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Discordance between Two Interferon-Gamma Release Assays in the Diagnosis of Latent Tuberculosis Infection in Healthcare Workers

Hiroshi Fujiwara[1†], Tomoyasu Nishimura[4†], Osamu Iketani[1], Yaoko Takano[1], Akiko Sakai[3], Naomi Kondo[3], Kazuko Ohtake[3], Shuji Oguchi[3], Nobuko Shimizu[3], Ayako Shibata[3], Masatoshi Wakui[2], Mitsuru Murata[2], Masaaki Mori[4], Satoshi Iwata[1] and Naoki Hasegawa[1*]

[1]*Center for Infectious Diseases and Infection Control, Japan*

[2]*Department of Laboratory Medicine, Keio University School of Medicine, Tokyo, Japan*

[3]*Central Clinical Laboratory, Keio University Hospital, Tokyo, Japan*

[4]*Health Center, Keio University, Kanagawa, Japan*

[*]***Corresponding author:*** *Naoki Hasegawa, Center for Infectious Diseases and Infection Control, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan, Tel: +81-3-5363-3710; Fax: +81-3-5363-3711; E-mail:* n-hasegawa@z8.keio.jp

[†]These authors contributed equally to this work.

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Abstract

Background: Interferon-gamma release assays (IGRAs) more accurately diagnose *Mycobacterium tuberculosis* (Mtb) infection than the tuberculin skin test. To prevent outbreaks in medical facilities, early detection and treatment of Mtb infection (including latent tuberculosis infection (LTBI)) is important in healthcare workers. Therefore, the IGRAs have considerable utility for Mtb infection control in medical facilities. In Japan, two IGRAs are commercially available, QuantiFERON®-TBGold In-Tube assay (QFT-GIT) and T-SPOT®.TB (T-SPOT). However, it remains unclear if diagnostic yields of LTBI by both IGRAs are equivalent in healthcare workers.

Methods: We performed both QFT-GIT and T-SPOT simultaneously in healthcare workers with a high risk of LTBI (excluding active tuberculosis) between December 2012 and February 2013.

Results: Among 313 subjects (excluding 2 cases with indeterminate T-SPOT), 6 (1.9 %) and 12 (3.8 %) were QFT-GIT positive and T-SPOT positive, respectively. There was no significant concordance of results between the QFT-GIT and the T-SPOT (p=0.064 and Kappa=0.43, 95% confidence interval 0.082-0.78). Among 10 discordant cases between two IGRAs, 8 cases had IGRAs' results near the cutoff values.

Conclusion: Without a diagnostic gold standard for LTBI, it is difficult for us to further assess which test is more accurate and more suitable for the diagnosis of LTBI. However, to diagnose LTBI of healthcare workers with IGRAs' results near the cutoff values, we should consider clinical context, such as contact level, as well as the results of IGRAs.

Keywords: Interferon-Gamma Release Assay; Latent Tuberculosis Infection; Healthcare worker

Abbreviations

IGRA: Interferon-Gamma Release Assay; Mtb: Mycobacterium tuberculosis; LTBI: Latent Tuberculosis Infection; QFT-GIT: QuantiFERON®-TB Gold In-Tube assay; T-SPOT: T-SPOT®.TB; TST: Tuberculin Skin Test; JSTB: Japanese Society for Tuberculosis; PBMCs: Peripheral Blood Mononuclear Cells; CDC: Centers for Disease Control and Prevention

Introduction

In Japan, approximately 20,000 people develop tuberculosis per year, and the incidence rate is approximately 16 per 100,000 people, which is 5 times that of the USA. Unfortunately, Japan has not taken yet its place among low incidence countries. Tuberculosis is a contagious, air-borne disease with the possibility of outbreaks of infection. In particular, medical facilities are at high risk for the outbreak of *Mycobacterium tuberculosis* (Mtb) infection, in part because of the population of immunocompromised patients. To prevent outbreaks of Mtb infection, patients with latent tuberculosis infection (LTBI) or active tuberculosis should be identified and treated with anti-tuberculosis drugs immediately in medical facilities.

Conventionally, the tuberculin skin test (TST) was widely used for indirect detection of Mtb infection, but TST use was limited by the high false-positive rate caused by bacilli Calmette-Guerin vaccination in Japan. The interferon-gamma release assays (IGRAs), which measure interferon-gamma produced by effector T lymphocytes stimulated with Mtb-specific antigens, are new assays developed this century for detection of Mtb infection. While chest X-ray and sputum culture test are useful to detect active pulmonary tuberculosis, the IGRAs are more accurate than the TST in detection of LTBI [**1**]. At the present time, IGRAs are recommended for LTBI identification by the Japanese Society for Tuberculosis (JSTB) [**2**] and two IGRAs are commercially available in Japan, QuantiFERON®-TBGold In-Tube assay (QFT-GIT, Qiagen, Hilden, Germany) and T-SPOT®.TB (T-SPOT, Oxford Immunotec, Abingdon, UK). However, it remains unclear if diagnostic yields of LTBI by both IGRAs are equivalent in healthcare workers. To evaluate the consistency of two tests in

diagnosis of LTBI, we performed both QFT-GIT and T-SPOT in subjects with a high risk of LTBI (excluding active TB).

Methods

Study population

Between December 2012 and February 2013, QFT-GIT and T-SPOT were simultaneously performed in 315 healthcare workers from Keio University Hospital, following approval from the Institutional Ethics Committee of the Keio University School of Medicine (2012-343). 71 healthcare workers were TB contacts and 244 were in the high risk group with possible Mtb contact. In total, there were 208 women and 107 men aged 20-65 years (mean 35). The healthcare workers were 129 nursing staff, 76 medical doctors, 42 medical processors, 37 pathological department staff, 17 laboratory staff, and 14 radiation technologists. The high risk group with possible Mtb contact was defined as the healthcare workers in the departments with the possibility of exposure to patients with active pulmonary TB or Mtb.

The interferon-gamma release assays

Two blood samples were collected simultaneously to perform the tests in-house according to the manufacturers' instructions. Briefly, blood samples for QFT-GIT were incubated within 4 hours after blood collection, and blood samples for T-SPOT were collected and after 16-32 hours were treated with T-cell Xtend® (Oxford Immunotec, Abingdon, UK) before processing in the assay. The QFT-GIT and T-SPOT results were interpreted according to the manufacturer's instructions. Our clinical laboratory performs IGRAs more than 20 times a week and exercises regular quality control of them.

Statistical methods

The concordance of results between the QFT-GIT and the T-SPOT were evaluated by the calculation of the kappa value and test of coincidence. A P value <0.05 was considered statistically significant.

Results

Among 313 subjects (excluding 2 cases with indeterminate T-SPOT), 6 (1.9 %) and 12 (3.8 %) were QFT-GIT positive and T-SPOT positive, respectively (Table 1). There was no significant concordance of results between the QFT-GIT and the T-SPOT (p=0.064 and Kappa=0.43, 95% confidence interval 0.082-0.78). Table 2 shows the characteristics of the 10 cases with discordant results between the two tests (2 cases with QFT-GIT positive/T-SPOT negative and 8 cases with QFT-GIT negative/T-SPOT positive).

		T-SPOT results		
		positive	negative	total
QFT-GIT	results			
	positive	4	2	6
	negative	8	299	307
	total	12	301	313

Table 1: Comparison of QFT-GIT results and T-SPOT results

	Age	**Sex**	**Occupation** [2]	**Group**[3]	**QFT-GIT**				**T-SPOT**				
	(Years)	(M/F)[1]			Interpretation	Antigen[4]	Mitogen[5]	Nil[6]	Interpretation	ESAT-6[7]	CFP-10[8]	Mitogen[9]	Nil[10]
1	45	M	MD	High risk	Positive	4.55	8.15	1.96	Negative	0	0	336	0
2	28	M	MD	High risk	Positive	0.9	9.72	0.05	Negative	1	1	412	0
3	43	M	MD	High risk	Negative	0.74	9.13	0.06	Positive	12	0	666	0
4	29	F	NS	High risk	Negative	0.19	11.87	0.39	Positive	-1	8	350	2
5	43	F	LS	High risk	Negative	0.18	10.65	0.06	Positive	28	13	686	0
6	43	F	NS	Contact	Negative	0.14	12.52	0.07	Positive	9	0	528	0
7	24	F	NS	High risk	Negative	0.17	11.47	0.02	Positive	1	7	533	0
8	51	M	MD	High risk	Negative	0.05	8.82	0.05	Positive	6	7	595	0
9	37	F	PS	High risk	Negative	<0.00	8.14	1.24	Positive	0	7	255	0
10	26	F	NS	High risk	Negative	<0.00	9.31	0.06	Positive	0	6	400	0

[1]M: Male, F: Female; [2]MD: Medical doctor, NS: Nursing staff, LS: Laboratory staff, PS: Pathological department staff; [3]Contact: Tuberculosis contacts, High risk: The high risk group with Mycobacterium tuberculosis (Mtb) contact; [4]The interferon gamma concnetration in plasma from blood stimulated with Mtb-specific antigens (ESAT-6. CFP-10 and TB7.7) minus Nil; [5]The interferon gamma concnetration in plasma from blood stimulated with mitogen minus Nil; [6]The interferon gamma concnetration in plasma from blood incubated without antigen, [7]The greater number of spots resulting from stimulation of peripheral blood mononuclear cells (PBMCs)

with ESAT-6 minus Nil; [8]The greater number of spots resulting from stimulation of PBMCs with CFP-10 minus Nil; [9]The number of spots resulting from stimulation of PBMCs with mitogen; [10]The number of spots resulting from incubation of PBMCs in culture media without antigens

Table 2: The characteristics of 10 cases with the discordance of results between QFT-GIT and T-SPOT

Discussion

Our study showed that the positive rate of two IGRAs was approximately 3%. Although it is difficult for us to determine whether the specificity of two IGRAs in the diagnosis of LTBI is appropriate since there is no gold standard for LTBI, Harada et al. reported that the positive rate of the IGRA was 3.1% for comparatively-young Japanese healthcare workerssimilar to our subjects [3]. With reference to this, the specificity of two IGRAs seemed reasonable in our study. Our further analysis indicated that the concordance between the results of two IGRAs was moderate in according to the criteria for the strength of agreement beyond chance for various ranges of kappa value [4]. Previous reports have highlighted differences in sensitivity and specificity in active tuberculosis between the QFT-GIT and the T-SPOT [5,6]. The cross-sectional comparison study among military recruits was performed to assess the agreement between T-SPOT and QFT-GIT for LTBI in USA [7]. It showed kappa value was 0.39, which was similar to our result. Although based on the same principle, there are some methodological differences between the QFT-GIT and the T-SPOT. The QFT-GIT is an enzyme-linked immunosorbent assay-based, whole-blood test that uses three Mtb-specific antigens, ESAT-6, CFP-10 and TB7.7 in an in-tube format. The interferon-gamma concentration is measured for diagnosis. The T-SPOT is an enzyme-linked immunospot assay performed on separated and counted peripheral blood mononuclear cells (PBMCs), and the number of interferon-gamma producing T lymphocytes is measured for diagnosis. PBMCs are stimulated with two Mtb-specific antigens, ESAT-6 and CFP-10 [8]. The methodological differences between the two tests may explain their discordant results. In addition, the ability to produce interferon gamma may vary among the individuals since it would be dependent on the conditions of Mtb-exposure and host immunity. It may also contribute to the discordance between two IGRAs. Therefore it is reasonable that the different characteristics of the tests could occasionally create discrepant results for LTBI diagnosis. Without a diagnostic gold standard for LTBI, it is difficult for us to further assess which test is more accurate and more suitable for the diagnosis of LTBI.

Table 2 showed that the 10 cases had no common characteristics of sex, age, occupation or group, indicating that there were no specific characteristics contributing to the discordance between two IGRAs. The T-SPOT utilizes a borderline zone of 5, 6 or 7 spots as recommended by the Department of Health and Human Services Centers for Disease Control and Prevention (CDC) [9]. The QFT-GIT utilizes the borderline zone of interferon-gamma concentration, 0.10-0.35 IU/ml as recommended by the JSTB [10]. According to the guidelines of the CDC and the JSTB, there were 8 borderline cases (4 QFT-GIT borderline/T-SPOT positive, 1 QFT-GIT borderline/T-SPOT borderline and 3 QFT-GIT negative/T-SPOT borderline) among 10 cases with discordant results between the two tests. Several studies have raised concerns about the variability of IGRAs' results near the cutoff values [11,12]. Therefore, to diagnose LTBI precisely, Dorman et al. advised to confirm the interpretation of IGRAs by repeating IGRAs [13]. Taken together, we have to make a careful determination of IGRAs' results near the cutoff values.

Although previous reports showed that indeterminate results were significantly more frequent with QFT-GIT than with T-SPOT [**5**], there are 2 cases with indeterminate T-SPOT results and no cases with indeterminate QFT-GIT results in our study. Generally, indeterminate results of IGRAs are associated with immunosuppression [**14**]. However, our cases with indeterminate T-SPOT results are more likely due to issues with technical issues or accuracy, since the QFT-GIT results of them argue against immunosuppression.

In conclusion, to evaluate the consistency of QFT-GIT and T-SPOT in the diagnosis of LTBI, both QFT-GIT and T-SPOT were performed simultaneously in healthcare workers. We found discordance in LTBI diagnosis between the two tests. When diagnosing LTBI of healthcare workers to prevent the outbreak, it is most important to find the cases with LTBI precisely and thoroughly, and prevent the development of tuberculosis immediately. From our perspectives, to diagnose healthcare workers with IGRAs' results near the cutoff values as LTBI, we should consider the clinical context (e.g. contact level, the infection rate of the contacts and so on) as well as the interpretation of IGRAs.

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