Feasibility of latent tuberculosis infection diagnosis by interferon-gamma release assay remote from testing facilities

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Abstract

Although the tuberculin skin test (TST) has been the mainstay of the diagnosis of latent tuberculosis infection (LTBI) for many decades, interferon-gamma release assays (IGRAs) are gaining acceptance and are more specific for this diagnosis. The characteristics of one such IGRA, the QuantiFERON®-TB Gold Whole Blood In-Tube, make it feasible for use in a remote setting. This study performed 62 IGRAs with this test on individuals testing positive by TST, in a clinical setting over 3,000 km from the testing laboratory. Of these, 42 patients (68%) recorded negative results, 19 (31%) were positive, with only 1 result (2%) indeterminate. Negative, and therefore discordant in this study, test results were more common in those known to have been previously vaccinated with bacille Calmette-Guérin. These results are consistent with other reports, indicating that this approach to testing is logistically feasible, and has the potential to complement LTBI screening to assist tuberculosis control programs in settings remote from the testing laboratory. *Commun Dis Intell* 2011;35(2):168–171.

Keywords: tuberculin test, tuberculosis, interferon-gamma release assay, rural health services

Introduction

Globally, around one third of the world's population, approximately 2 billion people, is thought to be infected with tuberculosis (TB).[1] Although tuberculosis rates are comparatively low in Australia, the Northern Territory has the highest notification rate of any jurisdiction at around 25 new cases per 100,000 population per year.[2] Tuberculin skin tests (TSTs) have been used for over a century in the diagnosis of latent tuberculosis infection (LTBI), as positive results are associated with increased risk of current or future active disease. However, there are several limitations of TSTs, including the inconvenience of returning to a health-care provider after 48–72 hours for the reading of the result and the subjectivity of this reading. Also, as TSTs use antigens also found in bacille Calmette-Guérin (BCG) and non-tuberculous mycobacteria, false positive results can occur.[3]

New interferon-gamma release assays (IGRAs) measure interferon-γ release from previously sensitised memory T-cells in response to antigens specific to *Mycobacterium tuberculosis,* but found in few non-tuberculous mycobacteria. Such tests, including QuantiFERON®-TB Gold Whole Blood In-Tube, have been found to have improved specificity for *M. tuberculosis* infection, with similar sensitivity to TSTs.[4] Despite this, availability of the test and adequate transfer of specimens must be considered before the test is adopted by a tuberculosis control program servicing a remote area. In particular, the need for incubation to be commenced within 16 hours of specimen collection and continued at 37ºC for a further 16 to 24 hours can be limiting in this setting. For reasons including these, the Northern Territory Centre for Disease Control Tuberculosis Unit continues to use TST as the mainstay of diagnosis of LTBI. This study aimed to determine whether this form of IGRA could be used to produce interpretable results in a location remote from the testing laboratory.

Methods

Subjects were prospectively enrolled from the Chest Clinic at the Centre for Disease Control (CDC), Darwin, with all adults eligible for inclusion if assessed for LTBI from August 2008 to December 2009. Patients were recruited when collection and transport could be co-ordinated with the local laboratory, located on the same campus as the CDC Chest Clinic. Study methods were approved by the Menzies School of Health Research Human Research Ethics Committee and subjects gave written, informed consent.

At the initial consultation, baseline demographic characteristics, indication for testing, results of chest x-ray, Indigenous status and BCG status were recorded. TST was then performed, followed by blood sampling for IGRA. TST was performed with intradermal injection of Tubersol® 0.1 mL (Sanofi Pasteur) and read after 48–72 hours, with results recorded as the transverse diameter of skin induration in millimetres. For the purpose of this study into immunocompetent adults, a positive result was defined as 10 mm or greater of induration regardless of BCG vaccination status.

Subjects recording a positive TST result proceeded to IGRA with the commercial kit QuantiFERON®-TB Gold Whole Blood In-Tube assay (Cellestis, Carnegie, Victoria). Approximately 5 mL of venous whole blood was obtained for testing, from which 1 mL of blood was instilled into each of three tubes (TB antigen, mitogen and nil control) and shaken for 5 seconds. On the same day (< 16 hours), the tubes were placed into incubation at 37°C for 16–24 hours at the Pathology Department of the Royal Darwin Hospital. The next day they were centrifuged at 3,000 revolutions per minute for 15 minutes. The specimens were then stored at 2°C to 8°C and sent refrigerated by air-freight to the Victorian Infectious Diseases Reference Laboratory, (North Melbourne Victoria, Australia) for enzyme-linked immunosorbent assay (ELISA) interferon-γ quantification according to the manufacturer's instructions. All individuals tested were seen again in the CDC Chest Clinic to receive counselling on the implications of their test result and options for treatment.

Multivariate logistic regression was performed for the binary outcome of a positive IGRA result, using the exposure variables of age, gender, BCG status, indication for testing, diameter of TST induration, presence of chest x-ray abnormalities and country of birth risk level for tuberculosis.

Results

A total of 62 subjects with positive TST results were tested with IGRA, with baseline characteristics displayed in Table 1. Patients were predominantly non-Indigenous, BCG vaccinated, young adults with an even gender distribution. Most were assessed in relation to employment, of which health care workers (22) and defence force personnel (16) were the largest groups. Other indications for assessment included external referral for assessment of tuberculosis risk (10) and follow up of a tuberculosis undertaking (5).[5] Chest x-rays were abnormal in 9 (15%) patients, with most changes being focal areas of minor scarring or granulomata. No patients were diagnosed with active tuberculosis. Of the 62 IGRAs obtained, 42 (68%) were negative, 19 (31%) positive and 1 (2%) indeterminate.

No exposure variables were independently associated with the outcome of a positive IGRA result on multivariate analysis ($P > 0.05$). Although not significant on multivariate analysis, a significantly lower proportion of patients with an established history of BCG vaccination was found to be IGRA positive than those without such a history ($P = 0.023$, $\chi 2$, Table 2). Although our guidelines state that 15 mm induration or greater is suggestive of true exposure, rather than BCG effect, this difference was not limited to those with less strongly positive TST results (10–14 mm induration), but was also seen in those with ≥ 15 mm induration.[7]

Table 1: Patient characteristics and interferon-gamma release assays results (n = 62)

Age, median (IQR)	36 (27–41)	
	n	%
Female gender	30	48
Indication for testing		
Health care worker	22	35
Other employment	20	32
Other indication	20	32
Country of birth*		
Australia	34	55
Other lower risk level country	6	10
Medium risk level country	0	
Higher risk level country	22	35
Indigenous status		
Indigenous	2	3
Non-Indigenous	60	97
Bacille Calmette-Guérin status		
Vaccinated	50	81
Unvaccinated	6	10
Uncertain	6	10
Diameter of tuberculin skin tests induration		
10 to 14 mm	23	37
15 to 19 mm	24	39
20 mm and greater	15	24
Chest x-ray abnormality present	9	15
Interferon-gamma release assays result		
Positive	19	31
Negative	42	68
Indeterminate	1	2

* Risk levels as defined by the Australian Government Department of Immigration and Citizenship.[6]

Discussion

Our results were broadly comparable to previous findings in use of IGRAs in less remote settings. In particular, this study reports a low proportion of indeterminate results suggesting that performance of the IFN-γ ELISA at a site remote from the testing laboratory is feasible. This finding is likely to relate to the short duration from venesection to incubation of generally less than 2 hours, as the reduction of this delay is associated with fewer indeterminate results.[8]

The lower proportion of positive IGRAs among BCG vaccinated individuals was also consistent with previous research.[9] However, the fact that patients with a history of BCG and negative IGRA were not primarily those with less strongly positive TSTs (10–14 mm) was unexpected. This finding may not be borne out with larger numbers, and as a reliable date

Table 2: Interferon-gamma release assays results by bacille Calmette-Guérin vaccination status and tuberculin skin tests size

Group	Interferon-gamma release assays result		
	n	Total	%
All tuberculin skin tests results			
BCG vaccinated	12	49	24
BCG unvaccinated*	7	12	58
$\chi^2 = 5.1$, $P = 0.023$			
Tuberculin skin tests 10–14 mm			
BCG vaccinated	3	16	19
BCG unvaccinated*	3	7	43
$\chi^2 = 1.5$, $P = 0.226$			
Tuberculin skin tests ≥15 mm			
BCG vaccinated	9	33	27
BCG unvaccinated*	4	5	80
$\chi^2 = 5.4$, $P = 0.021$			

Indeterminate result excluded from analysis.

* Includes bacille Calmette-Guérin (BCG) status uncertain.

for BCG vaccination could not be established for all subjects, the effect of timing of vaccination could not be assessed. This would be important given the high proportion of health care workers, particularly those on short-term placements, who may have received recent vaccination. However, the number of subjects enrolled limited our ability to study factors associated with increased rates of IGRA positivity.

IGRA tests, in particular QuantiFERON, have been shown to be feasible when used in hospital settings, and in clinics located in close proximity to laboratory testing facilities where processing is performed on the day of phlebotomy.[10] This observation has been expanded to groups including homeless, immigrant, refugee and intravenous drug-users and has been extended to the newer QuantiFERON®-TB Gold test.[11,12]

In the United States of America, a country where BCG has not been used as a recommended tuberculosis control strategy, the Centers for Disease Control and Prevention recommends use of IGRA in preference to TST in all BCG vaccinated individuals. These recommendations include programmatic considerations, in preferring IGRAs to TSTs when targeting groups considered less likely to return after the appropriate interval for TST reading. When delivering a tuberculosis control program over a wide geographical region, remote from testing facilities, guidelines also recommend consideration of transportation of specimens.[13] British guidelines recommend TST as the initial investigation for the diagnosis of LTBI, but confirmation of all positive results with an IGRA is recommended where available.[14] In Australia, the recommend initial investigation for LTBI remains the TST, but jurisdictions are encouraged to undertake further research to better define the role of IGRAs.[15]

The processing of samples for this study was consistent with the manufacturer's recommendation that tubes be incubated within 16 hours of collection and will then be stable for transport for up to 3 days.[16] Reports exist of effective IGRA testing in remote areas, although testing is most commonly performed locally in clinical research.[17] Ravn et al report satisfactory results in a remote Ethiopian setting in which specimens were transported by road for 6–8 hours then resting overnight before separation and testing for response to ESAT-6.[18] A report of a multicentre school-based screening program in Norway using QuantiFERON®-TB Gold In-Tube included rural areas; processing and storing specimens locally, before transport to a central testing facility.[19]

This study documents effective IGRA performance at a considerable distance from final testing, with results available within a reasonable time-frame to allow for effective decision-making. The finding of satisfactory results following local centrifugation and incubation, suggests IGRAs may be a feasible test to complement the Northern Territory tuberculosis control program. The use of IGRA in a low risk group with high rates of BCG vaccination would lead to fewer diagnoses of LTBI and a consequent reduction in the number of patients treated for this condition. However, for the test to be used successfully in remote communities, specimens would still require transportation to Darwin for centrifugation and incubation as early as possible within 16 hours of collection. This would present further logistical issues, the feasibility of which has not been tested. Using IGRAs in urban Darwin, but the TST in remote communities could also be considered but may present programmatic difficulties in training and use of two different diagnostic approaches.

The low rate of indeterminate results is not a consistent finding across all studies,[20] and may not be reliably replicated outside the research setting if familiarity with local processing is not maintained. As this study did not limit the interval between TST and IGRAs, some IGRA results may have been affected by a boosting effect.[21]

In conclusion, IGRA performance in urban Darwin is feasible and provides interpretable results with a low frequency of indeterminate results obtained. Further study will be required before IGRAs can be used in remote communities, away from basic laboratory facilities. However, use of these tests in urban areas, to complement LTBI screening in the Northern Territory tuberculosis control program, may be considered in future guidelines.

Acknowledgements

We are grateful to the nursing staff of the Darwin Centre for Disease Control Tuberculosis Unit for facilitating patient recruitment, as well as doctors Ben Rogers, Tom Snelling and Shaun Flint. We also thank the staff of the Victorian Infectious Disease Research Laboratory, including Norbert Ryan, for their assistance.

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