Journal of Microbiology, Immunology and Infection (2017) 50, 349—354

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Diagnostic utility of QuantiFERON—TB Gold In-Tube test in pediatric tuberculosis disease in Taiwanese children

Kin-Sun Wong [a,*], Yen-Chun Huang [b], Han-Chung Hu [c], Yhu-Chering Huang [d], Chang-Hui Wen [d], Tzou-Yien Lin [d]

[a] *Division of Pediatric Pulmonology, Department of Pediatrics, Chang Gung Children's Hospital, Taoyuan, Taiwan*
[b] *Department of Pediatrics, National Taiwan University Children's Hospital, Taipei, Taiwan*
[c] *Department of Pulmonary and Critical Care Medicine, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Linkou, Taiwan*
[d] *Division of Pediatric Infectious Diseases, Chang Gung Children's Hospital, Taoyuan, Taiwan*

Received 8 June 2015; received in revised form 13 July 2015; accepted 15 July 2015
Available online 28 August 2015

KEYWORDS
children;
QFG-IT;
tuberculin skin test

Abstract *Purpose:* To compare the performance of a commercial interferon-gamma release assay, QuantiFERON TB Gold-in-Tube (QFG-IT) with the tuberculin skin test (TST) in Taiwanese children for the diagnosis of active tuberculosis (TB).
Methods: A retrospective chart analysis of pediatric patients (<18 years of age) who underwent QFG-IT tests and TST for the confirmation of active TB between January 2008 and June 2014.
Results: The sensitivity of QFG-IT was 100% [95% confidence interval (CI): 63.1—100], versus sensitivity of 62.5% for TST (95% CI 24.5—91.5). The positive predictive value of QFG-IT was 100 (95% CI: 89.7—100), while the negative predictive value for TST was 86.9% (95% CI: 67—96.3). Among three patients with Bacillus Calmette—Guérin (BCG) osteitis, two patients with TST were positive, but all tested samples for QFG-IT were negative.
Conclusion: QFG-IT assay was more sensitive for the diagnosis of TB disease than TST in an intermediate burden population with universal neonatal BCG vaccination. The increased recognition of BCG induced osteitis in recent years has alerted physicians that BCG induced lesions should be suspected when TST is positive but QFG-IT is negative.

* Corresponding author. Division of Pediatric Pulmonology, Department of Pediatrics, Chang Gung Children's Hospital, 5 Fu Hsin Street, Taoyuan, Taiwan.
E-mail address: kswong768@gmail.com (K.-S. Wong).

http://dx.doi.org/10.1016/j.jmii.2015.07.012
1684-1182/Copyright © 2015, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Despite higher costs for QFG-IT than TST, they have additional value for the diagnosis of active TB and should be performed when a diagnosis of TB remains in doubt.
Copyright © 2015, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

In Taiwan, the incidence of tuberculosis (TB) in the year 2000 was 70/100,000 person/year in adults, and less than 5/100,000 in children younger than 5 years of age.[1] Since 1960, the national Bacillus Calmette—Guérin (BCG) vaccination program had been instituted in Taiwan with a coverage rate of 97% in infancy.[2] Tuberculin skin test (TST) has been useful for the diagnosis of TB infections and diseases, but is not able to discriminate between the two; furthermore, the distinction between TB infections and BCG vaccination is not possible using TST. Interferon-gamma (IFN-γ) release assays (IGRAs) have the advantage of a higher specificity than TST, at the same time IGRAs do not cross-react with BCG vaccination and most nontuberculous mycobacterial (NTM) strains.[3] IGRAs do not have a booster effect in patients who require repeated examinations. The inclusion of mitogen response in IGRAs has the added capability of exclusion of immunological anergy.

Commercial IGRAs can be used to distinguish the production of IFN-γ in response to *Mycobacterium tuberculosis* infection from the BCG vaccine.[4,5] IGRAs have been recommended for use in children with a positive TST who received BCG vaccinations, to avoid unnecessary chemoprophylaxis.[3] However, comparative studies using commercial QuantiFERON TB Gold-in-Tube (QFG-IT) assay and the TST in an endemic region where national neonatal BCG program were instituted are nonexistent. The objective of the present study was to compare the performance of a commercial IGRA (QFG-IT), with the TST in a cohort of pediatric patients in a high neonatal BCG vaccination uptake population. The recruited patients consisted of children with clinically suspected active TB in an intermediate-burden region. We calculated the sensitivity and specificity of QFG-IT for the diagnoses of TB diseases using microbiological culture and histo-pathological findings of granuloma formations as the golden standard. Demographic data, clinical manifestations, and laboratory data were analyzed.

Methods

Patient population

A computerized search of our database of pediatric patients (<18 years of age) who underwent QFG-IT tests were recruited for the confirmation of active TB between January 2008 and June 2014.

Inclusion criteria

All children aged between 2 months and 18 years who were investigated for active TB with the performance of QFG-IT tests were included.

Exclusion criteria

The patients who were referred solely for contact tracing after exposure to active TB were excluded when sputum, gastric lavage fluid for acid-fast bacteria (AFB) staining, and culture for TB were not performed.

Definition of study groups

Active TB: Cases of active TB were defined according to the following three categories: (1) definite TB: all children had culture-confirmed TB; (2) probable TB: children in this group had symptoms and signs of active TB but had no bacterial confirmation; abnormal radiography consistent with TB and/or response to TB therapy; and (3) possible TB: signs and symptoms consistent with active TB and existing risk factors for active TB, such as history of TB contact.

Tuberculin skin testing

Two units (0.2 mL) of purified protein derivative RT23 (Staten Serum Institute, Copenhagen, Denmark) were applied to the volar surface of the left lower forearm according to the intradermal Mantoux method. The transverse diameter of skin induration recorded in millimeters 48—72 hours later were retrieved from the medical chart. Induration >10 mm was considered positive.

Laboratory assays

Peripheral blood samples were obtained for IGRAs according to the manufacturer's recommendations. All blood samples were processed within 4 hours of blood collection. The commercially available QFG-IT assays (Cellestis, Carnegie, Australia) were used in all examinations.

The cut-off value for a positive IFN-γ production in response to the QFG-IT were interpreted as positive if the IFN-γ level in response to Early Secreted Antigenic Target 6 kDa (ESAT-6), Culture Filtrate Protein 10 kDa (CFP10), or tuberculosis antigen (TB 7.7) was 0.35 IU/mL and $\geq$25% of the background value. The values were interpreted as negative if the IFN-γ level was <0.35 IU/mL or <25% of the background value, with the IFN-γ level in response to mitogen (the positive control) reaching at least 0.5 IU/mL. The result was interpreted as indeterminate if the IFN-γ level was <0.35 IU/mL or <25% of background value with the IFN-γ level in response to mitogen <0.5 IU/mL, or if the negative control was positive, irrespective of the IFN-γ response to ESAT-6, CFP-10, or TB 7.7 was set at 0.35 IU of IFN-γ. Absolute levels of antigen specific IFN-γ were calculated by subtracting the value of the negative control from the antigen-specific value.

Ethics

The institutional review board of Chang Gung Memorial Hospital approved the retrospective review of patients' chest radiographs and medical records for this study (CGMH 103-5143B). Informed consent was waived because of the retrospective nature of this study.

Statistical analysis

Non-normally distributed variable were present as median and range. The performance of TST and QFG-IT were assessed by calculating their sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Statistical analyses were performed using Stat 11 (Stat, Inc, College Station, TX, USA).

Results

Between January 2008 and June 2014, 70 children who had undergone the QFG-IT test in the Chang Gung Children's Hospital for active TB disease surveillance were enrolled into the study. Twenty-three cases were excluded from analysis because they were studied solely as contact investigation of TB without further bacteriologic or pathologic confirmation (Figure 1).

A total of 47 cases of suspected TB infections underwent QFG-IT tests. Eight patients had proven active TB disease, seven of them were confirmed using bacterial isolation, and one had "probable" TB by the pathologic finding of granuloma formations in the knee (Table 1). The median age of this cohort of patients was 10.2 years (range, 2.5 months to 18 years). There are 27 male and 20 female patients in the study. Six (8.6%) patients had underlying diseases. Comorbid diseases included: antineutrophil cytoplasmic antibody-associated vasculitis and chronic kidney disease postrenal transplantation ($n = 1$); acute myeloblastic leukemia ($n = 1$); acute lymphoblastic leukemia with peripheral blood progenitor cell transplantation ($n = 1$), Hodgkin lymphoma ($n = 1$), common variable immunodeficiency with bronchiectasis ($n = 1$); transient hypogammaglobulinema of infancy ($n = 1$), diabetes mellitus ($n = 1$).

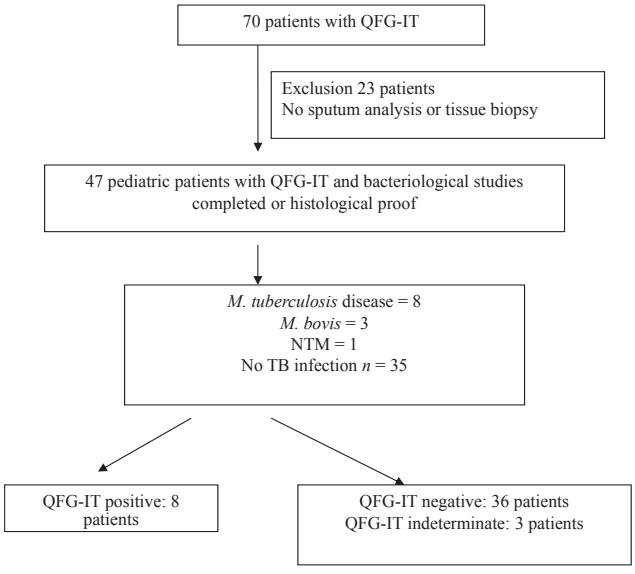

Figure 1. Flow chart of the study population. Of 70 patients with suspected pulmonary tuberculosis recruited, 47 were eligible for inclusion in the final analysis. QFG-IT = QuantiFERON-TB Gold in-Tube; NTM = nontuberculous mycobacterium; TB = tuberculosis.

The presenting symptoms for the evaluation of TB disease were: pneumonia ($n = 21$), pleural effusion ($n = 8$), lymphadenopathy/neck mass/axillary mass ($n = 7$), fever of unknown origin ($n = 6$), hemoptysis/bloody sputum ($n = 6$), bone or joint pain ($n = 4$), chronic cough >3 weeks ($n = 3$), respiratory distress or sepsis ($n = 2$), unexplained body weight loss ($n = 2$), single cases of abdominal distention, chest wall bulging, and seizure respectively.

Three patients had indeterminate QFG-IT results and were later confirmed not to have mycobacterial disease. The clinical profiles of the patients with definite TB disease and "probable" TB disease were presented in the Table 2. One patient suffered from acute lymphoblastic leukemia had allogeneic peripheral blood progenitor cell transplantation was diagnosed as having pulmonary nontuberculous mycobacterium (NTM) infection. Three patients had BCG related disease in the study. Case 10 presented with left knee pain for half a year before visiting our hospital. Case 11 was brought to our hospital due to weakness of lower limbs, which turned out to be BCG thoracic spondylitis (Figures 2 and 3). Case 12 was evaluated due to progressive parasternal protrusion for 1 month prior to referral (Figure 4).

The diagnostic results of TB infections in these 47 patients using TST, QFG-IT assays, and polymerase chain reaction (PCR) were tabulated in Table 3. TST has a sensitivity of 62.5%, specificity 57.1%, PPV of 62.5%, and NPV 87.5%. Regarding the QFG-IT test, the sensitivity, specificity, PPV, NPV were 100%, 97.6%, 88.9%, and 100% respectively (Table 2).

Discussion

In this retrospective study, 47 children were investigated for active TB by complete bacteriologic studies, as well as tests including TST, QFG-IT, and PCR. In this study only seven patients were bacteriologically-proven. The number was small because it reflected the true clinical conditions. IGRAs were not performed at extra expense for those patients with obvious clinical features of TB, consistent radiographic findings and positive AFB microscopy. Most patients in this study who underwent exhaustive examinations represented cases with complicating clinical conditions or equivocal laboratory results. The diagnosis of active TB has been difficult due to its paucibacillary nature.[4] The stringent bacteriology confirmation of TB in this study suggests that the diagnosis of TB disease in some children may be missed.

TST has been used to identify persons with TB infections but is notorious for false-positive and false-negative results.[6–9] The two most common causes of false positive

Table 1 Clinical features and laboratory results of 12 patients with a confirmed diagnosis of active tuberculosis.

Case	Age	Sex	Presentation	Diagnosis	TST	QFG-IT	AFB stain		TB culture		TB PCR	
							Sputum/ GA	other	Sputum/ GA	Other	Sputum/ GA	Other
1	15y	F	Fever for 6 d	Pul TB + TB pleurisy	neg	+	+	ND	+	ND	ND	PE neg-
2	17y 1mo	M	Cough for 1 mo	TB pleurisy	+	+	Neg	PE neg	+	PE neg	neg	PE neg
3	15y 6mo	M	Cough for 1mo	Pul TB	+	+	+	ND	+	ND	ND	ND
4	8y 11mo	F	Fever for 8 d	TB pericarditis, clavicle TB	neg	+	Neg	CE neg Bone neg	neg	CE neg Bone neg	ND	CE neg Bone +
5	16y 8mo	M	Abdominal distention	TB peritonitis	neg	+	Neg	Ascites +	neg	+	ND	Ascites +
6	8mo	F	Fever and seizure	TB meningitis	neg	+	Neg	CSF neg	+	CSF +	+	CSF neg
7	18y	M	Chronic cough	Pul TB	+	+	+	ND	+	ND	+	ND
8	16y	F	Abnormal screening CXR	Pul TB	+	+	Neg	ND	+	ND	ND	ND
9	15y 2mo	F	SOB, bloody sputum	Pul NTM APBPCT	ND	neg	+	ND	MAC	ND	complex neg	ND
10	3y	M	Left knee pain for half year	BCG arthritis	+	neg	ND	Tissue neg	ND	Tissue neg	ND	Tissue BCG +
11	1y 5mo	F	Bone pain and walking difficulty	BCG spondylitis, T7-8	+	neg	Neg	Tissue +	neg	Tissue BCG +	ND	Tissue BCG +
12	1y	M	Parasternal elevation	BCG sternal abscess	ND	neg	ND	ND	neg	Tissue BCG +	ND	Tissue BCG +

AFB = acid fast bacilli; APBPCT = allogeneic peripheral blood progenitor cell transplantation; BCG = Bacillus Calmette—Guérin; CE = cardiac effusion; CSF = cerebrospinal fluid; CVID = common variable immunodeficiency; GLF = gastric lavage fluid; ND = not done; neg = negative; NTM = nontuberculous mycobacterium; PCR = polymerase chain reaction; PE = pleural effusion; Pul = pulmonary; QFG-IT = QuantiFERON-TB Gold in-Tube; SOB = shortness of breath; TST = tuberculin test.

Table 2 Significance of laboratory results regarding mycobacterium infections.

	TST			QFG-IT			PCR		
	Pos	Neg[a]	ND	Pos	Neg[a]	ND	Pos	Neg[a]	ND
Mycobacterium tuberculosis disease ($n = 7 + 1$[b])	5	3	0	8	0	0	1	3	4
No *M. tb* infection ($n = 35$)	1	20	14	1	31 + 3*	0	0	15	20
M. bovis infection ($n = 3$)	2	0	1	0	3	0	3	0	0
NTM ($n = 1$)	0	0	1	0	1	0	0	1	0

[a] Indeterminates were considered negative in this study.
[b] Probable tuberculosis by biopsy with caseous necrosis but acid fast bacteria negative.
ND = not done; Neg = negative; NTM = nontuberculous mycobacterium; PCR = polymerase chain reaction; Pos = positive; QFG-IT = QuantiFERON-TB Gold in-Tube; TST = tuberculin skin test.

results are infections with NTM and prior BCG vaccination. The specificity of IGRAs has been reported to be higher than TST, because the antigens used are not found in BCG or most NTM infections, except for *M. kansaii*, *M. szulgai*, and *M. marinum*.[3,10] The sensitivity and specificity of TST of our study for BCG vaccinated children were 62.5% and 95.2%, respectively, with a comparable sensitivity of 50% to 70% and a higher specificity of 49% to 65% in literature.[6,9] In this study, an even higher sensitivity of 100% and specificity of 97.6% for the QFG-IT tests were found, as compared with previous reference of 60—80% and 89—100% of BCG-vaccinated children.[11—14] The high sensitivity and specificity of the QFG-IT tests can be attributed to highly selectivity of the patients of this study, because we only recruited highly probable TB patients so as to not include patients with latent TB infections.

IGRAs are the preferred test for TB infections in immunocompetent children ≥5 years of age with previous BCG vaccination. For children <5 years, 48 out of 50 children had good correlation between TST and QFT-IT had been reported.[14] In our study, only five (10.6%) patients were immunocompromised. No meaningful result regarding QFT-IT tests for young age and immunodeficiency conditions had been possible.

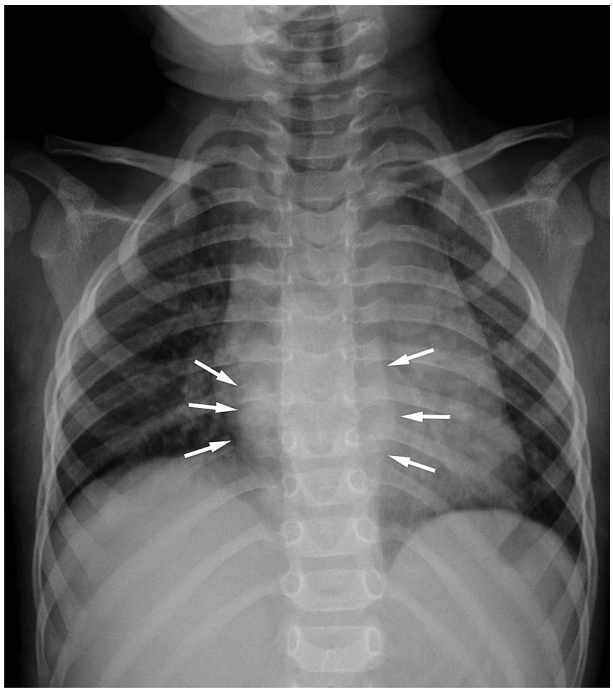

Figure 2. Bilateral paraspinal bulging between thoracic 6 and vertebrae 9 (arrows).

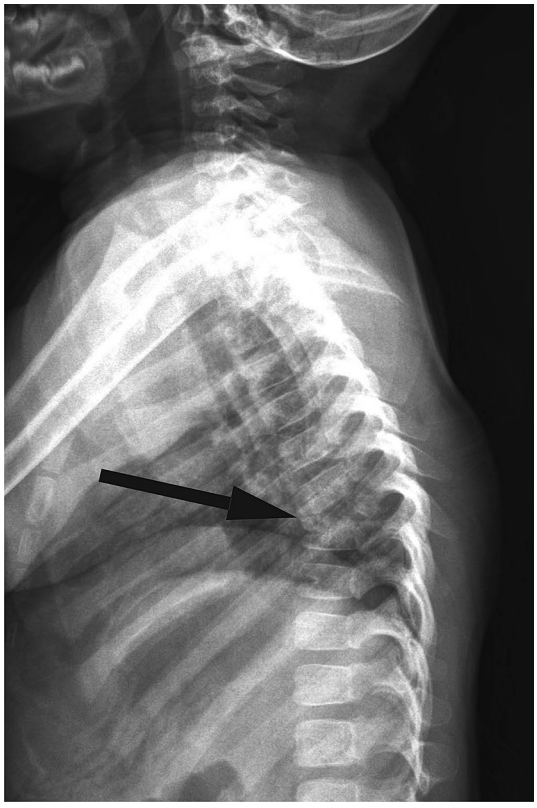

Figure 3. Chest lateral view reveals spondylitis of T7-8 with angular kyphosis and gibbus deformity (arrow).

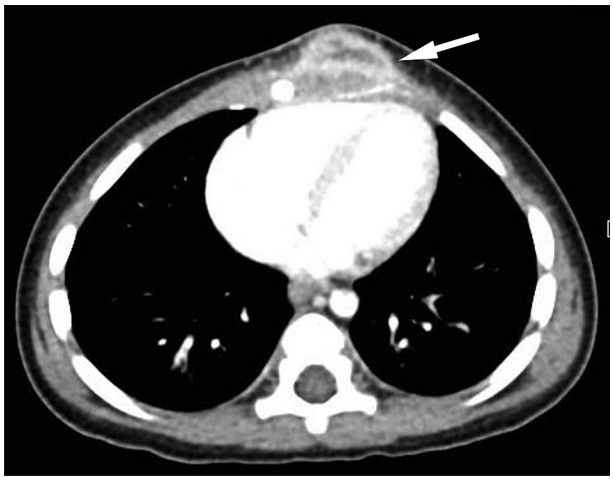

Figure 4. Chest computed tomography scan at low lung field reveals fusiform abscess at sternum with central necrosis (arrow).

IGRAs have not been able to discriminate between infections from disease.[3–6,15] In our study we excluded patients with latent TB infections, therefore the number of patients with false positive results decreases, resulting an increase of PPV.[5,16,17] The significance of negative IGRA tests remains controversial, however, the diagnosis of TB is very unlikely with a negative IGRA result.[12]

According to Table 1, nine specimens were sent for TB-PCR study, four of them were negative: pleural effusion ($n = 2$), pericardial effusion ($n = 1$), and cerebrospinal fluid ($n = 1$). Diagnosis of pleural TB is challenging due to its nonspecific clinical presentation and paucibacillary nature. Direct testing for AFB and culture of pleural fluid, lack sensitivity (<5% and 40%, respectively), but TB-PCR may increase sensitivity to 42%.[18] Most of the time, definitive diagnosis of pleural TB depends on demonstration of *M. tuberculosis* or caseous granulomas in pleural biopsy. Similarly, the sensitivity of PCR for TB pericarditis was only 15% with pericardial specimens but scored higher with tissue specimens (80%).[19] False-negative PCR results for exudative effusions reflected a low burden of *M. tuberculosis* organisms or hypersensitivity nature.

With an increasing awareness of vaccine strains osteomyelitis from neonatal BCG vaccination and modern molecular diagnostic technique, the diagnosis of BCG vaccine-induced complication become feasible.[20,21] Between 2008 and 2014, we identified three children with osteitis due to Tokyo-172 BCG strains by specific PCR tests in this case series.

Limitations

We acknowledge that a few limitations of our study exist. Firstly, our study focused on patients with high probability of TB disease in an endemic area with TB together with universal neonatal BCG program. Secondly, a proportion of TB cases may be missed by bacteriological culture and that would bias the estimates of sensitivity and specificity of the IGRA in this study. Thirdly, the number of patients below 2 years of age and immunodeficiency are low, therefore our

Table 3 Sensitivity, specificity, positive predictive values, and negative predictive values for tuberculin skin test and QuantiFERON-TB Gold in-Tube assay as the reference standard for tuberculous diseases.

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
TST	62.5 (24.5—91.5)	95.2 (76.2—99.9)	83.3 (41.7—99.6)	86.9 (67.0—96.3)
QFG-IT	100 (63.1—100)	97.1 (85.1—99.9)	88.9 (51.7—99.7)	100 (89.7—100)

CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value; QFG-IT = QuantiFERON-TB Gold in-Tube; TST = tuberculin skin test.

results may not be generalized to asymptomatic patients of young age and those living in areas of TB low-prevalence.

In conclusion, this study demonstrated that QFG-IT had a higher sensitivity of 100% compared with 62.5% for TST in a pediatric population of immediate-burden TB population with national BCG program. With increasing recognition of BCG osteitis, positive TST but negative QFG-IT is the first clue of extrapulmonary TB involvement of Tokyo-172 BCG strains.

Conflicts of interest

The authors declare no conflicts of interest with respect to the research, authorship, and publication of this article.

Acknowledgments

KS Wong, HC Hu, Yhu-Chering Huang, Lin TY contributed to conception, design, analysis and interpretation, drafting, and critical revision of the manuscript. Yen-Chun Huang, CH Wen contributed to data acquisition, analysis, and interpretation of the data.

References

1. Center for Disease Control. Tuberculosis Annual Report, 2001: Strategies for Tuberculosis Control. Taipei: Center for Disease Control, Department of Health, Taiwan.
2. Chan PC, Huang LM, Kuo HS. Is neonatal Bacillus Calmette-Guerin vaccination protective in Taiwan? *J Formos Med Assoc* 2008;**107**:195—7.
3. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recomm Rep* 2005;**54**:49—55.
4. Starke JR. Diagnosis of tuberculosis in children. *Pediatr Infect Dis J* 2000;**19**:1095—6.
5. Detjen AK, Keil T, Roll S, Hauer B, Mauch H, Wahn U, et al. Interferon-γ release assays improve the diagnosis of tuberculosis and nontuberculous mycobacterial disease in children in a country with a low incidence of tuberculosis. *Clin Infect Dis* 2007;**45**:322—8.
6. Connell TG, Ritz N, Paxton GA, Buttery JP, Curtis N, Ranganathan SC, et al. A three-way comparison of tuberculin skin testing, QuantiFERON-TB gold and T-SPOT.TB in children. *PLoS One* 2008;**3**:e2624.
7. Chan PC, Chang LY, Wu YC, Lu CY, Kuo HS, Lee CY, et al. Age-specific cut-offs for the tuberculin skin test to detect latent tuberculosis in BCG-vaccinated children. *Int J Tuberc Lung Dis* 2008;**12**:1401—6.
8. World Health Organization. *Guideline for national tuberculosis programs on the management of tuberculosis in children*. WHO/HTM/STB/2006.371. Geneva, Switzerland: WHO; 2006.
9. Comstock GW, Edwards LB, Philip RN, Winn WA. A comparison in the United States of America of two tuberculins, PPD-S and PPD RT 23. *Bull World Health Organ* 1964;**31**:161—70.
10. Belknap R, Daley CL. Interferon-gamma release assays. *Clin Lab Med* 2014;**34**:337—49.
11. Starke JR. Interferon- γ release assays for diagnosis of tuberculosis infection and disease in children. *Pediatrics* 2014;**134**: e1763—73.
12. American Academy of Pediatrics. Summaries of Infectious Diseases. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red Book: 2012 Report of the Committee on Infectious Diseases*. Elk Grove Village IL: American Academy of Pediatrics; 2012. p. 736—59.
13. Centers for Disease Control and Prevention. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection — United States, 2010. *MMWR Morbid Mortal Wkly Report* 2010;**59**:1—13.
14. Palvic I, Topic RZ, Raos M, Aberle N, Nodig S. Interferon-γ release assay for the diagnosis of latent tuberculosis in children younger than 5 years of age. *Pediatr Infect Dis* 2011;**30**: 866—70.
15. Kampmann B, Whittaker E, Williams A, Walters S, Gordon A, Martinez-Alier N, et al. Interferon-γ release assays do not identify more children with active tuberculosis than the tuberculin skin test. *Eur Respir J* 2009;**33**:1374—82.
16. Mandalakas AM, Detjen AK, Hesseling AC, Benedetti A, Menzies D. Interferon-gamma release assays and childhood tuberculosis systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2011;**15**:1018—32.
17. Lodha R, Mukherjee A, Saini D, Saini S, Singh V, Singh S, et al. Role of QuantiFERON-TB In-Tube test in the diagnosis of intrathoracic childhood tuberculosis. *Int J Tuberc Lung Dis* 2013;**17**:1383—8.
18. Trajman A, da Silva Santos Kleiz de Oliveira EF, Bastos ML, Belo Neto E, Silva EM, da Silva Lourenco MC, et al. Accuracy of polymerase chain reaction for the diagnosis of pleural tuberculosis. *Resp Med* 2014;**108**:918—23.
19. Cegielski JP, Devlin BH, Morris AJ, Kitinya JN, Pulipaka UP, Lema LE, et al. Comparison of PCR, culture, and histopathology for diagnosis of tuberculous pericarditis. *J Clin Microbiol* 1997;**35**:3254—7.
20. Jou RW, Huang WL, Su WJ. Tokyo-172 BCG vaccination complications, Taiwan. *Emerg Infect Dis* 2009;**15**:1525—6.
21. Kwon HJ, Chung BH, Choi BM, Park KU, Kim YK. Severe osteomyelitis as a complication of Tokyo-172 BCG vaccination. *J Korean Med Sci* 2012;**27**:221—4.