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Evaluation of a new interferon-gamma release assay and comparison to tuberculin skin test during a tuberculosis outbreak

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SUMMARY

Background: The tuberculin skin test (TST) is commonly used for the diagnosis of latent tuberculosis infection (LTBI) in non-bacille Calmette–Guérin (BCG) vaccination settings. In recent years, attention has been drawn to interferon-gamma release assays (IGRAs), especially in BCG-vaccinated populations. In this study, we evaluated the TST and a new whole blood IGRA in BCG-vaccinated individuals during a tuberculosis (TB) outbreak in China.

Methods: A TB outbreak occurred at a university in Dalian, China from March to November 2010. The TST and a whole blood IGRA were used to screen for TB infection. The correlation between exposure levels, TST, and the IGRA were evaluated.

Results: We found that agreement between the IGRA and TST was poor (kappa 0.182–0.290). IGRA positivity was associated with the level of exposure, and IGRA positivity and the level of exposure were risk factors for TB incidence. Neither the IGRA nor the TST alone picked up all TB incidences. However, if a 10 mm cutoff for the TST was used in the highest risk exposure group and IGRA positivity was used in the other risk groups, 19 of the 20 (95%) TB cases were identified.

Conclusions: A recommended preventive treatment regimen for China should be based on the level of exposure in conjunction with IGRA and TST test results.

1. Introduction

China is one of the world's 22 high burden countries for tuberculosis (TB) infection. As a consequence, TB outbreaks in settings such as those in which individuals remain in close contact for long periods of time are a serious public health problem. The screening methods commonly used during TB outbreaks in China are chest X-rays and the tuberculin skin test (TST). However, individuals who have received the mandatory bacille Calmette–Guérin (BCG) vaccine in China may have a cross-reaction to TST. In recent years, whole blood interferon-gamma release assays (IGRAs) have become a recommended method for the detection of latent TB infections (LTBI) in the USA and Japan.[1] In spite of this, IGRAs have not been fully evaluated in either BCG-vaccinated populations in high TB burden countries or in outbreak settings.

The TST, which is based on a delayed-type hypersensitivity response to purified protein derivative (PPD) from *Mycobacterium tuberculosis*, is still the primary method used in the diagnosis of LTBI in China. The sensitivity and specificity of the TST are dependent upon the cutoff value used to define a positive result in a given population. The specificity is increased (and sensitivity decreased) by progressively increasing the reaction size that is classified as a positive test result. The specificity of the skin test is variable and dependent primarily upon the likelihood of cross-reactions with non-tuberculous mycobacteria.[2] In addition, distinguishing between TST reactions caused by natural mycobacterial infections and those caused by BCG is challenging and can lead to the generation of false-positive results.[2,3] It has also been shown that repeated TST testing or two-step testing can boost BCG-induced sensitivity.[4,5] The TST also requires a second visit in which an experienced healthcare worker must read the diameter of the induration.

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In contrast, whole blood IGRAs use stimulator proteins derived from the RD1-region of *M. tuberculosis*, which is not present in BCG or in most non-tuberculous mycobacteria.[6,7] This reduces the chances of cross-reactivity and makes IGRAs more suitable for use in BCG-vaccinated populations. Studies have shown that IGRAs may be valuable for the diagnosis of recent infections.[8,9] Because active TB occurs mainly in the first year after exposure, IGRAs could be used to detect contact infections thereby limiting TB outbreaks. Another advantage of whole blood IGRAs is that they require only a simple blood draw at a single visit.

The aim of our study was to compare the TST and a new IGRA (ASACIR.TB; A.TB) during an outbreak in China. We examined the correlation between exposure levels, TST and IGRA results, and the observed incidence of TB during the outbreak. The outbreak occurred between March and November 2010 in Dalian, China. The 520 volunteer subjects were divided into six groups based upon their risk of exposure. Three methods were used for patient screening and LTBI detection: TST, A.TB, and chest X-ray. Since IGRAs have not been recommended as a TB diagnostic tool by the China Centers for Disease Control and Prevention (China CDC), the anti-TB treatment was administered to infected subjects as defined by a TST induration of $\geq$15 mm. This study was approved by the Ethics Committee of Dalian Tuberculosis Hospital and informed consent was obtained from all participants.

2. Materials and methods

2.1. Case review

The TB outbreak occurred among students enrolled at a university in Dalian in September 2007. The group included 520 students (361 male and 159 female) with an average age of 21.5 years. Male and female students lived in separate buildings. Among the members of this group, 98.7% had received a BCG vaccination.

The index TB case was a 21-year-old male diagnosed with active pulmonary TB (PTB) on March 6, 2010. The patient had coughing symptoms for 4 weeks before diagnosis. Between March and November of 2010, a total of 19 secondary cases of active TB were diagnosed.

2.2. Patient diagnosis

The index case appeared smear-positive. Of the 19 secondary cases, 10 were diagnosed as PTB cases: three appeared smear-negative but culture-positive, two were confirmed with TB by biopsy using a bronchoscope, and five (with the typical TB imaging characteristics but small lesions) were diagnosed with active TB after effective anti-TB treatment. In the nine remaining secondary cases of simple TB pleurisy, the lymphocyte and adenosine deaminase (ADA) levels were elevated in the pleural effusion. During anti-TB treatment all pleurisy cases experienced absorption of the pleural effusion.

2.3. Contact classification

The 520 volunteer subjects were divided into six groups based on their risk of exposure (Figure 1). Level 1 (L1) students lived in the same or consecutive rooms (R1–R11) and frequently studied in the same classroom as the index case. Level 2 (L2) students lived on the same floor (R12–R33) and frequently studied in the same classroom as the index case. Level 3 (L3) students lived on different floors in the same building and frequently studied in the same classroom as the index case. Level 4 (L4) students did not live in the same building but frequently studied in the same classroom as the index case (mainly females). Level 5 (L5) students lived on a different floor in the same building as the index case but studied in different classrooms (mainly males). Level 6 (L6) students did not live in the same building and studied in different classrooms (mainly females) to the index case. Based on the above criteria, there were 39 subjects in level 1, 82 in level 2, 116 in level 3, 93 in level 4, 126 in level 5, and 64 in level 6.

2.4. Screening methods

For TST screening 0.1 ml (5 IU) of PPD was injected under the palmar skin of the left upper forearm and the diameter of induration (DI) measured 72 h post injection. The first round of TST testing was carried out on 505 of the 520 subjects (97.1%) in September 2007. The second round of TST testing was carried out on 520 subjects (100%) in May 2010.

The A.TB IGRA was developed by Haikou VTI Biological Institute, Hainan, China. This assay uses Haikou VTI's patented technology (US patent number 7754219), which enables the intracellular delivery of full length culture filtrate protein 10 (CFP-10) and early secretory antigenic target 6 (ESAT-6) antigens to stimulate antigen-specific T cells through the classic major histocompatibility complex class 1 (MHC-1) pathway.[10,11] The assay was performed according to the user manual. In brief, negative control phosphate buffered saline (PBS) (N), positive control ConA (P), and the TB stimulators CFP-10 and ESAT-6 (T) were each mixed with 1 ml of fresh heparinized whole blood and incubated for approximately 24 h at 37 °C. The plasma was then collected and stored at 4 °C for up to 2 weeks or at −20 °C for long-term storage. The interferon-gamma levels in the plasma were then determined by ELISA. If N was $<$0.5 IU/ml and $(T-N)/(P-N) \geq 0.6$, or if N was $>$0.5 IU/ml and $(T-N)/(P-N) \geq 0.85$ the test was determined to be positive, otherwise the result was negative. Blood testing was performed following the second TST screening. All 520 subjects received the test.

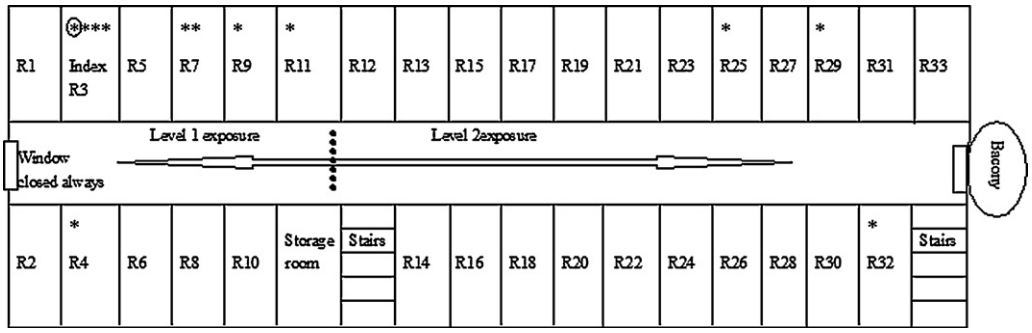

Figure 1. Floor plan showing rooms of level 1 and level 2 exposure; R, room. Students living in R1–R11 were grouped into level 1 exposure; students living in R12–R33 were grouped into level 2 exposure. Asterisks denote a TB case; the index case occurred in R3. Note: The window on the left was always closed.

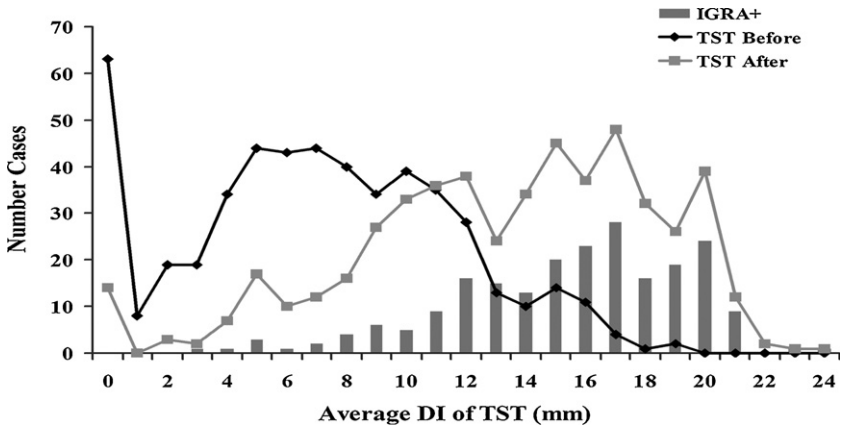

Figure 2. Trends in IGRA positivity and TST results before and after the outbreak (IGRA, interferon-gamma release assay; TST, tuberculin skin test; DI, diameter of induration). There was a significant difference between the DI values before and after the outbreak. The TST curve shifted significantly to the right, with the intersection of the two curves (before and after the outbreak) at the 10–11-mm interval. The trend of the TST curve was consistent with the IGRA positivity curve.

2.5. Anti-TB preventive treatment

Anti-TB treatment was administered based on a TST of ≥15 mm. Beginning in April 2010, 41 students voluntarily accepted anti-TB preventive treatment. In all cases a 6-month oral course of isoniazid was administered.

2.6. Statistical methods

Data were analyzed using SPSS 15.0. Kappa was used to evaluate concordance between the different methods. A p-value of <0.05 was considered statistically significant. In order to determine risk factors for TB incidence, SPSS 15.0 software was utilized to carry out the binary logistic regression analysis using secondary TB cases as the dependant variable and TST ≥15 mm, TST ≥10 mm, A.TB result, and the level of exposure as covariates.

3. Results

3.1. IGRA positivity was significantly associated with the level of exposure

In 2007, 505 of the 520 subjects in this study were examined by TST and the average DI was 7.1 ± 4.5 mm. During the outbreak in 2010, 520 subjects were retested by TST and the average DI was 13 ± 5 mm. There was a significant difference between the DI values before and after the outbreak ($p = 0.00$). After the outbreak, the TST curve shifted significantly to the right (see Figure 2), with the intersection of the two curves (before and after the outbreak) at the 10–11-mm interval.

Although the trend of the TST curve was consistent with that of the IGRA positivity curve, the agreement between the IGRA and TST was poor (Table 1). The overall kappa value between the IGRA and TST ≥10 mm was 0.182, and between the IGRA and TST ≥15 mm was 0.290. The overall TST positivity was 79.0% using 10 mm as the cutoff and 47.3% using 15 mm as the cutoff, while the IGRA positivity was 41.7%. The overall rate of concordance between the IGRA and TST was 64.8% at the 15 mm cutoff and 55.0% at the 10 mm cutoff.

The volunteers were classified into six groups based on the risk of exposure to the index case. Level 1 was at the highest risk of exposure. IGRA positivity was 71.8%, 62.2%, 47.4%, 40.9%, 23.8%, and 20.3% in groups L1 to L6, respectively (Table 2); IGRA positivity was significantly associated with the level of exposure ($p < 0.05$). However, using the 10 mm cutoff point, the positivity of the TST was 100%, 82.9%, 81.9%, 89.2%, 72.2%, and 56.3% in groups L1 to L6, respectively; and using the 15 mm cutoff, the positivity of the TST was 71.8%, 54.9%, 50%, 48.4%, 42.1%, and 26.6% in groups L1 to L6, respectively (Table 2).

3.2. Level of exposure and IGRA positivity are risk factors for TB incidence

In order to establish the risk factors for incidence, the volunteers who accepted preventive treatment ($n = 41$) and the

Table 1
The discordance between the ASACIR.TB IGRA and the TST

	Risk of exposure level						
	L1 (n = 39)	L2 (n = 82)	L3 (n = 116)	L4 (n = 93)	L5 (n = 126)	L6 (n = 64)	Sum
A.TB-positive							
TST ≥10 mm	28	43	51	36	28	10	196
TST <10 mm	0	8	4	2	2	3	19
TST ≥15 mm	20	29	35	28	21	6	139
TST <15 mm	8	22	20	10	9	7	76
A.TB-negative							
TST ≥10 mm	11	25	44	47	62	26	215
TST <10 mm	0	6	17	8	34	25	90
TST ≥15 mm	8	16	23	17	32	11	107
TST <15 mm	3	15	38	38	64	40	198
Concordance rate							
A.TB vs. PPD (10 mm)	71.8%	59.8%	58.6%	47.3%	49.2%	54.7%	55.0%
A.TB vs. PPD (15 mm)	59.0%	53.7%	62.9%	71.0%	67.5%	71.9%	64.8%

10 mm was used as the TST cutoff. A.TB, ASACIR.TB IGRA; TST, tuberculin skin test; PPD, purified protein derivative; IGRA, interferon-gamma release assay.

Table 2
Comparison between the ASACIR.TB IGRA and the TST in the different risk of exposure level groups

		L1 (n = 39)		L2 (n = 82)		L3 (n = 116)	
		Cases	TB (n = 9)	Cases	TB (n = 3)	Cases	TB (n = 5)
A.TB	Pos	28 (71.8%)	6	51 (62.2%)	3	55 (47.4%)	4
	Neg	11 (28.2%)	3	31 (37.8%)	0	61 (52.6%)	1
TST ≥10 mm	Pos	39 (100%)	9	68 (82.9%)	2	95 (81.9%)	3
	Neg	0	0	14 (17.1%)	1	21 (18.1%)	2
TST ≥15 mm	Pos	28 (71.8%)	5	45 (54.9%)	1	58 (50%)	2
	Neg	11 (28.2%)	4	37 (45.1%)	2	58 (50%)	3
		L4 (n = 93)		L5 (n = 126)		L6 (n = 64)	
		Cases	TB (n = 3)	Cases	TB (n = 0)	Cases	TB (n = 0)
A.TB	Pos	38 (40.9%)	3	30 (23.8%)	0	13 (20.3%)	0
	Neg	55 (59.1%)	0	96 (76.2%)	0	51 (79.7%)	0
TST ≥10 mm	Pos	83 (89.2%)	3	91 (72.2%)	0	36 (56.3%)	0
	Neg	10 (10.8%)	0	35 (27.8%)	0	28 (43.8%)	0
TST ≥15 mm	Pos	45 (48.4%)	3	53 (42.1%)	0	17 (26.6%)	0
	Neg	48 (51.6%)	0	73 (57.9%)	0	47 (73.4%)	0

10 mm was used as the TST cutoff. A.TB, ASACIR.TB IGRA; TST, tuberculin skin test; IGRA, interferon-gamma release assay.

index case were eliminated from the calculation. Binary logistic regression analysis (n = 478/519) was conducted with all 19 secondary cases as dependant variables; TST ≥15 mm, TST ≥10 mm, A.TB result, and level of exposure were the covariates. We found that the level of exposure (p = 0.00, odds ratio 2.642) and IGRA positivity (p = 0.04, odds ratio 3.359) were the risk factors for subsequent TB incidence.

3.3. Anti-TB preventive treatment and subsequent TB incidence

IGRAs are not included in the *Guidelines for tuberculosis control in the schools of China*, so the preventive therapy started at the end of April was based on a TST value ≥15 mm. Treatment consisted of the administration of oral isoniazid for a 6-month period. During the 12-month follow-up period there were no secondary cases in the 41 individuals in the high exposure groups (levels 1–4) who agreed to preventive therapy. Of the 478 individuals not taking preventative medication there were 13 subsequent TB cases during the peak incidence period (March to June). Eight of these cases had a TST of <15 mm. In the groups with relatively low exposure (L5 and L6), there were a number of TST ≥15 mm and IGRA-positive subjects, but no secondary cases were found during the 12-month follow-up period.

Of a total of 20 TB cases, 16 were detected by IGRA, 17 by TST using a 10 mm cutoff and 11 using a 15 mm cutoff. No assay by itself was able to identify all cases (see Figure 3).

4. Discussion

As a rule, a negative TST indicates that a person has not been affected by TB. In this study, two secondary TB cases had TST values of 0 mm and 3 mm but appeared IGRA-positive. These could

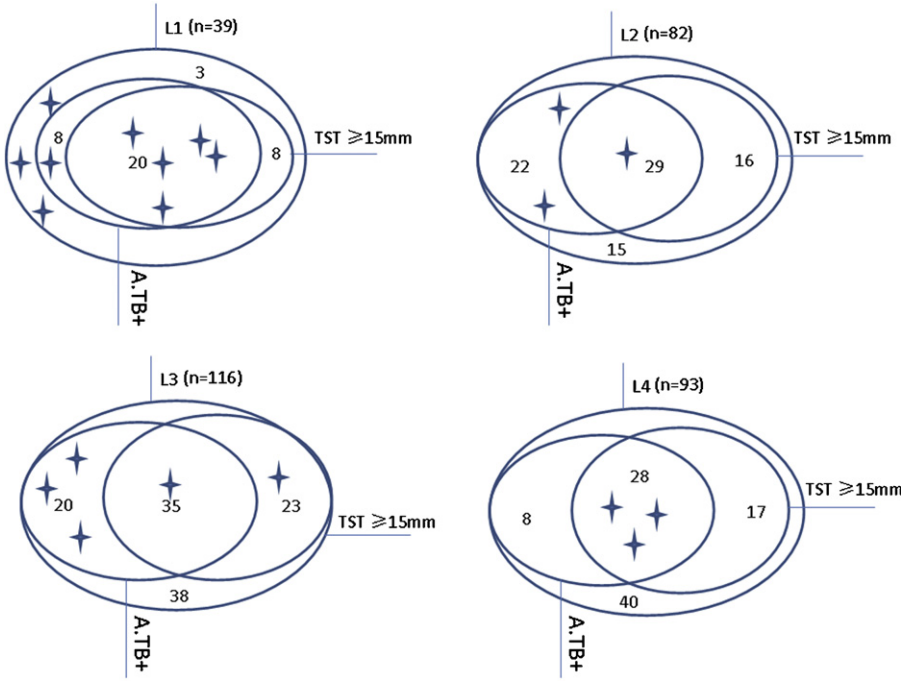

Figure 3. Relationship between TB incidence, ASACIR.TB IGRA, TST, and the level of exposure (L, level of exposure; A.TB, ASACIR.TB IGRA; IGRA, interferon-gamma release assay; TST, tuberculin skin test). The stars designate each TB case. Of a total of 20 TB cases, 16 were detected by IGRA and 11 by TST using a 15 mm cutoff. No assay by itself was able to identify all cases. Among the 520 students, 215 (41.3%) appeared IGRA-positive and 246 (47.3%) appeared TST-positive using a 15 mm cutoff.

be interpreted as false-negative TST results. As reported elsewhere, 5–25% of patients with active TB show a negative skin test (≥10 mm cutoff).[12,13] However, since the index case was reported on March 6 and these two cases were identified on April 14 and 21, the negative TST could have been due to a delayed response. Generally, from 2 to 12 weeks is required after primary infection for the skin test conversion to occur.[14]

In this study, 70.5% of those tested were IGRA-negative/TST-positive when using a TST cutoff of >10 mm, and 34.0% were IGRA-negative/TST-positive when using a TST cutoff of >15 mm. This suggests either a lack of sensitivity for the IGRA, as reported in previous studies,[15,16] or perhaps a lower sensitivity for older LTBI and a better sensitivity for recent infections. However, 8.8% of those tested were IGRA-positive/TST-negative when using a TST cutoff of <10 mm, and 35.3% when using a TST cutoff of <15 mm. This may contradict the assumption of a lower sensitivity for IGRAs. In addition, we found that IGRA positivity was significantly associated with the level of exposure, which may imply that it was more likely to suggest a recent infection, as reported elsewhere.[17–20]

A total of four TB cases appeared IGRA-negative/TST-positive, with indurations of 10–13 mm, which indicates an IGRA false-negative. An alternative interpretation is that the response to the A.TB stimulators was diminished by a high bacterial load.[21] Interestingly, three of the four cases had pleural effusion. As estimated by meta-analysis, 25% of TB pleurisy would be missed by IGRAs.[22] In our study, seven out of nine (77.8%) TB pleurisy cases were identified by IGRA. Certainly, this observation should be further confirmed by future studies in larger numbers of patients and in longitudinal studies with clinical outcomes. Therefore, in the high level contact group in a TB outbreak, a negative IGRA result may not exclude the possibility of the subsequent development of active TB.

The study revealed that the level of exposure and IGRA positivity were risk factors for the incidence of TB. Preventive treatment was administered based on a TST ≥15 mm, and after 6 months of treatment, no cases were reported from this group. However, of a total of 20 TB cases, nine (45%) had a TST of <15 mm, which suggests that the regimen currently used in China for the detection of TB during an outbreak is not sufficient.

Neither the IGRA nor the TST alone picked up all incidences of TB. However, if a TST of 10 mm was used as an indicator for the level 1 exposure group and IGRA positivity was used as an indicator for levels 2–4, this would pick up 95% (19/20) of the TB cases. The one missed case (TB pleurisy) had a TST of 20 mm and was from the level 4 exposure group, a group that had few infections (Figure 3). As mentioned previously, the case of TB pleuritis might be missed by IGRAs.

A limitation of this study is that a detailed evaluation of the occurrence of LTBI during the 2-year period from 2007 to 2010 (from student enrolment into the university to TB outbreak) has not been carried out, which could affect the conclusions drawn in this paper. Furthermore, the follow-up period was not long enough, which might have an impact on the conclusions.

In conclusion, the level of exposure and IGRA positivity were found to be risk factors for TB incidence during an outbreak in a high TB burden country. A preventive treatment regimen is recommended based on the level of exposure, in conjunction with the results of IGRA and TST tests.

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