Policy

Position statement on interferon-γ release assays for the detection of latent tuberculosis infection

Ivan Bastian, Chris Coulter and the National Tuberculosis Advisory Committee (NTAC)

Summary

Interferon-*y* release assays (IGRAs), such as the Quantiferon (QIFN) TB-Gold Plus assay (Qiagen, Hilden, Germany) and the T-SPOT.*TB* test (Oxford Immunotec Limited, Abingdon, United Kingdom), are marketed as a substitute for the tuberculin skin test (TST) for the detection of latent tuberculosis infection (LTBI). The relative merits of IGRAs and TST have been hotly debated over the last decade. The specificity of IGRAs has been optimised by using *Mycobacterium tuberculosis*-specific antigens. However, IGRAs are functional *in vitro* T-cell-based assays that may lack reproducibility due to specimen collection, transport, processing and kit manufacturing issues.

Longitudinal studies comparing the ability of IGRAs and TST to predict the future development of active tuberculosis disease (TB) are the ultimate arbiters on the respective utility of these assays. Three meta-analyses addressing this comparison have now been published and clinical experience with IGRAs is accumulating. The systematic reviews show that IGRAs and TST have similar (but poor) ability to identify patients with LTBI at risk of developing active TB disease. The improved specificity of IGRAs however may reduce the number of patients requiring preventative therapy.

Based on these meta-analyses, The National Tuberculosis Advisory Committee (NTAC) now recommends either TST or an IGRA for the investigation of LTBI in most circumstances. Both tests may be used in patients where the risk of progression to active TB disease is high and the disease sequelae potentially severe (eg. LTBI testing in immunocompromised patients or those commencing anti-tumour necrosis factor-α (TNF) therapy). Neither test should be used in the investigation of active TB disease (though TST and/or IGRA may be used as supplementary tests in paediatric cases). The choice of test for serial testing in healthcare workers (HCWs) remains controversial. A preference remains for TST in this circumstance because IGRAs have been bedevilled by higher rates of reversions and conversions when used for serial testing. These recommendations supersede all previous NTAC IGRA statements.

Background

Detection and treatment of latent tuberculosis infection (LTBI) is an increasingly important element of tuberculosis (TB) control efforts in Australia and other low-incidence countries.[1,2] *In vitro* T-cell based interferon-*y* release assays (IGRAs) are marketed as a substitute for the tuberculin skin test (TST) for the detection of LTBI.

The National Tuberculosis Advisory Committee (NTAC) has released position statements on the use of these assays (the last statement being in early 2012) and has undertaken to revise the recommendations on a regular basis.[3] As for the 2012 statement, the Committee has followed a template recommended in a survey of international IGRA guidelines by Denkinger et al.[4] Each Committee member reviewed one of the following sub-sections. The Committee then

discussed each member's literature review and proposed recommendation for each sub-section before reaching a consensus position.

The Committee has not formally graded the quality of the evidence supporting each recommendation but has cited meta-analyses where possible and has provided a few key references for each sub-section.

Summary of available commercial interferon-γ release assays

The methodology for TST and IGRAs has been described in detail elsewhere.[5,6] Briefly, tuberculin (or purified protein derivative-PPD) has been used as an *in vivo* test for LTBI for over 50 years.[5] Tuberculin is injected intradermally on the volar aspect of the forearm; the diameter of induration is read 48 hours later. Disadvantages of the TST include that the patient must return to the clinic for the result to be read (leading to large drop-out rates) and that the TST lacks specificity because the tuberculin preparation contains antigens that cross-react with BCG and non-tuberculous mycobacteria (NTM).[5,7] However, TST's long history of use has provided valuable research data and experience, particularly longitudinal data that provides important predictive information, that is slowly becoming available for IGRAs.[3]

The Quantiferon (QIFN) TB-Gold Plus assay (Qiagen, Hilden, Germany) is the most-commonly used IGRA in Australia. The specificity of this assay has been optimised by utilising pooled synthetic antigens, such as early secretory protein 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), from the *M. tuberculosis*-specific region of difference 1 (RD1).[8] Four tubes - two test tubes containing TB antigens, a positive control tube (containing mitogen), and a negative control tube - are inoculated with the patient's blood; incubated for 16–24 hours; the plasma is separated; and the IFN-γ concentration released from lymphocytes measured by an ELISA. Unlike previous versions of the Quantiferon assay, QIFN TB-Gold Plus has a second antigen tube with peptide configuration designed to assess CD8 T lymphocyte response as well as a CD4 response.

An alternative commercial assay, the T-SPOT.*TB* test (Oxford Immunotec Ltd, Abingdon, Oxfordshire, UK), is available but has not been marketed widely in Australia. An enzyme-linked immunospot (ELISPOT) methodology is used to enumerate activated effector T-cells that react to TB-specific peptides from ESAT-6 & CFP-10.[9] The assay is technically demanding requiring separation and counting of peripheral blood mononuclear cells, and subjective reading by a technician. However, some studies suggest that the T-SPOT.*TB* test is more sensitive than the QIFN tests, particularly in immunocompromised individuals.[10]

The antigens employed in both IGRA formats are absent from BCG and most NTM, but present in *M marinum*, *M. kansasii*, and *M. szulgai*.[7,8] The antigens may also be present in other unrecognised un-sequenced NTM. A small potential for cross-reaction with NTM therefore remains even with the IGRAs.

The following NTAC guidelines consider the QIFN and T-SPOT.*TB* tests as comparable assays and, unless specified, refer to these tests by the generic term "interferon-γ release assays". The choice of commercial IGRA that may be used is left with Australian laboratories and other healthcare professionals.

Review of recent literature and other national guidelines

Successive NTAC statements have noted the need for longitudinal studies estimating the performance of IGRAs in predicting the long-term progression to active TB disease in untreated individuals. The utility and interpretation of IGRAs depend on such studies. The last NTAC statement summarised two relevant meta-analyses that found that both IGRAs and TST were poor predictors of subsequent development of active TB disease.[3,11,12] For example, Rangaka et al analysed nine studies that reported incidence rates per person time of follow-up and

found TB rates in IGRA-positive patients were only 4-48 cases per 1,000 person-years and were even lower (2-24 cases per 1,000 person-years) in TST-positive patients.[12] The median follow-up in these nine studies was four years (IQR 2-6 years). A subsequent review paper by Pai et al extended the meta-analysis of Rangaka et al to include five additional papers.[10] Tuberculosis incidence rates varied between 3.7-84.5 cases per 1,000 person-years in IGRA-positive patients and between 2.0-32.0 cases per 1,000 person-years in IGRA-negative patients.

More recently, as part of the development of LTBI guidelines,[13] the World Health Organization (WHO) undertook a novel systematic review and meta-analysis of individual risk of progression to active TB following LTBI diagnosis with either TST or IGRA. The primary effect measure was the risk ratio (TB incidence in those with positive tests versus negative tests, in those who did not receive chemopreventative therapy). The overall pooled risk ratio (from 29 studies) for TST was 2.64 (95% CI 2.04-3.43) and 8.45 (95% CI 4.13-17.31) for IGRA. In a subgroup analysis including only studies where TST and IGRA were compared head-to-head (8 studies), the risk ratio for TST was 2.58 (95% CI 1.72-3.88) and IGRA was 4.94 (95% CI 1.79-13.65). While the overall risk ratio was significantly higher for IGRA, the difference was not statistically significant when limited to head-to-head comparison studies, and accordingly the WHO guidelines recommend that either TST or IGRA is appropriate for contact investigation.

These four important meta-analyses show that the ability of IGRAs to predict future active TB disease is poor but marginally better than TST (probably due to the improved specificity of IGRAs). Better biomarkers and an improved understanding of the spectrum of immune reactions that portends progression to active TB disease are required for targeting LTBI treatment programs.[10]

Some countries, such as the United States (US) and Japan, have been "early adopters" of IGRAs.[4,14] Other national guidelines have been updated to recommend both IGRAs and TST as acceptable tests for LTBI based on the accumulating evidence described above. For example, the European Centre for Disease Prevention and Control (ECDC) supervised a meta-analysis[11] and had an expert scientific committee review other literature.[15] They suggest that "IGRAs may be used as part of an overall risk assessment to identify individuals for preventive treatment", and provide detailed advice for specific patient groups and settings. Similarly, the Canadian Tuberculosis Standards were revised in 2014 based on a review of meta-analyses and other literature.[6] The revised Canadian guideline states, "Both the TST and IGRA are acceptable alternatives for LTBI diagnosis. Either test can be used for LTBI screening in any of the situations where testing is indicated…" with some exceptions listed.

With the increasing use of IGRAs, problems with test reproducibility have been recognised. Test variability of the QIFN assays has been studied more thoroughly than for the T-SPOT.*TB* test. QIFN results may vary due to pre-analytic factors (including faulty kit manufacturing, kit transport temperatures, blood volume inoculation, tube shaking, delayed tube incubation) and analytic factors (e.g. pipetting errors).[10,16] A systematic review found that under ideal conditions (i.e. repeat testing of an aliquot of the same sample) the QIFN interferon (IFN)- γ result could vary ± 0.26 IU/ml (95% CI, 0.23-0.29) if the initial test result fell between 0.25-0.8 IU/ml (with the manufacturer's recommended cut-off being 0.35 IU/ml).[16] If the QIFN test was repeated 4 weeks later (introducing more variation in specimen collection, transport and processing), 95% of the repeat test results would fall within ± 0.70 IU/ml (95% CI, 0.66-0.75) if the initial test result was between 0.25-0.8 IU/ml. The same systematic review highlighted that the blood volume inoculated into the QIFN tubes (range 0.8-1.2 ml) and delay before tube incubation were the major causes of QIFN variability.[16] Clinicians interpreting IGRA results must consider the variability of IGRAs, particularly for the serial testing of HCWs.

Diagnosis of active tuberculosis in adults

The previous NTAC statement in 2012 recommended against the use of IGRAs for the diagnosis of active TB disease in adults citing a meta-analysis by Metcalfe et al.[3,17] For diagnosing active TB disease, this review found that IGRAs had a pooled sensitivity of 69 - 83% in HIV non-infected subjects and 60 - 76% in HIV co-infected patients (i.e. equivalent to prior results for TST).[17] Also, like TST, IGRAs cannot distinguish between LTBI, active TB or past infection. Hence specificity for active TB was low: 52 - 61% in HIV non-infected and 50 - 52% in HIV infected subjects. Further meta-analyses on the use of IGRAs to diagnose extrapulmonary TB,[18] and in immunocompetent and immunosuppressed patients using IGRAs to test blood and other body fluids (e.g. pleural fluid),[19] have reached similar conclusions. The limited sensitivity and specificity of IGRAs means that these tests cannot be used to rule-in or rule-out active TB disease in adults, and have no place in the investigation of active TB disease in adults. Sadly, anecdotal experience amongst TB physicians and limited published data suggest that IGRAs are (mis)used for this purpose in Australia.[20]

Recommendation unchanged

TST and IGRAs have no place in the initial investigation of active TB disease in adults.

IGRA (like TST) cannot and should not be used to exclude suspected TB disease in adults.

Contact investigation in adults

Contact tracing and identification of LTBI following an exposure to active, infectious TB is an important component of TB control, particularly in low-TB incidence settings.[1,21] Various studies have provided different estimations for the progression rate to active disease two years after TST/IGRA conversion but the overall lifetime risk is generally described as 10%–15%. Treatment of LTBI with isoniazid reduces risk of future disease by 75%–90%.[22] Early identi-

fication of infected contacts and appropriate preventive treatment therefore has the potential to minimise future incident cases and ongoing transmission of infection. Amongst key limitations for effective contact investigation is the lack of a gold standard test that can identify LTBI, differentiate between active and latent infection, or predict patients at highest risk of progressing to active disease. Both TST and IGRAs detect a cellular immune response to *M. tuberculosis* antigens as an imperfect surrogate marker for LTBI. Specificity of IGRA for diagnosis of LTBI is higher than TST, particularly in the setting of previous BCG vaccination.[23]

Experience with use of IGRA in programmatic contact tracing has expanded since the 2012 NTAC IGRA recommendations. Some jurisdictions, particularly in the US, have phased out the use of TST in favour of IGRA.[24] No Australian jurisdiction has replaced TST with IGRA, however experience with local use in contact tracing has been reported in at least one state TB program.[25] In this study, the negative predictive value for subsequent development of active TB was 99.5%.

The four meta-analyses of longitudinal studies summarised in the above section show that both TST and IGRA can (poorly) stratify risk of active TB following exposure in TB contacts.[10-13] While a number of studies have suggested a higher risk of progression to active TB after positive IGRA, this difference is not significant in meta-analysis of head-to-head studies to date.[13] Accordingly, either TST or IGRA may be used for investigation of contacts of active TB. In some populations, particularly those contacts with a history of BCG vaccination, the improved specificity of IGRA may allow better targeting of preventative therapy. The specificity of TST is minimally affected by BCG immunisation administered before the age of one year, especially if immunisation occurred ≥ 10 years ago.[26] However, TST specificity is adversely affected if immunisation occurs after infancy or if BCG is repeatedly administered.[26]

Revised recommendations

Either TST or IGRA can be used in adults exposed to patients with active TB disease (i.e. in contact tracing). IGRA may be preferred in contacts with a history of multiple BCG immunisations, or immunisation with BCG after the age of one year.

Diagnosis of active tuberculosis in children

The 2012 NTAC position recommended that IGRAs (like TST) should only be as an adjunctive test to standard microbiological and radiological investigations in the investigation of active TB disease in children, and that IGRAs (like TST) cannot and should not be used to exclude suspected TB disease in children.

Studies of children with bacteriologically confirmed tuberculosis, including studies in low TB endemic settings, suggest a similar sensitivity of IGRA and TST.[27-31] A recent systematic review and meta-analysis reported that in children with microbiologically confirmed TB, sensitivity of TST, QIFN-Gold In Tube and T-SPOT. TB was 79%, 81% and 81% respectively with similar findings when stratified to low income countries (74%, 66% and 80% respectively) and high income countries (86%, 86% and 79% respectively).[30] It was concluded that IGRAs did not perform better than TST.

IGRAs (like TST) cannot and should not be used to exclude TB disease. Given the difficulty of establishing an accurate diagnosis of active TB in children, an IGRA (and/or TST) may provide additional evidence of *M. tuberculosis* infection in a child with suspected TB. A positive IGRA or TST result does not, however, discriminate between TB disease and LTBI. Neither test should be used as a replacement for standard microbiological and radiological investigations.

Recommendation unchanged

In the diagnosis of active TB in children, IGRAs (like TST) should only be used as an adjunctive test in addition to standard microbiological and radiological investigations.

IGRA (like TST) cannot and should not be used to exclude suspected active TB disease in children.

Diagnosis of latent tuberculosis infection in children

Detection of LTBI is undertaken in children at risk for active TB for whom preventive therapy is indicated. These include recent contacts of active cases and migrants from high TB incidence settings. The 2012 NTAC position statement recommended that IGRA does not replace TST for detection of LTBI in children and (like TST) cannot be used to exclude LTBI.[3] It was noted that IGRA may have additional value over TST in children that received BCG vaccination after the first year of life.

IGRAs (like TST) can be used to diagnose LTBI but a negative IGRA or a negative TST does not exclude LTBI. A large number of studies have compared the performance of IGRAs with TST as a marker of LTBI in children.[30,32] The absence of a recognised gold standard makes it difficult to estimate the 'true' sensitivity and specificity of IGRA or TST for the detection of LTBI. Therefore, defined exposure to *M. tuberculosis* has become an accepted quasi 'gold standard' on which to base comparative evaluations between TST and IGRA in children.[32]

Discordance between IGRA and TST results are common in children, with TST-positive and IGRA-negative (TST+/IGRA-) being the most common discordant pattern in the low TB endemic setting.[33] This discordance may be partly due to false-negative IGRA results. It may also be partly due to false-positive TST results due to previous BCG or infection with non-tuberculous mycobacteria.[26,33] Indeterminate IGRA results are also commonly reported in young children [34](< 5 years). Further, as with TST,

the timing of the IGRA is likely to be important (e.g. may be false-negative if the exposure is very recent). Therefore, a negative IGRA should not be used to exclude LTBI in children.

In settings with low rates of BCG immunisation, such as Australia, IGRA adds little over TST in the context of TB testing or contact investigation. In BCG-immunised children (usually immigrants) IGRA may have an advantage, as TST can yield false positive results in BCG vaccinated children (especially during the first 2-5 years of life, if vaccinated at birth).[26,35] Studies of immigrant children from regions with routine BCG immunisation suggest that IGRA may be a better test than TST to guide the use of preventive therapy.[36-39] LTBI testing as part of pre-migration testing has recently been introduced for children older than 2 years immigrating to Australia and the USA.[36] It is always important to explore potential close contact with a TB source case. The infectiousness of the source case, the proximity and duration of contact, and risk of the child contact to progress to disease (greatest in young children <2-5 years of age with recent TB exposure) are the most important factors in deciding the need for preventive therapy, irrespective of the IGRA or TST result.

The Australian Immunisation Handbook recommends that all individuals (except infants < 6 months of age) should undergo a TST before BCG vaccination.[40] Only immunocompetent persons with a TST induration < 5 mm should receive BCG. The rationale for this TST is to detect individuals already infected with *M. tuberculosis* or an NTM, or who have an immediate cutaneous reaction to TST. An adverse reaction to BCG may occur in this latter group.[41] The evidence for this pre-BCG TST is limited. While there is no literature on using IGRAs for such pre-BCG testing, TST must remain the preferred test for this purpose.

Revised recommendations

IGRA and TST are acceptable options for LTBI diagnosis, but neither is 100% sensitive or specific.

IGRA may be the preferred test for testing for LTBI in children with prior BCG vaccination.

Testing of immigrants

The evolving epidemiology of TB in Australia is driven mostly by migration of individuals from countries with a high burden of disease. Following arrival in Australia, disease amongst immigrants occurs most commonly as a result of reactivation of latent TB. In 2014, overseas-born people contributed 89% of the total TB case-load.[42] The TB incidence rate in the overseas-born population was 19.1 cases per 100,000 population. This rate is more than 17 times the incidence rate experienced in the Australian born population.

Post-arrival testing and treatment of LTBI in newly-arrived refugees has been shown to be a cost-effective measure, due to the prevention of TB transmission in the community and number of cases and deaths from TB averted.[43] Among countries that test for LTBI, there is heterogeneity in which immigrant subgroups are tested. A survey of 31 member countries of the Organisation for Economic Cooperation and Development (OECD) found 16 (55.2%) of 29 respondent countries tested for LTBI; the TB incidence threshold from country of origin for testing ranged from >20 cases per 100,000 to >500 cases per 100,000.[44] This wide variation likely reflects uncertainty about the optimal threshold at which to test. Setting the incidence threshold too low results in large numbers of immigrants needing to be tested, increasing costs and potentially overwhelming TB testing services. The most cost-effective policy option is likely to be to target at an intermediate incidence that balances the numbers of immigrants being tested against prevalence of LTBI in the immigrant population.

In 2014, Australia introduced LTBI testing of children in immigration detention facilities as well as offshore testing for migrants aged 2-10 years. More extensive LTBI testing of migrants to prevent disease may become an increasingly-important component of TB control within

Australia. The extent of this LTBI testing will depend on the policies, priorities and resources of the state and territory TB control services.

As outlined in previous sections, there has been increasing evidence since the 2012 NTAC position statement that IGRA is as sensitive and more specific than TST as a test for LTBI.[3] The survey of LTBI testing practices in OECD countries found that 6 (37.5%) of 16 countries used IGRA as part of their testing algorithm. Furthermore, studies from the USA indicate that IGRA-based testing is potentially more cost-effective and safer for children.[39]

Revised recommendation

If LTBI testing is performed for immigrants from high-incidence population settings after arrival, either IGRA or TST may be used.

Immunocompromised individuals with HIV infection

HIV infection significantly increases the risk that LTBI will progress to clinical disease. In TB non-endemic areas, HIV-positive patients co-infected with TB have an annual risk of 5-8% per year of progressing to active TB disease compared with a 10% lifetime risk in the general population.[45] Hence, when the risk of TB infection is high for a HIV-positive person, such as being a close household contact of an infective TB case, treatment for LTBI should be considered irrespective of the results of TST or IGRA testing. Before commencing LTBI treatment, a careful assessment to exclude active TB disease must be undertaken in all HIV-infected subjects, including culture of sputum or induced sputum because the chest X-ray appearance may be atypical or normal in the presence of culture-positive sputum.

In the absence of a history suggesting recent infection, all HIV subjects of all ages should be tested for LTBI with a view to instituting preventative treatment. Numerous studies summarised in a systemic review by Catamanchi *et al* confirm that the sensitivity of IGRA tests are reduced in HIV-infected subjects with similar

findings for the TST.[46] A lower CD4 count (<200 cells/µL) was associated with more negative and indeterminate results. The same meta-analysis suggested that the T-SPOT.*TB* test had greater sensitivity than the QIFN assay in HIV subjects when using active TB disease as a surrogate for LTBI to assess IGRA sensitivity.[46] Performance of IGRAs in detecting active TB cases however may not necessarily mirror their performance in detecting latently-infected subjects. Subsequent studies have produced discordant results reporting higher positivity rates and/or higher indeterminate rates with either the T-SPOT.*TB* test or the QIFN assay.[47-49] Two review articles have summarised these disparate studies by stating that neither IGRA test has been shown to be consistently more sensitive than TST in detecting LTBI in HIV-positive patients,[46] and that IGRA tests overall perform similarly to TST.[10]

Whether IGRA tests are useful in HIV-positive patients in predicting progression from latency to active disease is not well studied. Three studies found that IGRA-positive HIV-infected subjects were about three times more likely to develop TB than IGRA-negative patients.[10] A recent French study compared the results of QIFN, T spot and TST in 415 anti-retroviral-therapy (ART)-naïve HIV infected patients and followed their clinical progress for two years.[49] Of 47 patients with one or both IGRA tests positive, eight (14.5%) developed active disease, all within 4 months of enrolment. The eight cases of TB documented included two cases with a negative TST. No patient who had a negative result with both IGRA tests developed tuberculosis in the two-year follow up period. A systematic review and meta-analysis also found that a negative QIFN test implied a very-low short-to-medium risk of active TB.[50]

WHO recommends that either IGRA or TST can be used in a low-burden high-income country such as Australia.[13] Guidelines from some countries provide caveats to such a recommendation. Recognising that the number of false-negative and indeterminate tests increase when the CD4 count is low, UK national guidelines recommend that both IGRA and TST be performed concurrently when the CD4 count is (<200 cells/

µL), while Canadian and European guidelines suggest that concurrent testing may be helpful in immunocompromised subjects including HIV-positive individuals.[6,15,51]

A recent study from Taiwan suggested that an algorithm utilising HIV viral load, CD4 count and IGRA results improves the sensitivity and negative predictive value of testing, potentially reducing the number needing chemoprophylaxis.[52]

Revised recommendations

HIV infected subjects who have close household or other close prolonged exposure to an active infective TB case should be considered for treatment for latent TB without, or irrespective of, IGRA or TST testing on the assumption that transmission was likely, the risk of disease progression high and that existing diagnostic tests are imperfect for exclusion of latent infection.

HIV infected subjects with CD4 count (>200 cells/µL)

In the absence of recent significant close TB contact, all HIV infected subjects should be tested for LTBI. Where CD4 count is (>200 cells/µL), either TST or IGRA can be used.

HIV infected subjects with CD4 counts ≤200 cells/µL

All HIV infected subject presenting with advanced immuno-suppression (CD4 counts ≤200 cells/µL) should be assessed for active TB utilising chest X-ray and sputum examination (and other cultures depending on clinical findings).

Where there is no evidence of active disease and CD4 is less than 200 cells/µL, latent tuberculosis should be considered and both tests should be performed if the first test is negative or indeterminate.

Although the specificity of TST is lower than IGRA when there is a history of BCG vaccination, either a positive TST (>=5) or IGRA should

be considered an indication for preventative therapy in the setting of HIV infection, regardless of BCG history.

In the absence of either test being positive prior to commencement of ART, subsequent testing should be considered following restoration of immune function.

In keeping with recommendations on serial LTBI testing (see below), in HIV subjects where repeat exposure to TB is likely, TST may be subject to less conversions/reversions than IGRA tests and is the preferred investigation for repeated evaluation.

Immunocompromised individuals receiving anti-tumour necrosis factor-α therapy

Patients with immune-mediated inflammatory diseases (IMID) - such as rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, ulcerative colitis and Crohn's disease – are at increased risk of developing active TB disease due to their traditional immunosuppressive therapy (e.g. prednisolone) and particularly when receiving the newer immunomodulatory biological agents, such as tumour necrosis factor-α (TNF- α) inhibitors.[53] Jick et al reported that "low-dose" (< 15 mg/day) and "high dose" (≥15 mg/day) prednisolone was associated with active tuberculosis with odds ratios of 2.8 (95% CI 1.0–7.9) and 7.7 (95% CI 2.8–21.4), respectively.[54] Five TNF- α inhibitors are available in Australia: infliximab, adalimumab, etanercept, certolizumab and golimumab. The TNF- α inhibitors have been associated with 4-20 fold increases in active TB disease with infliximab and adalimumab carrying a greater TB risk than etanercept.[53] TB risk in those taking TNF- α inhibitors is higher if they are also on corticosteroids, methotrexate or azathioprine.[55,56]

The "standard of care" is therefore to test for LTBI before beginning treatment with TNF- α inhibitors.[55] LTBI testing in IMID patients is problematic because they are often already on prednisolone therapy (which can confound LTBI testing) and controversy surrounds the choice of

test (i.e. TST or IGRA). Smith et al summarised 14 studies comparing TST and IGRAs in a total of 1,630 patients with a variety of IMIDs.[57] The lack of a "gold standard" for LTBI again confounded these studies, which therefore relied upon correlating TST and IGRA results; five publications also studied the association of test results with TB risk factors by multivariate analysis. The summary of these 14 studies was that IGRAs could not be demonstrated to be superior to TST for LTBI testing in IMID patients.[57] Higher-level evidence of the efficacy of IGRAs in IMIDs is also lacking (such as a formal meta-analysis or longitudinal studies of the risk of active TB in IGRA-positive and -negative patients).

Several societies and organisations in high-income countries with a low incidence of TB have published guidelines for LTBI testing in IMID patients.[53,57] These guidelines generally recommend TST and/or IGRA. Emphasis is also placed upon the importance of an extensive clinical history looking for TB risk factors (eg. exposure to a TB patient; residence in a TB-endemic country; working or living in congregate settings such as hospitals, jails or homeless shelters) and on a chest X-ray (looking for fibronodular opacities suggestive of inactive TB). For example, the Australian Rheumatology Association recommends a case history risk assessment, chest x-ray within last three months, and either two step TST skin test or IGRA.[55]

Recommendation unchanged

Either TST or IGRA are acceptable for LTBI testing in IMID patients. IGRA may be preferred if there is a history of BCG immunisation after age one year. Both TST and IGRA may be performed if the risk of LTBI is considered high; a diagnosis of LTBI would be made by a positive result in either test.

The TB exposure history and chest X-ray are central in interpreting the TST/IGRA result and in determining the overall risk of LTBI in IMID patients.

Other immunocompromised individuals

Other immunocompromised populations (eg. pre-organ transplantation, patients with end-stage renal failure on dialysis) are also at increased risk of TB reactivation. For example, the incidence of post-transplant TB is 1.2%-6.4% in non-endemic countries, which is 20-74 fold higher than the general population.[58] Testing for LTBI is therefore indicated in these groups. Unfortunately, published comparisons of IGRAs and TST in these populations are limited and there is a high rate of indeterminate IGRA results in these groups.[58-60] There is also a lack of higher-level evidence of the efficacy of IGRAs in these "other immunocompromised patient groups". Hence, NTAC makes the same recommendations for LTBI testing in these "other immunocompromised" individuals as for IMID patients pre- anti-tumour necrosis factor-α therapy.

Recommendation unchanged

Either TST or IGRA are acceptable for LTBI testing in other immunocompromised patients. IGRA may be preferred if there is a history of BCG immunisation after age one year. Both TST and IGRA may be performed if the risk of LTBI is considered high; a diagnosis of LTBI would be made by a positive result in either test.

The TB exposure history and chest X-ray are central in interpreting the TST/IGRA result and in determining the overall risk of LTBI in immunocompromised patients.

Serial testing of healthcare workers

These new NTAC guidelines provide an overall recommendation that either TST or IGRA may be used for the detection of LTBI in most settings. The regular (annual) serial testing of HCWs is one situation where the choice of investigation remains controversial.[14] While IGRAs have advantages including convenience and specificity, high rates of conversions and reversions have been reported leading to more-costly follow-up of test-positive subjects.[10,61]

These conversions and reversions tend to occur more frequently when the initial QIFN result is close to the cut-off (0.35 IU/ml).[61,62] The manufacturer does not recommend a "grey zone" but the literature suggests that IFN-γ results of 0.25-1.0 IU/ml should be interpreted with caution.[62] The Committee therefore still prefers TST for the serial testing of HCWs. If an IGRA such as QIFN is used, NTAC recommends that the laboratory report the numeric IFN-γ result (IU/ml) as well as the "positive" or "negative" interpretation. Depending on the clinical circumstances of the HCW, the clinician may choose to repeat the IGRA test if the initial result falls within a pre-determined "grey zone".

Recommendation unchanged

The problem of defining an appropriate cut-off point has resulted in a trend towards more cautious use of IGRAs for HCW testing. For the present, TST remains the preferred test for serial HCW testing in Australia with IGRA's role limited to supplementary testing as a specificity tool.

Indeterminate results

IGRAs can produce un-interpretable (termed "indeterminate") results either due to inappropriately high or low IFN-γ response in the negative or positive controls, respectively. The rate of indeterminate results has varied between studies, between populations, and between assays.[47,61,63] Advice on the handling of indeterminate results is conflicting. Kobashi et al found that indeterminate IGRA results are more common among immunosuppressed patients, and subsequent IGRA testing one month later in this patient group is often indeterminate again.[63] Hence, when an initial IGRA result is indeterminate, a TST may be the preferred sequential test. In contrast, the Canadian guidelines recommend repeat testing of immunocompromised patients with an initial-indeterminate result.[3] There is insufficient evidence to favour an alternative IGRA test as a supplementary assay following an initial indeterminate IGRA result. Repeated indeterminate results are considered a marker of

anergy. The clinician must then determine the patient's LTBI status based on TB exposure history and other results.

The handling of indeterminate results highlights an important principle. IGRAs should be performed in cooperation with clinicians experienced in the diagnosis and management of TB and LTBI. The investigation and management of such patients should occur in liaison with the relevant state or territory TB service. Problematic IGRA results, including indeterminate reactions, can then be assessed expertly in the patient's clinical setting.

Cost-effectiveness analyses

While international studies have attempted to define the performance and utility of IGRAs, NTAC notes a continuing absence of high-quality cost-effectiveness analyses (CEAs) of IGRAs internationally and more particularly under Australasian TB program conditions. Three meta-analyses of IGRA CEAs have all bemoaned the methodologic flaws and the variability in test parameters and cost estimates, and warned that any IGRA CEA results be viewed with caution.[64-66]

Both NTAC and the state-based TB services encourage further clinical and economic evaluation of IGRAs, particularly independent cost-benefit analyses on the use of IGRAs using states' and territories' preferred protocols of investigating LTBI in Australia. Such analyses are needed to determine the relative economic outcomes of changing from TST to IGRAs taking into account the structure of TB services and program delivery in Australia.

This NTAC position statement supersedes all previous NTAC IGRA recommendations. NTAC is committed to ongoing monitoring of new diagnostic tests that may be of value in TB control. This IGRA position statement will remain under ongoing review and will be revised when significant developments occur in this field.

Transparency declaration

Corresponding author:

Dr Ivan Bastian

Clinical Microbiology Consultant

Microbiology Infectious Diseases (MID) Directorate, SA Pathology

Ivan.Bastian@sa.gov.au

References

1. World Health Organization. Toward tuberculosis elimination: an action framework for low-incidence countries. Document WHO/HTM/TB/2014.13. Geneva: WHO, 2014.

2. National Tuberculosis Advisory Committee. The strategic plan for control and elimination of tuberculosis in Australia: 2016 – 2020. (Manuscript in preparation).

3. National Tuberculosis Advisory Committee. Position statement on interferon-γ release assays in the detection of latent tuberculosis infection. *Commun Dis Intell Q Rep* 2012; 36: 125-31.

4. Denkinger CM, Dheda K, Pai M. Guidelines on interferon-γ release assays for tuberculosis infection: concordance, discordance or confusion? *Clin Microbiol Infect* 2011; 17: 806-14.

5. Lee E, Holzman RS. Evolution and current use of the tuberculin test. *Clin Infect Dis* 2002; 34: 365-70.

6. Pai M, Kunimoto D, Jamieson F, Menzies D. Diagnosis of latent tuberculosis infection, chapter 4. In: Canadian Tuberculosis Standards – 7th edition. Canadian Thoracic Society and The Public Health Agency of Canada, 2013. Available at: http://www.respiratory-guidelines.ca/sites/all/files/Canadian_TB_Standards_7th_Edition_ENG.pdf

7. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* 2000; 356: 1099-104.

8. Quantiferon TB-Gold Plus package insert. Qiagen, February 2016. Available at: http://www.quantiferon.com/wp-content/uploads/2017/04/English_QFTPlus_ELISA_R04_022016.pdf

9. T-SPOT.*TB* package insert. Oxford Immunotec Limited, 2013. Available at: http://www.oxfordimmunotec.com/international/wp-content/uploads/sites/3/PI-TB-IVD-UK-v2f288.pdf

10. Pai M, Denkinger CM, Kik SV, et al. Gamma interferon release assays for the detection of *Mycobacterium tuberculosis* infection. *Clin Microbiol Rev* 2014; 27: 3-20.

11. Diel R, Loddenkemper R, Nienhaus A. Predictive value of interferon-γ release assays and tuberculin skin testing for progression from latent TB infection to disease state: a meta-analysis. *Chest* 2012; 142: 63-75.

12. Rangaka MX, Wilkinson KA, Glynn JR, et al. Predictive value of interferon-γ release

assays for incident active tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; 12: 45-55.

13. World Health Organization. Guidelines on the management of latent tuberculosis infection. Document WHO/HTM/TB/2015.01. Geneva: WHO, 2015.

14. Alexander TS, Miller MB, Gilligan P. Point-Counterpoint: Should interferon gamma release assays become the standard method for screening patients for *Mycobacterium tuberculosis* infections in the United States? *J Clin Microbiol* 2011; 49: 2086-92.

15. European Centre for Disease Prevention and Control. Use of interferon-gamma release assays in support of TB diagnosis. Stockholm: ECDC, 2011. Available at: http://ecdc.europa.eu/en/publications/Publications/1103_GUI_IGRA.pdf

16. Tagmouti S, Slater M, Benedetti A, *et al.* Reproducibility of interferon gamma (IFN-γ) release Assays. A systematic review. *Ann Am Thorac Soc* 2014; 11: 1267-76.

17. Metcalfe JZ, Everett C, Steingart KR et al. Interferon-γ release assays for active pulmonary tuberculosis diagnosis in adults in low- and middle-income countries: systematic review and meta-analysis. *J Infect Dis* 2011; 204 Suppl 4: S1120-9.

18. Fan L, Chen Z, Hao XH, et al. Interferon-gamma release assays for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. *FEMS Immunol Med Microbiol* 2012; 65: 456-66.

19. Sester M, Sotgiu G, Lange C, et al. Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2011; 37: 100-11.

20. Tsang T, Waring J. retrospective study on the appropriate implementation of Quantiferon Gold Assay in a tertiary setting. *Respirology* 2010; 14(S1): TP180.

21. Erkens C, Kamphorst M, Abubakar I, et al. Tuberculosis contact investigation in low prevalence countries: a European consensus. *Eur Respir J* 2010; 36: 925-49.

22. Herrera V, Perry S, Parsonnet J, Banaei N. Clinical application and limitations of interferon-γ release assays for the diagnosis of latent tuberculosis infection. *Clin Infect Dis* 2011; 52: 1031-7.

23. Getahun H, Matteelli A, Chaisson RE, Raviglione M. Latent *Mycobacterium tuberculosis* Infection. *N Engl J Med* 2015; 372: 2127-35.

24. Grinsdale J, Ho C, Banouvong H, Kawamura L. Programmatic impact of using QuantiFERON®-TB Gold in routine contact investigation activities. *Int J Tuberc Lung Dis* 2011; 15: 1614-20.

25. Goebel K, Tay E, Denholm J. Supplemental use of an interferon-gamma release assay in a state-wide tuberculosis contact tracing program in Victoria: a six-year review. *Commun Dis Intell* 2015; 39: 191-6.

26. 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.

27. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children: a systematic review and meta-analysis. *Pediatr Infect Dis J* 2011; 30: 694–700.

28. Mandalakas AM, Detjen AK, Hesseling AC, et al. Interferon-gamma release assays and childhood tuberculosis: systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2011; 15:1018–1032.

29. Kampmann B, Whittaker E, Williams A, Walters S, Gordon A, Martinez-Alier N, et

al. Interferon-gamma release assays do not identify more children with active tuberculosis than the tuberculin skin test. *Eur Respir J* 2009; 33: 1374–1382.

30. Sollai S, Galli L, de Martino M, Chiappini E. Systematic review and meta-analysis on the utility of Interferon-gamma release assays for the diagnosis of Mycobacterium tuberculosis infection in children: a 2013 update. BMC Infect Dis 2014; 14 Suppl 1: S1-6.

31. Chiappini E, Bonsignori F, Mazzantini R, et al. Interferon-gamma release assay sensitivity in children younger than 5 years is insufficient to replace the use of tuberculin skin test in western countries. *Pediatr Infect Dis J* 2014: 33: 1291-3.

32. Mandalakas AM, Kirchner HL, Walzl G, et al. Optimizing the detection of recent tuberculosis infection in children in a high tuberculosis–HIV burden setting. *Am J Resp Crit Care Med* 2015; 191: 120-130.

33. Connell TG, Ritz N, Paxton GA, et al. A three-way comparison of tuberculin skin testing, QuantiFERON-TB gold and T-SPOT.TB in children. *PLoS ONE* 2008; 3: e2624

34. Connell TG, Tebruegge M, Ritz N, et al. Indeterminate interferon-gamma release assay results in children. *Pediatr Infect Dis J* 2010; 29: 285–286.

35. Seddon JA, Paton J, Nademi Z, et al. The impact of BCG vaccination on tuberculin skin test responses in children is age dependent: evidence to be considered when screening children with tuberculosis infection. Thorax Published Online First: [22 June 2016] doi:10.1136/thoraxjnl-2015-207687

36. Taylor EM, Painter J, Posey DL, et al. Latent tuberculosis infection among immigrant and refugee children arriving in the United States: 2010. J Immigrant Minority Health 2015 Sep 12 [Epub ahead of print]

37. Méndez-Echevarría A, González-Muñoz

M, Mellado MJ , et al; Spanish Collaborative Group for Study of QuantiFERON-TB GOLD Test in Children. Interferon-γ release assay for the diagnosis of tuberculosis in children. *Arch Dis Child* 2012; 97: 514–516.

38. Spicer KB, Turner J, Wang SH, et al. Tuberculin skin testing and T-SPOT.TB in internationally adopted children. *Pediatr Infect Dis J* 2015; 34: 599–603.

39. Howley MM, Painter JA, Katz DJ, et al; Tuberculosis Epidemiologic Studies Consortium. Evaluation of QuantiFERON-TB Gold in-tube and tuberculin skin tests among immigrant children being screened for latent tuberculosis infection. *Pediatr Infect Dis J* 2015; 34: 35–39.

40. Australian Technical Advisory Group on Immunisation (ATAGI). The Australian immunisation handbook 10th ed (2015 update). Canberra: Australian Government Department of Health, 2015. Available at: http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/7B28E8751 1E08905CA257D4D001DB1F8/$File/Aus-Imm-Handbook.pdf

41. Tarlo SM, Day JH, Mann P, Day MP. Immediate hypersensitivity to tuberculin. *Chest* 1977; 71: 33-7.

42. Toms, C., Stapledon, R., Coulter, C., Douglas, P., National Tuberculosis Advisory Committee for CDNA & Australian Mycobacterium Reference Laboratory Network. Tuberculosis notifications in Australia, 2014. *Commun Dis Intell 41*, E243–E259 (2017)

43. Pareek M, Watson JP, Ormerod LP, et al. Screening of immigrants in the UK for imported latent tuberculosis: a multicentre cohort study and cost-effectiveness analysis. *Lancet Infect Dis* 2011; 11: 435–444.

44. Pareek M, Baussano I, Abubakar I, et al. Evaluation of immigrant tuberculosis screening in industrialized countries. *Emerg Infect Dis* 2012; 18: 1422-9.

45. Selwyn PA, Hartel D, Lewis VA, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. *N Engl J Med* 1989; 320: 545-50.

46. Cattamanchi A et al. Interferon-Gamma Release Assays for the Diagnosis of Latent Tuberculosis Infection in HIV-Infected Individuals: A Systematic Review and Meta-Analysis. *J Acquir Immune Defic Syndr* 2011; 56:230–238.

47. Ramos JM, Robledano C, Masiá M, et al. Contribution of interferon gamma release assays testing to the diagnosis of latent tuberculosis infection in HIV-infected patients: a comparison of QuantiFERON-TB Gold In Tube, T-SPOT.TB and tuberculin skin test. *BMC Infect Dis* 2012; 12: 169.

48. Cheallaigh CN, Fitzgerald I, Grace J, et al. Interferon gamma release assays for the diagnosis of latent TB infection in HIV-infected individuals in a low TB burden country. *PLoS One* 2013; 8(1): e53330.

49. Bourgarit A, Baron G, Breton G, et al. Latent tuberculosis infection screening and 2-year outcome in antiretroviral-naive HIV-infected patients in a low-prevalence country. *Ann Am Thorac Soc* 2015; 12: 1138-45.

50. Santin M, Muñoz L, Rigau D. Interferon-γ release assays for the diagnosis of tuberculosis and tuberculosis infection in HIV-infected adults: a systematic review and meta-analysis. *PLoS One* 2012; 7(3): e32482.

51. National Institute for Health and Care Excellence (NICE). Tuberculosis guideline. Available at: https://www.nice.org.uk/guidance/ng33.

52. Lee SS, Lin HH, Tsai HC, et al. A Clinical Algorithm to Identify HIV Patients at High Risk for Incident Active Tuberculosis: A Prospective 5-Year Cohort Study. PLoS One. 2015 Aug 17;10(8):e0135801. doi: 10.1371/journal.pone.0135801. eCollection 2015.

53. Winthrop KA. The risk and prevention of tuberculosis: screening strategies to detect latent tuberculosis among rheumatoid arthritis patients who use biologic therapy. *Int J Adv Rheumatol* 2010; 8: 43–52.

54. Jick SS, Lieberman ES, Rahman MU, et al. Glucocorticoid use, other associated factors, and the risk of tuberculosis. *Arthritis Rheum* 2006; 55: 19–26.

55. Tymms K, Therapeutics Committee, Australian Rheumatology Association. Screening for latent tuberculosis infection (LTBI) prior to use of biological agents in Australia. Available at: http://rheumatology.org.au/downloads/SCREENINGFORLATENTTUBERCULOSISINFECTION.pdf

56. Lorenzetti R, Zullo A, Ridola L, et al. Higher risk of tuberculosis reactivation when anti-TNF is combined with immunosuppressive agents: a systematic review of randomised control trials. *Ann Med* 2014; 46: 547-54.

57. Smith R, Cattamanchi A, Steingart KR, et al. Interferon-gamma release assays for diagnosis of latent tuberculosis infection: evidence in immune-mediated inflammatory disorders. *Curr Opin Rheumatol* 2011; 23: 377-84.

58. Theodoropoulos N, Lanternier F, Rassiwala J, et al. Use of the QuantiFERON-TB Gold interferon-gamma release assay for screening transplant candidates: a single-center retrospective study. *Transpl Infect Dis* 2012; 14: 1-8.

59. Jafri SM, Singal AG, Kaul D, Fontana RJ. Detection and management of latent tuberculosis in liver transplant patients. *Liver Transpl* 2011; 17: 306-14

60. Triverio P-A, Bridevaux P-O, Roux-Lombard P, et al. Interferon-gamma release assays versus tuberculin skin testing for detection of latent tuberculosis in chronic haemodialysis patients. *Nephrol Dial Transplant* 2009 24: 1952–6.

61. Joshi M, Monson TP, Woods GL. Use of interferon-gamma release assays in a health care worker screening program: Experience from a tertiary care centre in the United States. *Can Respir J* 2012; 19(2): 84-88

62. Daley CL, Reves RR, Beard MA, et al. A summary of meeting proceedings on addressing variability around the cut point in serial interferon-γ release assay testing. *Infect Control Hosp Epidemiol* 2013; 34: 625-30.

63. Kobashi Y, Sugiu T, Mouri K, et al. Indeterminate results of QuantiFERON TB-2G test performed in routine clinical practice. *Eur Respir J* 2009; 33: 812-5.

64. Nienhaus A, Schablon A, Costa JT, Diel R. Systematic review of cost and cost-effectiveness of different TB-screening strategies. *BMC Health Serv Res* 2011; 11: 247

65. Oxlade O, Pinto M, Trajman A, Menzies D. How methodologic differences affect results of economic analyses: a systematic review of interferon gamma release assays for the diagnosis of LTBI. *PLoS One* 2013; 8(3): e56044.

66. Campbell JR, Sasitharan T, Marra F. A systematic review of studies evaluating the cost utility of screening high-risk populations for latent tuberculosis infection. *Appl Health Econ Health Policy* 2015; 13: 325-40.

Submit an Article

You are invited to submit your next communicable disease related article to the Communicable Diseases Intelligence (CDI) for consideration.

More information regarding CDI can be found at: http://health.gov.au/cdi.

Further enquiries should be direct to: cdi.editor@health.gov.au.