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Validity of interferon-γ-release assays for the diagnosis of latent tuberculosis in haemodialysis patients

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Abstract

Haemodialysis patients are at higher risk of developing active tuberculosis (TB) infection. However, tuberculin skin tests (TST) have limitations and the diagnostic usefulness of interferon-γ-release assays (IGRAs) remains unclear in immunocompromised hosts including haemodialysis patients. Haemodialysis patients were enrolled from a dialysis centre in Korea, an intermediate TB-burden country with a high bacille Calmette–Guérin (BCG) vaccination rate. The QuantiFERON-Gold TB In tube test® (QFT) and the T-SPOT TB test® (TSPOT) were performed, along with the TST. We stratified patients to low- and high-risk groups, according to the risk factors for latent TB. Association between each of the three diagnostic tests and the risk of latent TB was analysed. One hundred and sixty-seven patients were enrolled. The positive rates for the TST, the QFT and TSPOT were 23.5, 45.9 and 60.4%, respectively. Previous BCG vaccination increased the TST-positive rate in the low-risk group (OR 4.438), whereas it affected neither QFT nor TSPOT. The positive QFT rates were 41.2 and 62.5% in the low- and high-risk groups, respectively. The QFT was associated with the high-risk group (OR 2.578), whereas the TST was not. The positive TSPOT rates were 58.9 and 65.7% in the low- and high-risk groups, respectively. The frequency of indeterminate results was higher for the QFT (12.6%) compared with the TSPOT (4.8%). In conclusion, the IGRAs can be useful for the diagnosis of latent TB infection in haemodialysis patients.

Keywords: BCG, haemodialysis, immunocompromised, interferon-γ-release assays, latent tuberculosis
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Introduction

Tuberculosis (TB) infection is still an important public health issue worldwide. Both the incidence and mortality of TB infection are higher in haemodialysis (HD) patients compared with the general population [1]. Moreover, HD patients have a higher risk of reactivation of latent TB due to their compromised cell-mediated immunity [2] and dialysis units can be a source of TB spread [3]. Therefore, it is important to identify and treat latent TB infection among HD patients to prevent the reactivation and spread of TB in dialysis units [2]. The tuberculin skin test (TST) has been used as the standard diagnostic tool for latent TB infection. However, its sensitivity is low in immunocompromised hosts including HD patients [4–6]. In individuals who have been vaccinated with bacille Calmette–Guérin (BCG) the TST has an increased false-positive rate [7]. Therefore, the TST has potential limitations for the diagnosis of latent TB infection in HD patients.

Recently, interferon-γ-release assays (IGRAs) have been useful for the diagnosis of latent TB in immunocompetent hosts [6]. Reports on the IGRAs include the QuantiFERON-TB test (QFT) and the T-SPOT-TB test (TSPOT) [8]. These tests are more convenient than the TST, because they are less invasive and do not require a follow-up visit. In addition, these tests are not affected by a previous BCG vaccination and are less influenced by a non-tuberculosis mycobacterial infection, because TB-specific antigens are used [6,8]. Their sensitivity for active TB infection has been estimated to be 70–90% [6]. However, there is limited information on their diagnostic usefulness in HD patients. Recently, three groups reported that the IGRAs were better diagnostic tools compared with the TST for latent TB infection in HD

patients, but all studies were performed in low TB-burden countries [4,9,10]. Therefore, in this study, two IGRAs (QFT and TSPOT) were simultaneously compared with the TST for their diagnostic efficacy for latent TB infection in Korea, an intermediate TB-burden country.

Patients and Methods

Study population

The study participants were recruited from 1 March to 30 April 2008. They had received HD for at least 3 months at the Gil Medical Centre, Gachon University of Medicine and Science. Those patients who had taken empirical anti-TB medications and patients taking anti-TB medication for active TB infection were excluded. The study protocols were approved by the local Institutional Review Board (200804-01-I033) and all participants gave informed consent. This study was registered at the International Standard Randomized Controlled Trial Number Registry (http://www.controlled-trials.com/ISRCTN52111135).

Clinical data

A history of TB infection and contact with patients who had active TB was determined by patient report. Patients who had a microbiologically or radiologically confirmed TB infection were considered to have a history of TB infection. A close contact was defined as a household contact or a work contact, in the same room for > 8 h a day. The comorbidity and medication history of immunosuppressants were also noted. Patients who reported BCG vaccination in the past or had a BCG vaccination scar confirmed by physicians were regarded as patients with a previous BCG vaccination. Standard chest X-rays (CXR) were requested for all participants and the most current film, within 6 months before the study enrolment, was reviewed for patients who refused another CXR. All CXR were reviewed by the same pulmonary radiologist, blind to the patients' clinical information. The CXR findings were classified into three categories: normal, inactive TB lesions and active TB lesions. Fibronodular lesions in the upper lobe, calcified mediastinal lymph nodes, pleural thickening and any other lesions consistent with a previous TB infection were regarded as inactive TB lesions [9,11]. In addition, sputum acid fast bacteria (AFB) staining, sputum TB culture and sputum TB PCR were performed.

The interferon-γ-release assays and the tuberculin skin test

Whole blood was extracted just before dialysis for the two IFN-γ tests. The QFT was performed according to the manufacturer's instructions (Cellestis Ltd., Carnegie, Victoria, Australia) [12]. The TSPOT was also performed according to the manufacturer's instructions (Oxford Immunotec, Oxford, UK) [9]. Results of each test were classified as positive, negative or indeterminate, as previously described [13]. Within a week after the IGRAs, 2-TU of purified protein derivative RT23 (Statens Serum Institute, Copenhagen, Denmark) was intradermally injected on the volar side of the forearm contralateral to the patient's vascular access. Two physicians, blind to the patients' clinical information, measured the main diameter of the induration after 48 h independently. The positive criterion was $\geq$ 10 mm size of the mean values of two measurements.

Statistical analysis

The high-risk group for latent TB infection consisted of patients with a history of close contact with TB patients, old TB lesions on CXR, or a history of TB infection. The low-risk group was defined as patients who did not have any of the three risk factors for latent TB. According to this definition, association of each of the three diagnostic tests with the high-risk group was analysed using multiple logistic regression. Association of a previous BCG vaccination with the diagnostic tests in the low-risk group was also analysed. The agreement among test results of the TST, QFT and TSPOT was assessed using κ coefficients. All analyses were performed using SPSS software (version 12.0; SPSS Inc, Chicago, IL, USA).

Results

Characteristics of the study population

In total 167 patients were enrolled. Their clinical characteristics are summarized in Table 1. Twenty-one patients (12.6%) had a history of TB infection and all of them received appropriate treatment with anti-TB medication. All patients were ethnic Koreans and none had either anti-human immunodeficiency virus antibodies or non-mycobacterial tuberculosis infections. The AFB smear test, TB PCR test and TB culture test were negative in all subjects. Fourteen patients (8.4%) had a history of contact with active TB patients and 11 patients among them had a history of close contact (Table 1). Thirty-eight patients (22.8%) belonged to the high-risk group for latent TB infection (Table 2).

Overall results of the tuberculin skin test, the QuantiFERON-TB test and the T-SPOT-TB test

Positive rates of TST, QFT and TSPOT were 23.5, 45.9 and 60.4%, respectively (Table 2). The positive rate of TST was

TABLE 1. Clinical characteristics of the study population

	Mean ± standard deviation	Range
Age (year)	54.1 ± 14.4	17.0 – 88.0
Duration of haemodialysis (months)	60.8 ± 57.5	3.0 – 336.0
Serum albumin (g/dL)	3.85 ± 0.36	2.25 – 4.65
	Number of events	**Proportion of event (%)**
Sex (M:F)	71:96	42.5:57.5
Cause of end-stage renal disease		
Diabetes mellitus	67	40.1
Hypertension	18	10.8
Glomerulonephritis	12	7.2
Others	11	6.6
Unknown	59	35.3
Diabetes mellitus	80	47.9
History of cancer	12	7.2
Cardiac disease	46	27.5
Cerebrovascular accident	13	7.8
History of TB infection	21	12.6
Pulmonary TB infection	15	–
Extrapulmonary TB infection	6	–
History of contact with TB patients	14	8.4
Inactive TB lesions on chest X-rays	17	10.2
History of BCG vaccination or BCG scar	111	67.3
Immunosuppressant medication	9	5.4

still low (34.6%) even at a threshold of 5 mm induration. The positive rates in all three tests were higher in patients with a cured TB infection (Table 2). The frequency of indeterminate results was higher for the QFT (12.6%) compared with the TSPOT (4.8%).

Association of the diagnostic tests with the risk of latent TB infection

The results of the three diagnostic tests according to the risk groups of latent TB infection were analysed. The positive TST rates were similar in the low- and high-risk groups (23.8% vs. 22.2%). The positive QFT rate increased to 62.5% in the high-risk group (Table 2). The positive TSPOT rates were high in both groups (58.9% vs. 65.7%, Table 2). Taken together, these results suggested that the QFT had a trend towards an increasing response rate according to the increase in the risk of latent TB infection, whereas the TST had a low response rate and the TSPOT had a high response rate regardless of the risk of latent TB infection. The overall trends were similar for the data that excluded patients with cured TB infection (Table 2).

Table 3 shows the results from investigating the association of the three diagnostic tests with the risk of latent TB infection using the logistic regression analysis. The QFT was independently associated with the high-risk group (adjusted OR 2.578; 95% CI 1.063–6.254; p 0.036, Table 3). However, neither the TST nor the TSPOT was significantly associated with the high-risk group (Table 3). The overall trends were similar in the analysis after excluding patients with a cured TB infection, even though the statistical significance decreased (Table S1).

Association of a previous BCG vaccination with the diagnostic tests in the group at low risk of latent TB infection

In order to assess impact of a previous BCG vaccination on the false-positive rate of the diagnostic tests, the association of a history of previous BCG vaccination with the diagnostic test results in the low-risk group was analysed. A history of previous BCG vaccination increased the risk of the positive TST rate significantly (adjusted OR 4.438; 95% CI 1.154–17.071, p 0.030, Table 4) and therefore might increase the false-positive rate in TST. However, neither the QFT nor the TSPOT was affected by a previous BCG vaccination in the low-risk group (Table 4).

Agreement among the tuberculin skin test, the QuantiFERON-TB test and the T-SPOT-TB test

Overall, the degree of agreement among the three tests was poor (TST vs. QFT, $\kappa = 0.276$; TST vs. TSPOT, $\kappa = 0.163$; QFT vs. TSPOT, $\kappa = 0.273$). The concordance rates between the TST and both IGRAs were low regardless of

TABLE 2. Results of the tuberculin skin tests, the QuantiFERON-TB tests and the T-SPOT-TB tests

Risk factor	Tuberculin skin test (≥10 mm) (+)	QuantiFERON-TB test (+)	T-SPOT-TB test (+)
Inclusion of patients with a cured TB infection			
Total patients (*n* = 167)	38 (23.5%)	67 (45.9%)	96 (60.4%)
Low-risk group (*n* = 129)	30 (23.8%)	47 (41.2%)	75 (58.9%)
High-risk group (*n* = 38)	8 (22.2%)	20 (62.5%)	23 (65.7%)
Patient with a cured TB infection (*n* = 21)	6 (30%)	11 (68.8%)	15 (71.4%)
Exclusion of patients with a cured TB infection			
Total patients (*n* = 146)	32 (22.5%)	56 (43.1%)	81 (58.7%)
Low-risk group (*n* = 129)	30 (23.8%)	47 (41.2%)	75 (58.9%)
High-risk group (*n* = 17)	2 (12.5%)	9 (56.3%)	8 (57.1%)

Results were expressed by both number and proportion (in parentheses) of the positive response.
TB, *Mycobacterium tuberculosis*.

TABLE 3. Association of the diagnostic tests with the high-risk group of latent TB infection

Variable	Univariable logistic regression analysis		Multivariable logistic regression analysis	
	OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age	0.977 (0.950–1.006)	0.123		
Sex	0.725 (0.325–1.617)	0.432		
Diabetes mellitus	0.532 (0.233–1.215)	0.134		
History of cancer	0.421 (0.051–3.463)	0.421		
Cardiac disease	1.117 (0.464–2.687)	0.805		
Previous BCG vaccination	0.551 (0.242–1.256)	0.156		
Immunosuppressant	1.143 (0.225–5.805)	0.872		
Serum albumin	0.628 (0.226–1.747)	0.373		
Tuberculin skin test	1.129 (0.454–2.806)	0.795	1.251 (0.458–3.417)[a]	0.663
QuantiFERON-TB test	2.433 (1.072–5.522)	0.033	2.578 (1.063–6.254)[a]	0.036
T-SPOT-TB test	1.125 (0.498–2.543)	0.777	1.155 (0.481–2.773)[a]	0.747

TB, *Mycobacterium tuberculosis*; BCG, bacille Calmette–Guérin.
[a]Adjusted for age, sex, diabetes mellitus, history of cancer, cardiac disease, previous BCG vaccination, immunosuppressant and albumin.

TABLE 4. Association of a previous BCG vaccination with the diagnostic tests in the low-risk group of latent TB infection

Variable	Univariable logistic regression analysis		Multivariable logistic regression analysis	
	OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Tuberculin skin test				
Previous BCG vaccination	4.667 (1.293–16.848)	0.018	4.438 (1.154–17.071)[a]	0.030
QuantiFERON-TB test				
Previous BCG vaccination	0.976 (0.418–2.279)	0.954	1.068 (0.422–2.703)[a]	0.890
T-SPOT-TB test				
Previous BCG vaccination	1.464 (0.626-3.426)	0.380	1.332 (0.542-3.277)[a]	0.532

TB, *Mycobacterium tuberculosis*; BCG, bacille Calmette–Guérin.
[a]Adjusted for age, sex, diabetes mellitus, history of cancer, cardiac disease, immunosuppressant and albumin.

the risk of latent TB infection due to the poor sensitivity of the TST in the HD patients. However, good agreement was observed between the QFT and the TSPOT in patients with a cured TB infection ($\kappa = 0.538$, Table 5). Thirty-eight patients (27.3%) gave negative results for both the QFT and the TSPOT and 49 patients (35.3%) gave positive results for both tests, whereas 52 patients (37.4%) gave discordant results (Table 5).

Discussion

Latent TB infection was studied among four different risk groups in the general Korean population [14]. In the low-risk group, the positive response rates of the TST and the QFT were 51% and 4%, respectively. In the close contact group the positive response rates increased to 71% and 44%, respectively. Consistent with these results, the QFT results were proportional to the risk of latent TB infection in our data. However, the positive QFT rate in the low-risk group in our study was close to the value in the close contact group in the general population (41.2% vs. 44%). This result can be attributed to the fact that HD patients belong to a high-risk group compared with the general population, regardless of additional risk factors [1].

The positive TST rate was much lower in the HD patients, even in Korea with a high BCG vaccination rate and an intermediate TB burden [15]. A previous BCG vaccination

TABLE 5. Comparison between the QuantiFERON-TB test and the T-SPOT-TB test

		T-SPOT-TB test		
	QuantiFERON-TB test	Negative result	Positive result	κ coefficient
Total patients	Negative result	38 (27.3%)	39 (28.1%)	0.273
	Positive result	13 (9.4%)	49 (35.3%)	
Patients with a cured TB infection	Negative result	3 (18.8%)	2 (12.5%)	0.538
	Positive result	1 (6.3%)	10 (62.5%)	

TB, *Mycobacterium tuberculosis*.

increased the positive TST rate in the group at low risk of latent TB infection, a surrogate marker for the false-positive rate. Therefore, both the low positive TST rate and the possibility of false-positive results diminish the usefulness of the TST for the diagnosis of latent TB infection in Korean HD patients. However, a previous BCG vaccination did not influence the results of the QFT or the TSPOT.

There are several previous studies on IGRAs in HD patients [4,9,10]. In the Canadian study, the positive TSPOT rate was 35.5% [9]. In the American study, the positive rates of the QFT and the TSPOT were 22% and 28%, respectively [10]. The positive rates of both the QFT and the TSPOT in these studies were lower than the 45.9% (QFT) and 60.4% (TSPOT) found in this study. These results may reflect the relative TB burden in each country. Taken together, these data suggested that the IGRA is also effective in detecting latent TB infection in HD patients in countries with an intermediate to high TB burden.

Currently, there is no established standard diagnostic method for latent TB infection. Therefore, we stratified patients to the low- and high-risk groups for latent TB infection instead of defining latent TB itself, in order to analyse the association between the three diagnostic tests and the risk of latent TB infection. The QFT results were significantly associated with the high-risk group. In contrast, the positive TSPOT rates were high even in the low-risk group. These results suggest that the TSPOT could be sensitive enough to detect hidden latent TB infection in the low-risk group [6]. Considering both low sensitivity and low specificity of TST in HD patients, we would recommend the use of IGRAs as the screening test in HD patients, including renal transplantation candidates before transplantation. Long-term follow-up studies to assess the predictive ability of the IGRAs for future active TB infection are needed in order to confirm their validity in these patients. There was no significant risk factor for the discordant results between the QFT and the TSPOT, perhaps due to the low number of cases. Interestingly, most of the discordant results had a pattern of a negative result for the QFT with a positive result for the TSPOT, consistent with a higher sensitivity of the TSPOT [6,16]. Further, large-scale studies are needed to reveal the causes of the discordant results. The QFT gave a higher rate of indeterminate results than the TSPOT, consistent with the findings in previous reports [9,12,17].

The patients with a history of cured TB infection were not candidates for anti-TB medications for latent TB infection, because the risk of progression to active TB infection is low in this group [18,19]. However, this group can give a positive response on the IGRAs [8,20]. On analysis of the data, omitting those from the cured TB patients, the overall trends were found to be similar.

In summary, the TST was not useful for the diagnosis of latent TB infection in Korean HD patients. Both the QFT and the TSPOT were more sensitive compared with the TST and were not affected by a previous BCG vaccination. In conclusion, the IGRAs can be useful for diagnosis of latent TB infection, a common and important problem in HD patients.

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Transparency Declaration

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Association of the diagnostic tests with the high-risk group of latent TB infection after exclusion of patients with a cured TB infection.

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