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Evaluation of a domestic interferon-gamma release assay for detecting *Mycobacterium tuberculosis* infection in China

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SUMMARY

Interferon-gamma release assays (IGRAs) have been demonstrated to be useful in the diagnosis of *Mycobacterium tuberculosis* (MTB) infection. However, IGRAs have not been recommended for clinical usage in most low-income countries due to the shortage of clinical data available resulting from their high test cost. Recently, a cheaper domestic TB-IGRA was approved in China. In this study, we compared TB-IGRA with QuantiFERON-TB Gold In-Tube (QFT-GIT) for MTB infection diagnosis in 253 active TB patients, 48 non-TB lung disease patients, 115 healthcare workers and 216 healthy individuals. The proportion of positive TB-IGRA results in active TB patients, patients with non-TB lung disease, healthcare workers and healthy individuals was 88.3%, 27.1%, 40.9% and 17.6%, respectively, which was similar to the results of QFT-GIT, with an overall agreement of 95% ($\kappa = 0.89$) and a high correlation between their responses ($r = 0.85$, $p < 0.001$) being observed. In conclusion, the TB-IGRA has comparable clinical performance with QFT-GIT.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), remains a global threat to public health with approximately 8.6 million new cases and 1.3 million deaths in 2012 [1]. It is estimated that one-third of the world's population is infected with MTB, and the majority have a latent infection. Persons with latent TB infection (LTBI) are clinically asymptomatic but have a 5%–10% lifetime risk that latent mycobacteria will become active and cause TB [2]. LTBI screening and prophylactic treatment can substantially reduce the risk of the development of disease and are important TB control approaches [3].

MTB infection can elicit a robust adaptive cell-mediated immune response, which has been employed to identify MTB infection, especially LTBI. There are two approaches currently used to determine the adaptive immunity, the tuberculin skin test (TST) and the gamma interferon (IFN-γ) release assay (IGRA). The TST measures delayed-type hypersensitivity reactions to a crude mixture of MTB antigens which are also present in bacillus Calmette-Guérin (BCG) and non-tuberculous mycobacteria (NTM). IGRA is based on measurement of IFN-γ secreted from T cells previously exposed to MTB when stimulated *in vitro* with the MTB-specific antigens, such as ESAT-6 and CFP-10. Both antigens are encoded by RD1, a genomic region present in *M. tuberculosis* but lacking in all *Mycobacterium bovis* BCG vaccine strains and most of the NTM [4]. There are two commercial IGRAs currently available, the QuantiFERON-TB Gold In-Tube test (QFT-GIT) (Cellestis, Carnegie, Australia) and the T-SPOT.*TB* assay (Oxford Immunotec, Oxford, UK).

With higher sensitivity and specificity for detecting MTB infection compared with TST, IGRAs have been widely used to diagnose MTB infection under national guidelines in many developed countries, such as the USA, UK and Japan [5]. However, in most developing countries, including China, the clinical utilization of IGRAs is not recommended due to insufficient evidence of their

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performance in high TB burden settings [6,7]. Hitherto, the available clinical data are too limited to formulate guidelines for how IGRAs are used in the clinical situation in China. The high test cost of QFT-GIT and T-SPOT.TB is one of restrictive factors of their clinical usage in developing countries [8].

Recently, in China, a domestic TB IFN-γ release assay (TB-IGRA) (Beijing Wantai, Beijing, China), which is cheaper than the two imported IGRAs, QFT-GIT and T-SPOT.TB, was licensed by the China Food and Drug Administration (CFDA). The objective of this study is to evaluate the performance of TB-IGRA in populations at various levels of risk for MTB infection, compared with the QFT-GIT in China. A high quality and cheap assay will be beneficial to the clinical utilization of IGRA and accumulating more clinical data for demonstrating its clinical value in developing countries with high TB burden.

2. Results and discussion

The diagnosis of MTB infection is very important to control TB epidemics, such as the investigation and control of TB outbreaks [9], surveillance of high risk populations [10,11], and epidemiological study of LTBI [12,13]. Because there is no diagnostic gold standard for MTB infection, it is difficult to accurately evaluate the performance of an assay to diagnose MTB infection, especially LTBI. In low-incidence settings, active TB patients and low-risk individuals with no known TB exposure are often used as surrogates of confirmed positive and negative MTB infected populations to estimate the assay's sensitivity and specificity, respectively [14]. However, in high-incidence settings, such as China, there is no suitable population that can be considered as the surrogate of subjects without MTB infection, due to the relatively high frequency of TB exposure in the population. One alternate method is to investigate whether the assay's results in different populations reflected their risks of MTB infection. In this study, three populations with different MTB infection risks were recruited to evaluate the performance of TB-IGRA for MTB infection diagnosis, active TB patients with confirmed MTB infection, healthcare workers (HCWs) with increased MTB infection risk due to frequent TB exposure, and healthy individuals with normal risk.

From September 2010 to July 2011, a total of 331 suspected active TB patients, 115 HCWs working in TB wards and 216 healthy individuals visiting the Center of Physical Examination were recruited in the Third People's Hospital of Kunming City, Kunming, Yunnan, China. Diagnosis of active TB among the recruited patients was made on the basis of all clinical, radiological, microbiological and histopathological information collected after recruitment and response to anti-TB therapy for at least 3 months. Among the 331 suspected TB patients, 255 active pulmonary TB patients (PTBs) and 28 extra-pulmonary TB patients (EPTBs) were eventually confirmed, and 48 were diagnosed with non-tuberculosis lung disease. The 28 EPTBs consisted of 14 cases of pleural tuberculosis, 7 cases of tuberculous peritonitis, 4 cases of nephrotuberculosis, 2 cases of spinal tuberculosis and 1 case of tubercular lymphadenitis. Of the 48 patients with non-tuberculosis lung disease, 35 were pneumonia, and the others included lung cancer (n = 9), pneumoconiosis (n = 2), chronic bronchitis (n = 1) and hydrothorax (n = 1). This study received ethical approval from the Ethics Committee of the School of Public Health, Xiamen University. Written Informed consent was obtained from each participant.

The demographic characteristics of the subjects in this study are shown in Table 1. The number of male subjects was greater than the number of females in the populations of PTBs, EPTBs, non-tuberculosis lung disease and healthy individuals, but more females were found among HCWs. More than half of HCWs were nurses (67 out of 115), all of whom were female. Of the HCWs, the working years in TB high risk situation were from 1 to 40, with the mean of 14.27 ± 9.40.

At enrollment, all subjects were tested with TB-IGRA and QFT-GIT according to the manufacturer's instructions. Briefly, for TB-IGRA, one milliliter of fresh heparinized venous whole blood was added separately into each two-milliliter Eppendorf centrifuge tube containing nil for negative control (N), mitogen for positive control (P) and TB antigen (a recombinant fusion protein of CFP-10 and ESAT-6) (T). After hour incubation at 37 °C, each tube was centrifuged, and the concentration of IFN-γ in the plasma was measured using the ELISA method. The IFN-γ value (pg/ml) for the TB antigen and mitogen were corrected for background by subtracting the value of N, namely T-N and P-N. As recommended by the manufacturer, the result of the test was interpreted as positive (N ≤ 400 pg/ml, T-N ≥14 pg/ml and ≥25% of N), negative (N ≤ 400 pg/ml and P-N ≥14 pg/ml, T-N < 20 pg/ml or T-N ≥ −4 pg/ml but <25% of N) or indeterminate (N > 400 pg/ml, or N ≤ 400 pg/ml and P-N < 14 pg/ml). For QFT-GIT, one millimeter of whole blood was collected separately in each heparin-containing tube pre-coated with nil for negative control, mitogen for positive control and TB antigen (peptides from ESAT-6, CFP-10 and TB7.7 [Rv2654c]). After 24 h incubation at 37 °C, the sample tubes were centrifuged, and the plasma was collected for measuring the IFN-γ concentration by ELISA. The result were interpreted as positive, negative or indeterminate on the basis of the manufacturer's recommended cutoff value (IFN-γ ≥0.35 IU/ml) [15].

Valid results of TB-IGRA and QFT-GIT were available from all 662 subjects and are shown in Table 1. There were 6 indeterminate results, 1 for TB-IGRA and 5 for QFT-GIT. The positive rate of TB-IGRA was 88.6%, 85.7%, 27.1%, 40.9% and 17.6% in PTBs, EPTBs, non-tuberculosis lung disease, HCWs and healthy individuals, respectively. Those were similar to the results of QFT-GIT with positive rates of 86.7%, 85.7%, 29.1%, 41.7% and 15.7% in the corresponding populations. The positive rates of the two IGRAs in different populations manifested a positive correlation with their risk of MTB infection. The positive rates were highest in the population diagnosed with active TB (88.3% of TB-IGRA and 86.6% of QFT-GIT), followed by the populations with increased MTB

Table 1
Characteristics of study population and the results of TB-IGRA and QFT-GIT.

Population	No.	M/F*	Mean age ± SD (years)	TB-IGRA		QFT-GIT	
				Positive (%)	IND†	Positive (%)	IND
Active TB patients	283	182/101	40.8 ± 17.2	250 (88.3%)		245 (86.6%)	
PTBs	255	164/91	41.0 ± 17.2	226 (88.6%)		221 (86.7%)	3
EPTBs	28	18/10	36.8 ± 15.7	24 (85.7%)		24 (85.7%)	
Non-TB lung disease	48	28/20	48.9 ± 16.1	13 (27.1%)	1	14 (29.2%)	2
HCWs	115	40/75	36.2 ± 9.8	47 (40.9%)		48 (41.7%)	
Healthy individuals	216	122/94	30.9 ± 8.7	38 (17.6%)		34 (15.7%)	

* M/F indicates the ratio of male/female.
† IND indicates the indeterminate results of IGRAs.