Guidelines

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

National Tuberculosis Advisory Committee

Summary

In vitro T-cell based interferon-*y* (IFN-*y*) release assays (IGRAs), the QuantiFERON-TB Gold In-Tube test (QFN-GIT) (Cellestis Limited, Carnegie, Victoria, Australia) and the T-SPOT. TB test (T-Spot) (Oxford Immunotec Limited, Abingdon, United Kingdom), are marketed as a substitute for the tuberculin skin test (TST). The specificity of these immunoassays has been optimised by using *Mycobacterium tuberculosis*-specific antigens. IGRAs are more specific in patients with previous Bacille Calmette-Guérin (BCG) immunisation or exposure to non-tuberculous mycobacteria (NTM).

There have been a plethora of comparative studies of TST and IGRAs, several meta-analyses in specific patient groups, and a few longitudinal studies of the predictive ability of IGRA-positive results for the development of active tuberculosis (TB) disease. A summary of these studies is that IGRAs have not been clearly demonstrated to be superior to TST. The National Tuberculosis Advisory Committee (NTAC) also notes a continuing absence of cost-effectiveness studies of IGRAs under Australasian TB program conditions. Furthermore, TST remains a familiar test with a long history of use and longitudinal data that provides important predictive information that is not yet available with IGRA.

TST therefore remains the preferred test for latent tuberculosis infection (LTBI) in most patient groups. IGRAs may be used as supplemental tests to improve specificity in screening immunocompetent subjects and in addition to TST in immunocompromised patients considered at high risk of LTBI. The specific recommendations in various patient groups are listed in the body of the text.

Background

Detection and treatment of LTBI is considered to be an increasingly important element of TB control efforts in Australia and other low-incidence countries. IGRAs are marketed as a substitute for the TST for the detection of LTBI.

NTAC has released position statements on the use of these assays (the last statement being in September 2009) and has undertaken to revise the recommendations on a regular basis. A MedLine search for 'interferon gamma release assay tuberculosis' articles in English between August 2009 and August 2011 found 197 new publications. To address this large body of literature, the Committee has followed a template recommended in a survey of international IGRA guidelines by Denkinger et al.[1] Each Committee member reviewed one of the following sub-sections. The Committee then discussed the member's proposed recommendation for each sub-section before reaching a consensus position.

Denkinger et al[1] suggested using an evidence-based grading system though the ability to grade the quality of research studies remains controversial.[2] The quality of the IGRA literature is disparate and some of the publications are not relevant to a high-income country such as Australia with a low incidence of TB. The Committee therefore has not formally graded the quality of the evidence but has cited meta-analyses where possible and has provided a few key references for each sub-section.

Summary of available commercial interferon-*y* release assays

Tuberculin (or purified protein derivative-PPD) has been used as an *in vivo* test for LTBI for over 50 years.[3] Tuberculin is injected intra-cutaneously 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 NTM.[3,4] However, TST's long history of use has provided valuable research data and experience, particularly longitudinal data that provide important predictive information that is not yet available with IGRAs.[3]

The United States' Food and Drug Administration have approved three *in vitro* IGRAs that attempt to address these disadvantages of the TST. The spe-

cificity of these immunoassays 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).[5,6] The assay formats have been summarised in the 2010 United States Centers for Disease Control and Prevention and the 2011 European Centre for Disease Control and Prevention guidelines.[7,8] The currently-available assay, the QFN-GIT, comprises three tubes: a test tube containing antigens from ESAT-6, CFP-10 and part of the sequence of TB7.7; a positive control tube (containing phytohaemagglutinin); and a negative control tube. The three tubes are inoculated with the patient's blood; incubated for 16–24 hours; the plasma is separated; and the IFN-γ concentration measured by an ELISA.

An alternate commercial assay, the T-Spot test, is available but has not been marketed widely in Australia. In the T-Spot test, peripheral blood mononuclear cells (PBMCs) are separated from whole blood and distributed to a microtitre plate (250,000 cells/well) containing test wells (ESAT-6 and CFP-10), and positive- and negative-control wells. Following 16–20 hours incubation, an enzyme-linked immunospot assay (ELISpot) is used to detect increases in the number of cells that secrete IFN-γ (represented as spots in each test well) after stimulation with/without antigen. The T-Spot test is technically demanding requiring PBMC separation and a subjective reading of the ELISpot assay by a technician. However, some studies suggest that the T-Spot test is more sensitive than the Quantiferon tests, particularly in immunocompromised individuals.

The antigens employed in both IGRA formats are absent from BCG and most NTM, but present in *M. marinum, M. kansasii,* and *M. szulgai*.[4] 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.

Diagnosis of active tuberculosis in adults

The previous NTAC statement recommended that TST and IGRAs had no place in the initial investigation of active TB disease. There are limited new data that have bearing on the role of IGRAs in the diagnosis of active TB.

A meta-analysis of the role of IGRAs (i.e. the T-Spot and QFN-GIT assays) for diagnosing active TB disease found the 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).[9] Also, like TST, IGRAs cannot distinguish between LTBI, active TB or past infection. Hence, specificity for active TB is low: 52%–61% in HIV non-infected and 50%–52% in HIV infected subjects. Anecdotal experience amongst TB physicians in Australia and limited published experience[10] suggest that IGRAs are over-used in acute clinical settings where the diagnosis of active TB is being considered.

Recommendation

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

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.[11] 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%.[12] Early identification of infected contacts and appropriate preventive treatment therefore has the potential to minimise future incident cases and ongoing transmission of infection. The limitation 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. There have been two recent meta-analyses comparing the ability of TST and IGRAs to predict progression to active TB disease in patients without active disease at baseline.[13,14] The analysis by Rangaka et al[14] included 15 studies from countries with a low- and high-incidence of TB. The association of a positive IGRA result with subsequent development of active TB disease was weak, with a relative risk of 2.1 (95% CI 1.42–3.08) and similar to a positive TST result, which had a relative risk of 1.60 (95% CI 0·94–2·72) at the 10 mm cut-off. Only four studies fulfilled the inclusion criteria in the meta-analysis by Diel et al.[13] two studies involved screening HIV patients, while the other two consisted of large contact investigations among local and immigrant populations in Germany and The Netherlands. In one contact investigation study, a positive IGRA result had a positive predictive value (PPV) for progression to active TB disease of 14.6% (95% CI 6–29%) compared with TST's PPV of 2.3% (95% CI 0.7–5.2%). The other contact investigation study found no dif-

ference between QFN-GIT, T-Spot or TST with the respective PPVs being: 2.8% (95% CI 0.9–6.4%), 3.3% (95% CI 1.2–7.6%) and 3.1 (1.4–5.8%).

IGRAs therefore have not been clearly demonstrated to be superior to TST for detection of LTBI in contact investigations. In the absence of a clear choice between IGRAs and TST for contact investigations, a number of different approaches have been suggested ranging from TST alone to IGRA as the sole test with a variety of intermediary recommendations. Many guidelines recommend a sequential approach with TST performed as the first test, followed by IGRA as a confirmatory test in the event of a positive TST test. This approach may limit the costs associated with follow up of false-positive TST and unnecessary treatment of LTBI. Logistics and patient preferences must also be considered.

Recommendation

TST remains the test of choice for investigation of contacts of active TB. TST has similar specificity to IGRAs in a non-BCG vaccinated cohort, therefore IGRAs do not add additional value in this group.

In TST-positive subjects at low risk of LTBI and at low risk of progressing to active disease, an IGRA may be used as a supplementary test in a two-step process to confirm LTBI. The improved specificity of IGRA in this circumstance in subjects who have had previous BCG or NTM exposure may allow better targeting of preventative therapy.

IGRAs may be a preferred option where resources, distance or other factors make TST impractical to administer.

Diagnosis of active tuberculosis in children

The 2007 NTAC statement made no specific recommendations regarding the use of IGRA in children. As of July 2011, over 30 guidelines (some including children) that incorporate IGRA in diagnostic algorithms for either LTBI or TB disease are available worldwide.[1]

In children with confirmed TB disease in low TB endemic settings, studies suggest a similar sensitivity of IGRA and TST of between 50% and 90%.[15,16] Therefore, IGRA and TST cannot and should not be used to exclude TB disease. Combining the results of IGRA and TST is associated with a small overall increase in sensitivity in several studies.[17] Given the difficulty of establishing an accurate diagnosis of TB disease in children, results of IGRA (and/or TST) may provide additional evidence of *M. tuberculosis* infection in a child with suspected TB disease. 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

IGRA (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 TB disease in children.

Contact tracing in children

Given the absence of a recognised gold standard, estimating the 'true' sensitivity and specificity of IGRA or TST for the detection of LTBI in children is difficult. However, a recently published hierarchy of reference standards for the evaluation of IGRA for the detection of LTBI is informative.[18] Within this hierarchy, the weakest standard is concordance with the TST. Analysis of results of IGRA and TST with respect to defined exposure to *M. tuberculosis* is a better method to assess the accuracy of these tests. Although not all individuals exposed to a smear-positive TB contact will subsequently become infected, this has become an accepted quasi 'gold standard' on which to base comparative evaluations between TST and IGRA. The predictive ability of IGRA for the development of TB disease and the likely efficacy of preventive treatment based on the results of IGRA represent the highest quality standards but remain largely unstudied in children. A negative TST or IGRA does not exclude LTBI.

Almost 40 studies have compared the performance of IGRA with TST as a marker of LTBI in children. The design of most studies has been cross sectional, comparing results of IGRA with the TST in children screened for LTBI for a variety of indications. Results suggest that discordance between IGRA and TST results are common in children (most often TST positive/IGRA negative in the low TB endemic setting) and this may be due to false negative IGRA results. A high rate of indeterminate IGRA results has been reported in young children (< 5 years) in several recent studies.[19] Further, as with TST, the timing of the IGRA is likely to be important (e.g. may be false negative if the contact is less than 1 weeks ago). Therefore, a negative IGRA alone should not be used to exclude LTBI, especially in young children.

In settings with low rates of BCG immunisation such as Australia, IGRA add little over TST in the context of TB screening or contact investigation. In BCG immunised children (usually immigrants), IGRA may have an advantage as the TST can yield false positive results as a result of prior BCG

immunisation. A positive TST is highly specific for LTBI when the child received BCG immunisation as a neonate, which is usual practice. The majority of false positive TST results occur in children immunised after one year of age.[20,21,22]

Infectiousness of the source case and risk of disease in the child contact remain the most important factors in deciding the need for preventive therapy, irrespective of the IGRA or TST result.

Recommendation

IGRA does not replace TST for detection of LTBI in children and (like TST) cannot be used to exclude LTBI. IGRA may have additional value over TST in children that received BCG vaccination after the first year of life.

Screening 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 2008, overseas-born people contributed 86% of the total TB case-load. The TB incidence rate in the overseas-born population was 20.4 cases per 100,000 population. This rate is almost 19 times the incidence rate experienced in the Australian born population.[23]

Identification and treatment of people with LTBI to prevent disease is a key component of TB control within Australia. Post-arrival screening 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.[24]

In 2011, the NICE Clinical Guideline on TB diagnosis and management addressed the issue of diagnosis of LTBI in people who are recent arrivals from countries of high TB prevalence.[25] The conclusion was that IGRAs in this group appeared to be the most cost-effective diagnostic strategy, however, a dual testing strategy utilising both tuberculin skin testing and supplemental IGRA assessment was recommended as TST was a less expensive strategy that would be more effective in low incidence areas and, in particular, there were still issues over the operation of the IGRA tests and inter-subject variability.[25]

Recommendation

TST and supplemental IGRA assessment for people identified with a positive TST is the recommended diagnostic strategy in immunocompetent immigrants from countries where TB is highly prevalent.

Immunocompromised individuals with HIV infection

The utility of IGRAs in HIV-infected individuals has not been clarified. Their use is potentially hampered by the relative or absolute anergy demonstrated by patients with CD4 cell counts < 200 cells per mm^3, although some studies have suggested that IGRAs (especially the T-Spot test) may be less affected by CD4 count than the TST. A recent meta-analysis failed to come to a conclusion regarding the superiority or inferiority of IGRA in comparison to TST.[26]

Furthermore it is unclear whether the QFN-GIT and T-Spot test are equivalent. In the context of diagnosis of active TB (using *M. tuberculosis* culture positivity as the gold standard), the above meta-analysis found the sensitivity of the T-Spot test was 72% (95% CI, 62–81%) and of QFN-GIT was 61% (95% CI, 47–75%) in low–middle-income countries, and 94% (95% CI, 73–100%) and 67% (95% CI, 47–83%) respectively in high-income countries.[26] Thus the sensitivity and specificity of IGRAs vs TST in HIV-infected individuals is unclear. Furthermore, none of these tests (including TST) can be considered definitive for proving or discounting latent or active TB in HIV-infected individuals.

Recommendation

TST remains the test of choice for detection of LTBI in HIV-infected individuals. However, recognising the lowered sensitivity of TST in immunocompromised patients, an IGRA may be used as a supplementary test. An HIV-infected individual would be diagnosed with LTBI if either the TST or IGRA is positive.

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.[27] Jick et al[28] reported that 'low-dose' (< 15 mg per day) and 'high dose' (≥ 15 mg per day) prednisolone was associated with active tuberculosis with an odds ratios of 2.8 (95% CI 1.0–7.9) and 7.7 (95% CI 2.8–21.4), respectively. Five TNF-α inhibitors are available in Australia: infliximab, adalimumab, etanercept, certolizumab, 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.[27]

The 'standard of care' is therefore to screen for LTBI before beginning treatment with TNF- α inhibitors. LTBI screening in IMID patients is problematic because they are often already on prednisolone therapy (which can confound LTBI screening) and controversy surrounds the choice of screening test (i.e. TST or IGRA). Smith et al[29] recently summarised 14 studies comparing TST and IGRAs in a total of 1,630 patients with a variety of IMIDs. 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 screening in IMID patients. 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 screening in IMID patients.[27,29] 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 (e.g. 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[30] recommends a case history risk assessment, chest X-ray within last three months, and either two step TST skin test or IGRA.

Recommendation

Either TST or IGRA are acceptable for LTBI screening in IMID patients. IGRA may be preferred if there is a history of BCG immunisation after one year of age. 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 (e.g. 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.[31] Screening 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.[31–33] 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 screening in these 'other immunocompromised' individuals as for IMID patients pre-anti-tumour necrosis factor-α therapy.

Recommendation

Either TST or IGRA are acceptable for LTBI screening 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

Screening for LTBI in a low-prevalence the healthcare-worker (HCW) population using the traditional TST can be problematic due to the potential confounding effects from previous BCG vaccination and the booster effect from repeat tests in the previously sensitised that can result in false conversions. The IGRA offers improved specificity in relation to the BCG vaccinated and lack of a booster or sensitising effect from repeat testing. IGRAs therefore have potential advantages in HCW screening over the TST.

A recent systematic review of IGRA testing in HCWs from low-prevalence countries found that the IGRA predicts lower rates of LTBI than TST when used in a single screening situation.[34] The higher specificity of the IGRA in the BCG vaccinated is the suggested explanation and may result in fewer HCWs being recommended preventive therapy.

However, interpretation of IGRA results when used for serial testing has raised several questions, particularly regarding the threshold to distinguish new infection from non-specific variation.[35,36] Studies using IGRA in low prevalence populations suggest that the use of a single cut point (0.35 IU/ml) to separate negative from positive is problematic.[34,37–39] The IGRA can vary non-specifically close to this cut-point resulting in high conversion and reversion rates being observed. Gandra et al[37] from the University of Illinois College of Medicine at Peoria, report that screening 6,530 HCWs by QFN-GIT cost $436,096 compared with $78,360 by TST. The increased expense was caused by direct screening costs and additional indirect costs such as extra follow-up visits and investigations for HCWs with borderline-positive QFN-GIT test results and additional chest radiographs.

The dilemma of conversion/reversion results with IGRAs has prompted consideration of alternative definitions for a 'new infection' in HCWs including an absolute increase over baseline or use of a 'grey zone' with a higher cut-point.[37,38,39]

Recommendation

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

Indeterminate results

IGRAs can produce uninterpretable (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 (i.e. more common in children and immunosuppressed patients) and between assays.[40,41] Some international guidelines provide suggestions on the management of indeterminate reactions. For example, the Canadian guidelines recommend repeat testing of immunocompromised patients with an initial-indeterminate result.[40] 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 only be carried out by 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.

Concluding remarks

While international studies have attempted to define the performance and utility of IGRAs, NTAC notes a continuing absence of cost-effectiveness studies of IGRAs under Australasian TB program conditions. 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 immunoassays taking into account the structure of TB services and program delivery in Australia.

The World Health Organization has released recently a policy statement on the use of IGRAs in low– and middle-income countries.[41] This document was based on commissioned systematic reviews of studies from low- and middle-income countries supplemented by the input of an Expert Group. The recommendations are therefore not directly applicable to high-income low-incidence countries such as Australia. However, NTAC notes that the WHO IGRA recommendations match these NTAC guidelines.

This NTAC position statement and recommendations will remain under ongoing review and will be revised as new peer-reviewed published data becomes available. NTAC is committed to ongoing monitoring of new diagnostic tests that may be of value in TB control.

Transparency declaration

Some members of the Committee declared receipt of limited funding assistance from Oxford Immunotec, Cellestis Limited and CSL Limited to support investigator-led research and/or to attend an Australian IGRA conference in 2000.

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