The greater use of both immunosuppressive agents, such as azathioprine, methotrexate and oral steroid therapy—which also can increase the risk of active TB,¹¹ and anti-TNF α agents argues for a systematic approach to screening individuals with IBD who are being considered for treatment with anti-TNF α agents.

Conventional screening regimen

In some studies, screening for, and treatment of, LTBI before anti-TNF α treatment has decreased the incidence of active TB by more than 80%.¹² It is recommended by the British Thoracic Society.⁶ As discussed above, patients with LTBI are, by definition, asymptomatic from their *M tuberculosis* infection, though may have many systemic symptoms associated with IBD that can mimic active TB. The diagnosis of LTBI is based on a combination of assessments, all of which try to determine the chance that a given person may have been infected with *M tuberculosis* at some point in their lives, and if at all possible select those who are at greatest risk of progressing to active disease. At present there is no ‘gold standard’ test to diagnose LTBI.

Current screening involves taking a clinical history focusing on risk factors for TB infection, obtaining a chest radiograph within 3 months of the planned start of anti-TNF α therapy, and performing a TST (table AI). However, in many BCG-vaccinated individuals, the TST is positive—giving it a low specificity in countries such as the UK, where BCG is still widely given.¹³ It also has poor sensitivity in patients receiving immunosuppressive treatment; and is not recommended for use in such situations.⁶ As many patients with IBD will have received immunosuppression that can impair T-cell responses (defined by recently published ECCO guidance¹¹ as corticosteroid dose >20 mg/day in the past month, or other agents in the past 3 months), the TST is yet more limited in its value.¹⁴ Other problems associated with TST are highlighted in table AII. Although the BTS guidelines date from only 5 years ago, newer tests are now widely available which provide further options for TB risk assessment before anti-TNF drugs are used.

Interferon γ (IFN γ) release assays

Alternative cellular immune-based screening tests have been developed to detect *M tuberculosis* infection using *M tuberculosis* antigens (such as ESAT-6 and CFP-10, plus also TB 7.7 in the QuantiFERON-TB Gold In Tube assay) which were lost from the genome of BCG when it was developed as a live, attenuated vaccine. In BCG vaccinated populations such as that in the UK, these tests are generally, therefore, more specific than the TST.^{15 16} They do, however, have some cross-reactivity with other mycobacteria; though apart from *M kansasii*, these are usually of little clinical consequence outside specific clinical settings.

IFN γ release assays (IGRAs) are based on the principle that the T cells of individuals who have been exposed to TB (and hence its antigens) will produce IFN γ when they are rechallenged with mycobacterial antigens contained within the test kit.⁵ Two commercial assays are currently available. Both are blood-based but use different test platforms. The QuantiFERON-TB Gold In Tube (QFT-IT) (Cellestis Limited, Carnegie, Australia) uses an overnight whole-blood ELISA; while T SPOT-TB (Oxford Immunotec, Oxford, UK) measures IFN γ production from a fixed number of blood mononuclear cells via an ex vivo overnight enzyme-linked immunospot (ELISPOT) assay.

Both tests have a number of advantages over the TST: results are usually available within 24–48 h, they are more specific than the TST in BCG-vaccinated populations; no follow-up visit is required (as a negative result can often be dealt with over the phone, or at the next clinic appointment) and operator variability in reading and interpreting the results is reduced.⁵ Both tests are approved by the US Food and Drug administration and are included in the UK TB NICE guidelines.^{15 16} However, although similar they are not the same test. Hence, while the term ‘IGRA’ is used to describe both assays, they may provide slightly different results when used in routine clinical practice.¹⁷ This is not surprising, if one considers that although they both measure the amount of IFN γ produced after antigen-specific T-cell stimulation, QFT-IT uses a fixed volume of blood, while T SPOT-TB estimates cytokine production from a fixed number of mononuclear cells. Hence in circumstances of T-cell depletion (as may be seen in lymphopenic states associated with immunosuppression), the latter test is likely to be somewhat more sensitive.

Clinical use of IFN γ release assays

We are only beginning to understand how to use these tests in routine practice. Table AIII outlines the information generally required by clinicians. This is based on data supplied by an immunology laboratory, together with company literature. Some laboratories may offer only one type of assay; and the clinician needs to understand the limitations of the particular test that they have available for use. What is obvious from table AIII is that an ‘overnight’ test result is often not available the next day; and it is recommended that the clinician liaises closely with their laboratory team to determine how samples from particular clinics, where patients with IBD are being assessed for anti-TNF α therapy, will get to the laboratory at a time when they can be routinely processed. Our personal experience is that Friday clinics are a problem in this regard.

It is important to remember that a positive test result does not indicate active TB disease, or even predict whether or not that individual (if they are asymptomatic) is going to develop active disease in the future. It is a test of host immune response to specific

mycobacterial antigens. As mycobacteria can remain for a long time in tissue sites, even subjects who have had previous treatment for TB years earlier may be persistently IGRA positive for the rest of their lives. Thus, IGRAs in their current form cannot be used to monitor treatment response. It is strongly recommended that results are discussed with suitably experienced TB clinicians (see later).

Each time that a test is run on a blood sample, a negative control (which contains no stimulating antigen) and a positive control (with mitogen) are also tested against the blood sample. Indeterminate results occur when either the negative control records a positive result, (ie, the blood sample's T cells release IFN γ without specific stimulation) or the mitogen does not stimulate the cells as expected (and no IFN γ is produced). Indeterminate results are particularly common in the populations where a clear-cut result would be most helpful, such as the immunosuppressed, patients in an intensive care unit, children and older adults. Generally, a high negative result tends to arise from an individual's personal factors such as place of birth (often in a TB endemic country); while low mitogen may arise from poor sample preparation or qualitative defects in the lymphocyte response.

Indeterminate results are generally more common with QFT-IT, though usually occur at a maximum frequency of 10% of samples. T SPOT-TB results are given as the number of spot-forming units counted on the test plate. An indication of the sensitivity of this test is that a positive result is six or more spots over the background (taken to be the negative control). Although most positive results are considerably above this value, some score between five and seven spots (the former being just negative, the latter just positive). These results are termed 'borderline' and should be repeated, or further clinical risk assessment performed and a decision taken on whether or not to offer treatment for LTBI.

Cost effectiveness of IGRAs

The choice of assay used is often based on the population being tested, the indication for testing and local availability. However, IGRAs are a new test, and need to be evaluated for efficacy and cost effectiveness. Each test costs between £30 and £100 to perform, with QFT-IT generally being at the lower end of the range, as it is a lot less labour intensive than T SPOT-TB.

Health economic evaluations have been undertaken in a variety of TB screening settings (eg, contact tracing, occupational health, new entrant screening) and using various modelled scenarios. Generally, screening for LTBI using TST followed by either T-SPOT TB or QFT-IT is less costly than using TST alone, independent of the TST cut-off point used, as it prevents overuse of TB chemoprophylaxis caused by increased

false-positive results of the TST in BCG vaccinated subjects.¹⁹ In addition, Hardy *et al*²⁰ found that for immigrants from high-risk countries QuantiFERON-TB Gold blood testing followed by chest x-ray examination is feasible for TB screening, cheaper than screening using the current NICE guidance and identifies more cases of LTBI.

As discussed above, the picture changes in immunosuppressed populations where IGRAs *may* more accurately identify truly *M tuberculosis* infected patients that the TST would have missed. Hence it has been proposed that a single step IGRA (here using a positive T-SPOT TB result) may reduce the burden of disease and the costs associated with reactivation of TB by giving preventive TB treatment to all such patients.¹⁹

Current guidelines

At present there is little UK guidance that incorporates IGRA testing in subjects having pre- anti-TNF α assessment. NICE are in the process of reviewing the use of IGRAs for detecting latent TB (2010). The draft guidance suggests that IGRAs should be carried out in immunocompromised patients where possible but that screening procedures vary depending on experience and availability of testing (NICE 2010). Recently published Swiss recommendations²¹ suggest that screening for latent TB before anti-TNF α should be based on a combination of history of risk factors, chest x-ray and IGRA. TST is not recommended owing to the limitations discussed above. This approach deviates somewhat from the BTS recommendations in that the latter placed great emphasis on a clinical assessment, which balanced the annual risk of developing active TB (using country of birth, and age) against the chance of hepatotoxicity on treatment for LTBI.⁶ The proposed IBD biologics audit, which will initially run along side the third national IBD audit, and then become a wider, long-term register, should provide insight into UK screening practice and enable evaluation of the cost effectiveness of IGRA testing in the IBD population.

The authors recently produced a pre-anti-TNF α screening pathway for UK patients with IBD (figure 1). This took the pragmatic view that some element of TB risk assessment would be performed by the IBD team; but that it was better for patients to be referred on to the TB service if there were any doubt. This simple strategy appears to work well in practice (Greveson *et al* 2010—abstract DDW, unpublished); and so far has not led to a major clinical workload for either IBD or TB teams. This to some extent relies on relatively low numbers of high-risk patients, and also the assumption that an IGRA will detect all those subjects who have LTBI (ie, that it is 100% sensitive). As discussed earlier, these assumptions are not necessarily consistently true, though the protocols can take account of these problems. It is now being rolled out to other specialist disciplines using anti-TNF α agents.

If TB suspected during anti-TNF treatment: Refer to TB service for assessment +/- TB treatment. Consider benefit/risk of continuing anti-TNF treatment

Note: Indeterminate = test failure; Equivocal = borderline result ie close to cut off positive/negative result

Figure 1 Suggested screening protocol for tuberculosis (TB) infection before anti-tumour necrosis factor α (anti-TNF α) treatment. CXR, chest x-ray; IGRA, interferon γ release assay.

The future of TB screening in IBD

Apart from the debate about the clinical- and cost-effectiveness of IGRA in a population with a low incidence of TB, such as many parts of the UK, other areas of contention include the following: (a) should we carry out repeat screening on subjects maintained on anti-TNF α therapy? And if so, how often? Is yearly re-evaluation too frequent? (b) Once a patient stops anti-TNF α agents, how fast does the increased risk of TB activation fall? Or does it remain

at the same level if any form of immunosuppression is continued? (c) When does the risk of TB decline after anti-TNF? and finally (d) If a patient needs to start LTBI treatment, when can they safely start anti-TNF α agents? For this last point there is some guidance, as the BTS have recommended that treatment for LTBI is completed before starting anti-TNF α agents. However, depending on the severity of the IBD, this may be unrealistic—and even then, anti-TB drug regimens are far from 100% protective.

Key messages

- ▶ IFN γ release assays (IGRAs) are new blood tests which detect mycobacterial infection (usually due to M tuberculosis). They cannot distinguish between active, latent or treated TB.
- ▶ There are two commercial kits currently available which use different detection methodology: QuantiFERON TB Gold In Tube (ELISA) and the T SPOT-TB (ELISPOT).
- ▶ IGRAs are generally more sensitive and specific than tuberculin skin tests and do add some value to current screening procedures.
- ▶ Recommended assessment includes: clinical evaluation—in particular, a history of risk factors for TB infection, a chest x-ray and IGRA.
- ▶ The choice of specific IGRA is often determined locally.
- ▶ Keep in contact with the laboratory that is performing the IGRA to ensure minimal sample wastage (ie, blood sent out of test-processing hours); and also speak to TB clinicians with an interest.
- ▶ Discuss this with your service commissioners as these tests can become expensive (especially if screening is planned to be repeated regularly—for example, annually).

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Appendices

Table AI Risk assessment for active and latent tuberculosis

Risk assessment
Clinical examination
History of previous TB assessed
CXR within 3 months of starting anti-TNF α treatment
Skin test (TST) if no immunosuppression used
High-risk groups
Recent immigrants from high TB incidence area
Injecting drug users
HIV infected
Prisoners and prison staff
Homeless and hostel workers
Mycobacteriology laboratory workers
High-risk medical conditions

CXR, chest x-ray; TB, tuberculosis; TNF α , tumour necrosis factor α ; TST, tuberculin skin testing.

Table AII Problems and pitfalls with tuberculin skin testing (TST)

Placing and reading of TST (needs training)
Subjects must return for results to be read (~30% do not)
Subjectivity and operator bias in reading TST
PPD manufacturer and batch variation in response
Cross-reactivity to BCG (>10% false positive TST)
Cross-reactivity to NTM (~10% reactive NTM, ~30% TST positive)
Boosting of responses with repeat testing (especially if BCG immunised)
TST insensitive in active TB (20–40% TST negative)
Anergy and HIV (~50% TB/HIV+TST negative)

NTM, non-tuberculosis mycobacteria—for example, *M kansasii*, *M avium* complex.

Table AIII Practical information on the use of interferon γ release assay (IGRA)

IGRA	QuantiFERON-TB Gold In Tube ELISA	T-SPOT TB ELISPOT
Antigens used	ESAT-6, CFP-10, TB7.7	ESAT-6, CFP-10
Incubation	16–24 h	24 h
Sample requirements	<ul style="list-style-type: none"> ▶ Adults and children: Blood drawn directly into three separate tubes (nil control tube, TB antigen tube and a mitogen tube). 1 ml each tube ▶ Shake each tube vigorously 10 times to ensure full coating ▶ Sample must be delivered to laboratory Mon–Thurs 9:00–16:00 ▶ Sample must be delivered to laboratory within 12 h of venepuncture ▶ Sample must be stored at room temperature 	<ul style="list-style-type: none"> ▶ Adult and child >10 years: 2×full 6 ml blood lithium heparin tube ▶ Children <10 years: 1×6ml blood lithium heparin tube ▶ Sample must be taken Mon–Thurs ▶ Sample must be delivered to laboratory on the same day, in particular Thursday (or no later than 12:00 the following day—if T-cell Xtend also available) ▶ Sample must be stored at room temperature
Results and interpretation	Results usually available in two working days (though samples can be batched for efficiency) As much clinical detail as possible needed on request form to aid interpretation.	Results available in two working days As much clinical detail as possible needed on request form to aid interpretation