Usefulness of the Combination of Tuberculin Skin Test and Interferon-Gamma Release Assay in Diagnosing Children with Tuberculosis

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Japan is still designated as a medium incidence country, worldwide. Although the definitive diagnosis of tuberculosis is made by detecting pathogen from tissue specimens, collecting those from children is physically difficult. Therefore, we must frequently depend on the results of tuberculin skin test (TST) or interferon-gamma release assay (IGRA). However, the value of IGRA in diagnosing tuberculosis in infants remains unclear. We implemented TST and IGRA concurrently and examined the significance of combining these two tests by analyzing cases where conflicting results were evident from other tests. Subjects included 52 children (24 boys, 28 girls) age 15 years or younger (4.0 ± 3.4 years) who visited our hospital for close examination after medical checkup for tuberculosis contact tracing. The children underwent TST, T-SPOT®.*TB*, chest plain radiography, and chest computed tomography with a view to tuberculosis diagnosis during a 26-months period from January 2013 to March 2015. T-SPOT results were positive in 9/52 cases (17.3%), and TST results were positive in 12/52 cases (23.1%). Conflicting results were observed in 7/52 cases (13.5%), and all cases were treated as latent tuberculosis infection or incident cases of tuberculosis. The value of T-SPOT in tuberculosis diagnosis in children is still being established. However, there are a number of cases where conflicting results for T-SPOT and TST are indicated. Thus, it is currently desirable to use T-SPOT and TST concomitantly for children at an early age, especially for children below the age of 5 years, as a diagnostic measure in complementing other techniques.

Introduction

The number of tuberculosis cases in children is decreasing in Japan with a similar decrease in adults. In recent years, fewer than 100 registered patients under 15 years old are diagnosed with tuberculosis annually (Tuberculosis Surveillance Center 2016). Accordingly, it is very rare for general pediatricians to see tuberculosis cases. However, the prevalence of tuberculosis among all age groups was 15.4 diagnosed cases for every 100,000 people in 2014 (Izumi et al. 2017); consequently, Japan is still designated as a medium incidence country, worldwide. Hence, tuberculosis cannot yet be considered a rare disease in Japan. The source of infection in most instances of infant tuberculosis is adults, especially family members who have developed open tuberculosis. Therefore, it is important that latent tuberculosis infection (LTBI) cases be treated properly if the number of infant patients with tuberculosis is to remain small, given the prevalence of tuberculosis in Japan.

The definitive diagnosis of tuberculosis is made by detecting *Mycobacterium tuberculosis* from infected tissue specimens (Joos et al. 2006). However, collecting specimens from children is often physically difficult, and the positive rate in culture tests is also low when compared with adults (Cruz and Starke 2007). For those reasons, the tuberculin skin test (TST) has been used as a diagnostic

method for tuberculosis infection. TST is sensitive, but also reacts to Bacille de Calmette et Guerin (BCG). Therefore, the false-positive rate is a problem associated with TST. Consequently, the interferon-gamma release assay (IGRA) is often used as a technique to compensate for the shortcomings of TST. However, it has been suggested that the same sensitivity to IGRA in adults may not be present in infants without mature cell-mediated immunity (Jenum et al. 2014); namely, IGRA has excellent specificity but depends on the patient immune response.

Although some data suggest that IGRA is now useful for testing infants because IGRA technology has improved (Bianchi et al. 2009; Hesseling et al. 2009; Cruz et al. 2011; Kobashi et al. 2012), most of these studies have been conducted in patients who had already developed tuberculosis. Thus, it is unclear whether sensitivity is similar for cases with LTBI, where the patient still has a limited number of bacteria. In addition, it is inconclusive whether diagnosis of tuberculosis infection in children can be completely changed from use of the existing TST to the more recently developed IGRA.

To answer this clinical question, we implemented TST and IGRA for concurrent diagnosis of tuberculosis infection in infants and examined the significance of combining these two tests by analyzing cases where conflicting results were indicated using other tests.

Materials and Methods

Study population

Patients included 52 children age 15 years or younger who, at the request of a welfare and health center, visited Yokohama City University Medical Center for close examination after medical checkup for tuberculosis contact tracing. Patients underwent TST, T-SPOT®.*TB* (T-SPOT; Oxford Immunotec Limited, Abingdon, United Kingdom), chest plain radiography and chest plain computed tomography (CT) with a view to tuberculosis diagnosis during a 26-month period from January 2013 to March 2015. We explained the significance and purpose of the simultaneous TST and T-SPOT testing, and obtained informed consent from all patients and their parents. Medical information, including the diagnostic result of TST, BCG vaccination history, findings from chest plain CT, tuberculosis diagnosis (uninfected, LTBI, pulmonary tuberculosis) and treatments, was collected from a retrospective examination of electronic health records. This research has been approved by the ethics committee of Yokohama City University.

Tuberculin skin test

TST was performed using the Mantoux method by an experienced pediatrician. Injection of 10 U of purified protein derivative was administered to the volar surface of each patient's forearm. The transverse redness and induration diameter was assessed 48 to 72 hours later by the ruler method. In Japan, routine BCG vaccination was started since 2005; therefore, most Japanese children are TST positive according to American Thoracic Society and Centers for Disease Control and Prevention recommendations. Thus, unique standards are in place to consider the potential for false-positive results caused by BCG vaccination in Japan. The criteria for TST determination used in this study were compliant with the standards of the Japanese Society for Tuberculosis (Table 1) (The Japanese Society for Tuberculosis 2006). A positive TST result was defined as an induration > 15 mm or redness > 30 mm according to those criteria.

Interferon-gamma release assay (IGRA)

Two IGRA methods, T-SPOT and QuantiFERON®-TB Gold In-Tube assay (Cellestis Inc, Carnegie, Australia), are currently commercially available in Japan. We selected T-SPOT because this method has higher sensitivity in immunosuppressive conditions (Komiya et al. 2010; Cattamanchi et al. 2011) and requires less blood volume. At least 5 ml of venous blood was collected into a heparinized blood-collecting tube which was performed along with blood cell count and biochemical examination, and the blood samples were outsourced for the assay. Peripheral blood mononuclear cells (PBMCs) were separated from the collected whole blood and adjusted to a prescribed cell number. Then, PBMC specimens were added to microplate wells with immobilized anti-IFN-γ antibody, and reacted with two kinds of specific *Mycobacterium tuberculosis* antigens (ESAT-6 and CFP-10) for a period of between 16 and 20 hours. Wells were washed, and then labeled antibody reagent was added. The wells were washed again to remove unbound antibody, and then substrate was added. Finally, the number of T-cells producing IFN-γ could be recognized as spots. The presence or absence of infection was determined based on the number of spots.

Imaging test

Front and lateral chest plain radiography as well as chest plain CT was implemented in all cases, as a previous series had suggested nodular shadows on chest CT images and evidence of germ discharge without obvious abnormal findings on chest plain radiographs (Ushiki et al. 2014). Therefore, chest CT was performed in all cases to maximize the sensitivity. Experienced radiologists read the images. Before each reading, the radiologist was informed about patients who were being examined for tuberculosis contact tracing.

Table 1. Criteria for significant reaction based on the results of the tuberculin test.

		history of exposure to infectious pulmonary tuberculosis	
		−	+
history of BCG vaccination	−	induration ≧ 15 mm or redness ≧ 30 mm	induration ≧ 5mm or redness ≧ 10 mm
	+	induration ≧ 20 mm or redness ≧ 40 mm	induration ≧ 15 mm or redness ≧ 30 mm

Diagnosis

There is no gold standard test for LTBI; therefore, we have no choice but to confirm positive T-SPOT or make a diagnosis based on the degree of exposure or duration of contact in a comprehensive way, regardless of the of T-SPOT or TST results. Diagnosis of pulmonary tuberculosis was made by a positive T-SPOT or TST result and abnormal findings on chest CT determined to not be any other respiratory infection. There were no pulmonary tuberculosis cases confirmed by culture or PCR.

Results

The subjects included 24 boys and 28 girls who were 15 years old or younger. The mean age was 4.0 ± 3.4 years. Five patients (9.6%) were definitively diagnosed as uninfected for TST and T-SPOT negative with limited duration of contact with confirmed patient and low exposure risk, 40 patients (76.9%) were diagnosed as LTBI, and seven (13.5%) were diagnosed as incident cases of tuberculosis (Table 2). The details of LTBI cases are shown in Table 3. T-SPOT positive results comprised 9/52 patients (17.3%), and TST positive results comprised 12/52 patients (23.1%). A total of 7/52 patients (13.5%) were positive for both T-SPOT and TST, and 38/52 patients (73.1%) were negative for both T-SPOT and TST. There were 7/52 cases (13.5%) with conflicting results. These included 2/52 (3.8%) T-SPOT positive/TST negative cases, and 5/52 (9.6%) T-SPOT negative/TST positive cases. The details of cases with conflicting results are shown in Table 4.

One T-SPOT positive/TST negative case was a 1-year old boy who had nodular shadows observed by CT. He was diagnosed with pulmonary tuberculosis and received multidrug therapy (2HRZ/4HR: 2 months of isoniazid, rifampicin and pyrazinamide and 4 months of isoniazid and rifampicin). Furthermore, one T-SPOT negative/TST positive case was an 11-month old girl who had nodular shadows observed by CT. She was diagnosed with pulmonary tuberculosis and received multidrug therapy (2HRZ/4HR). All other cases with conflicting results were diagnosed as having LTBI and were internally administered isoniazid for 6 months.

Of the 12 TST positive cases, the mean ages of seven T-SPOT positive cases and five T-SPOT negative cases were 8.0 ± 9.4 and 1.8 ± 2.4 years, respectively; indeed, the mean age of the latter group was significantly lower (p = 0.025).

Discussion

Making a definitive diagnosis of latent tuberculosis infection is not straightforward. When TST and T-SPOT assays were concurrently implemented for children who visited our hospital for a medical checkup for tuberculosis contact tracing, 7/52 cases (13.5%) indicated conflicting results. If LTBI goes without treatment in younger children, there is a much higher possibility of severe tuberculosis developing (Marais et al. 2004). Therefore, when conflicting positive and negative results are obtained, it is better to adopt a precautionary stance and consider any conflicting result as a false-negative, thus providing the opportunity for proper treatment. This is preferable to considering a conflicting result as a false-positive.

There were two cases where a T-SPOT positive/TST negative pattern were observed in the present study. These results could be construed as false-positive by T-SPOT or false-negative by TST. For the former test, the T-SPOT is conducted with a reaction system using ESAT-6 and CFP-10, which are human tuberculosis-specific antigens and are not affected by BCG (Lewinsohn 2009). Although some acid-fast bacilli, such as *M. kansasii* have been shown to cause T-SPOT positivity, a positive result by T-SPOT strongly suggests *M. tuberculosis* infection regardless of the TST result considering the pretest probability. For the latter instance, it may be that there was a technical error in the TST assay. Because TST is determined by intradermal inoculation, the result is a false-negative when the inoculation is done subcutaneously. In addition, the causes of false-negative results in TST include administration of corticosteroids and anergic conditions due to severe tuberculosis (Huebner et al. 1993). However, no immunosuppressive

Table 2. Number of tuberculosis diagnosis by the results of T-SPOT and TST.

		T-SPOT		total
		negative	positive	
TST	negative	38 (73.1%)	2 (3.8%)	40 (76.9%)
		5 uninfected 33 LTBI	1 LTBI 1 pulmonary tuberculosis	5 uninfected 34 LTBI 1 pulmonary tuberculosis
	positive	5 (9.6%)	7 (13.5%)	12 (23.1%)
		4 LTBI 1 pulmonary tuberculosis	2 LTBI 5 pulmonary tuberculosis	6 LTBI 6 pulmonary tuberculosis
total		43 (82.7%)	9 (17.3%)	52 (100%)
		5 uninfected 37 LTBI 1 pulmonary tuberculosis	3 LTBI 6 pulmonary tuberculosis	5 uninfected 40 LTBI 7 pulmonary tuberculosis

TST, tuberculin skin test; LTBI, latent tuberculosis infection.

Table 3. Details of 40 LTBI cases which were diagnosed based on some circumstances.

Age	Sex	T-SPOT			TST (mm)			BCG	Duration of exposure (hours)	Living together with confirmed patient	Other infected patients among screened population
		ESAT-6	CFP-10		induration	redness	double redness				
2y	F	0	0	Negative	Unknown	15 × 5	-	Negative	Done	30	No	No
1m	M	0	0	Negative	0 × 0	0 × 0	-	Negative	Not done	9	No	No
7m	F	2	1	Negative	0 × 0	3 × 3	-	Negative	Not done	45	No	No
5y	M	3	0	Negative	0 × 0	0 × 0	-	Negative	Not done	240	No	No
8m	M	0	0	Negative	4 × 4	4 × 4	-	Negative	Not done	Unknown	No	Yes
4y	F	−1	−1	Negative	9 × 6	22 × 18	-	Negative	Done	42	No	Yes
6y	F	0	0	Negative	7 × 7	23 × 18	-	Negative	Done	192	No	No
8m	M	0	0	Negative	0 × 0	2 × 2	-	Negative	Done	180	No	No
3y	F	0	0	Negative	5 × 5	13 × 11	-	Negative	Done	12	No	No
3y	F	1	0	Negative	0 × 0	10 × 6	-	Negative	Done	68	No	No
10y	F	1	0	Negative	0 × 0	5 × 4	-	Negative	Done	> 3months	Yes	Yes
8y	M	1	0	Negative	4 × 3	16 × 12	-	Negative	Done	> 3months	Yes	Yes
4y	M	1	0	Negative	0 × 0	7 × 6	-	Negative	Done	13	No	No
1y	M	0	0	Negative	4 × 3	8 × 6	-	Negative	Done	13	No	No
4y	M	1	0	Negative	3 × 3	11 × 5	-	Negative	Done	> 3months	Yes	Yes
5m	F	0	0	Negative	9 × 8	21 × 16	-	Negative	Done	> 3months	Yes	Yes
1y	F	0	0	Negative	8 × 6	20 × 15	-	Negative	Done	8	No	No
2y	M	0	0	Negative	1 × 1	10 × 9	-	Negative	Done	8	No	No
2y	M	1	0	Negative	4 × 4	9 × 9	-	Negative	Done	2months	Yes	No
7y	F	0	0	Negative	0 × 0	16 × 15	-	Negative	Done	> 3months	Yes	No
8y	M	0	0	Negative	7 × 6	16 × 16	-	Negative	Done	> 3months	Yes	Yes
5y	M	0	0	Negative	0 × 0	6 × 6	-	Negative	Done	> 3months	Yes	Yes
10y	F	0	1	Negative	10 × 9	21 × 16	-	Negative	Done	24	No	No
5y	M	0	0	Negative	6 × 6	8 × 8	-	Negative	Done	24	No	No
1y	F	2	−2	Negative	9 × 7	22 × 22	-	Negative	Done	> 3months	Yes	No
8m	F	0	0	Negative	9 × 7	23 × 22	-	Negative	Done	> 3months	Yes	No
3y	F	0	0	Negative	5 × 5	8 × 8	-	Negative	Done	24	No	No
8y	F	0	0	Negative	0 × 0	5 × 4	-	Negative	Done	> 3months	Yes	Yes
4y	M	0	1	Negative	0 × 0	5 × 4	-	Negative	Done	> 3months	Yes	Yes
3y	M	0	0	Negative	0 × 0	7 × 6	-	Negative	Done	> 3months	Yes	No
4y	F	0	0	Negative	0 × 0	4 × 4	-	Negative	Done	120	Yes	No
4y	M	1	1	Negative	5 × 4	11 × 9	-	Negative	Done	72	No	No
6y	M	0	0	Negative	4 × 4	9 × 9	-	Negative	Done	2months	Yes	No
12y	M	43	> 50	Positive	11 × 11	11 × 11	36 × 27	Positive	Done	> 3months	Yes	No
13y	F	35	> 50	Positive	18 × 15	43 × 32	-	Positive	Unknown	> 3months	Yes	Yes
5y	F	8	26	Positive	11 × 11	25 × 16	-	Negative	Done	> 3months	Yes	Yes
4y	F	0	0	Negative	11 × 11	30 × 21	-	Positive	Done	> 3months	Yes	No
1y	F	2	0	Negative	17 × 15	33 × 22	-	Positive	Done	> 3months	Yes	No
2y	F	0	0	Negative	7 × 7	7 × 7	21 × 19	Positive	Done	24	No	No
1y	M	1	0	Negative	17 × 16	18 × 18	-	Positive	Done	56	No	No

LTBI, latent tuberculosis infection; TST, tuberculin skin test; BCG, Bacille de Calmette et Guerin.

agents were administered, and severe tuberculosis was not clinically suspected in the two cases in this study.

There were five cases where T-SPOT negative/TST positive was observed. This pattern may be caused by a false-negative result of the T-SPOT or a false-positive result of the TST. First, for the former suggestion, false-negative results of IGRA in young children who have no established cell-mediated immunity have been viewed with suspicion. A meta-analysis that examined the efficacy of T-SPOT in diagnosis of tuberculosis in children also pointed out the possibility that T-SPOT cannot accurately detect LTBI in children younger than 5 years old (Mandalakas et al. 2011). A possibility of false-negative results by T-SPOT due to young age was also fully assumed in our study because the mean age of the group of patients that were T-SPOT negative and TST positive was low. It is possible that a low specificity against acid-fast bacilli is possible if we consider the latter scenario. TST returns positive results against acid-fast bacilli, which include a number of non-tuberculous mycobacteria (Haimi-Cohen et al. 2001). In addition, cross-sensitivity with BCG is often a problem, especially in the Japanese population (Wang et al. 2002). BCG vaccination is regularly offered in Japan; therefore, the rate of BCG vaccination is very high in Japan. Accordingly, diagnosis of tuberculosis infection using TST should be made with caution. The Japanese Society for Tuberculosis has issued tentative standards that define strict criteria for determining positive cases as shown in Table 1. However, such stan-

Table 4. Details of cases where T-SPOT and TST results conflicted.

	Case No.	Age	Sex	T-SPOT		TST (mm)			BCG	CT findings	Culture	Diagnosis	Treatment
				ESAT-6	CFP-10	induration	redness	double redness					
T-SPOT positive and TST negative	1	5y	F	8	26	11 × 11	25 × 16	-	Done	n.p.	Not done	LTBI	6H
	2	1y	M	36	2	7 × 5	13 × 11	-	Done	nodule	Negative	Pulmonary TB	2HRZ + 4HR
T-SPOT negative and TST positive	3	4y	F	0	0	11 × 11	30 × 21	-	Done	n.p.	Not done	LTBI	6H
	4	1y	F	2	0	17 × 15	33 × 22	-	Done	n.p.	Not done	LTBI	6H
	5	2y	F	0	0	7 × 7	7 × 7	21 × 19	Done	n.p.	Not done	LTBI	6H
	6	1y	M	1	0	17 × 16	18 × 18	-	Done	n.p.	Negative	LTBI	6H
	7	11m	F	1	2	16 × 14	10 × 6	26 × 24	Done	nodule	Negative	Pulmonary TB	2HRZ + 4HR

TST, tuberculin skin test; BCG, Bacille de Calmette et Guerin; CT, computed tomography; LTBI, latent tuberculosis infection.

dards cannot exclude the effect of BCG with absolute certainty. The reason for the low mean age of the group indicated as T-SPOT negative/TST positive might be because the reaction to TST was strong in young children due to the short period post-BCG vaccination.

As mentioned above, both false-positive and false-negative results of TST cannot be ignored, and the sensitivity and specificity of these tests are not remotely acceptable as independent. However, TST is both inexpensive and easy to use, so its value is still evident in developing countries (Detjen et al. 2007; Tieu et al. 2014). The World Health Organization also recommends that priority be given to TST over IGRA, especially in countries with low-income and a high disease burden. There were 12/52 cases (23.1%) determined as being TST-positive in this study. Nodular shadows or swelling of the hilar area and mediastinal lymph nodes were observed by chest CT in six cases (50%). Of the 40 TST-negative cases, however, similar findings by chest CT were observed only in four cases (10%). These data are insufficient to fully discuss the sensitivity of TST for tuberculosis infection because no abnormal findings can be obtained by chest CT in LTBI, and there is no gold standard for diagnosis of LTBI. However, when the TST result was positive, abnormal findings were observed on CT in half the cases. In other words, considering the logistical ease of TST, its importance cannot be denied.

In conclusion, it is a responsible and inevitable decision to provide treatment for LTBI when at least one of these tests is positive because it is difficult to accurately identify the causes of conflicting results in TST and T-SPOT. Diagnosis of tuberculosis infection in children is difficult, and there are public health concerns that could significantly increase the risk of contracting the disease. Consequently, a certain degree of over-diagnosis cannot be avoided.

This study has several limitations. First, it was conducted in a single facility and with a small number of cases. Because infant tuberculosis has become rarer, a larger sample of cases from multiple facilities needs to be analyzed. Second, it is not possible to make a definitive diagnosis of LTBI. It is unclear whether the cases that indicated conflicting results by T-SPOT and TST in this study were truly LTBI.

The value of T-SPOT in tuberculosis diagnosis in children is being established. However, there are a significant number of cases indicating conflicting results when both T-SPOT and TST are used. It is currently desirable to use T-SPOT and TST concomitantly for children at an early age, especially for those below the age of 5 years, as complementary diagnostic measures.

Author Contributions

Conflict of Interest

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