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ORIGINAL ARTICLE

Role of the QuantiFERON-TB gold in tube in ruling out tuberculosis in end stage renal disease patients receiving hemodialysis

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Abstract *Background:* It is difficult to diagnose tuberculosis (TB) in dialysis patients because of the high rate of extrapulmonary TB in these patients compared with the general population. Recently, a new diagnostic test called QuantiFERON(QFT) has been developed and shown promise as a diagnostic tool for active TB diseases and latent TB infection. The aim of the present study was to analyze the performance of QuantiFERON-TB Gold in tube (QFT-G) in end stage renal disease patients receiving hemodialysis.

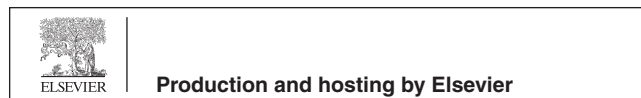
Methods: QuantiFERON Gold in tube (QFT-GIT) were prospectively performed in 50 end stage renal disease (ESRD) cases undergoing hemodialysis (HD), including 6 patients with active TB and evaluated the utility of this test in dialysis patients.

Results: Among 50 dialysis patients, positive QFT results occurred in 10 (20%), negative QFT results occurred in 25 (50%) and indeterminate QFT results occurred in 15 (30%). All six active TB patients had positive QFT results, and none of the 25 patients with negative results had active TB. Among 7 patients with a history of active TB, 2 (28.5%) had positive results. Although the indeterminate rate was relatively high, no patient with an indeterminate result had active TB. Among 30 cases after excluding the patients with previous TB and indeterminate results, the sensitivity of the QFT is 100% (6 of 6) and the specificity is 91.6% (22 of 24 cases).

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Conclusions: Our data suggest that the QFT test is a useful supplementary tool for the diagnosis of active TB even in dialysis patients. Negative and indeterminate results on this test may be used to exclude the presence of active TB.

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Introduction

Tuberculosis (TB) remains a major health problem not only in developing countries but in developed countries as well. The incidence of TB is higher in end-stage renal disease (ESRD) patients owing to impaired host defense mechanisms, particularly immune suppression, which is more important in endemic regions [1,2]. Diagnosis is difficult and delayed since extra pulmonary involvement is more common than isolated pulmonary involvement and nonspecific symptoms mimicking uremia are seen frequently. Uremia itself is known to cause impaired cellular immunity, which is the major host defense mechanism against TB infection. Moreover, patients on hemodialysis (HD) are at increased risk of developing active tuberculosis after primary infection, activation of quiescent disease, or reactivation of old TB infection. Nosocomial transmission of TB has also been reported in patients under long-term dialysis [3]. Diagnosis of TB in immune-compromised patients and patients on HD could be difficult and often missed. This is due to vague presentation with inconclusive chest X-rays and negative tuberculin skin tests (TST). The TST remains the standard method for identifying TB infection in screening of latent TB infection (LTBI) in chronic renal failure patients and in patients receiving hemodialysis. In fact, ESRD is known to be a risk factor for skin test anergy; although the rate of anergy is quite variable, recent reports suggest that about 32% to 40% of HD patients are anergic [4]. A new diagnostic method, QuantiFERON-TB gold in tube is an *in vitro* test for *Mycobacterium* (M) TB infection. It measures interferon gamma (IFN- γ) secreted by T-cells which are stimulated with mixtures of synthetic peptides including the early secretory antigenic target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10) that are specific to M. TB. After incubation of whole blood with the mixture of synthetic peptides, an ELISA detects the release of IFN- γ from lymphocytes. Because the target peptides are secreted by M. TB and *Mycobacterium bovis*, but not by strains present in the Bacille Calmette Guérin (BCG) vaccine and by most nontuberculous mycobacteria, QFT-G may correlate better with exposure to M. TB than the TST [5]. In this study we aimed to analyze the performance of the QFT-G in cases with ESRD receiving hemodialysis and evaluate the value of the test in the diagnosis of tuberculosis among patients on hemodialysis.

Subjects and methods

Study population

Between June 2009 and December 2011, QFT was tested in 50 patients with end-stage renal disease on hemodialysis aged 18 or older. They were HIV negative and hospitalized for reasons including suspected active TB, fever or inflammation of unknown origin, unilateral pleural effusion or abnormal lung shadows. Physicians decided to use the QFT depending on

the medical situation (usually suspected TB disease, fever of unknown origin or the need to rule out TB). Patients who underwent the QFT test were enrolled in the present study.

QuantiFERON test

Blood samples were obtained just before dialysis in haemodialysis patients. The QFT test was performed according to the manufacturer's recommendations, and test results were evaluated according to the guidelines of the Centers for Disease Control and Prevention for using the QFT test [6] as follows: the test consisted of a negative control (a 'nil' well; whole blood without antigens or mitogen), a positive control (a 'mitogen' well; whole blood stimulated with the mitogen phytohaemagglutinin) and two sample wells (whole blood stimulated with either ESAT-6 or CFP-10). Whole-blood specimens were incubated for 18 h (overnight) at 37 °C in a humidified atmosphere. The IFN- γ level of the nil well was considered to be the background value and was subtracted from the values for the mitogen well and the antigen-stimulated wells. The test result was considered positive, and TB infection suspected if the IFN- γ level in the sample well after stimulation with ESAT-6 and/or CFP-10 was >0.35 IU/ml (after subtraction of the value for the nil well) irrespective of the result for the positive control well. The test result was considered negative with no possibility of TB infection if the IFN- γ level was <0.35 IU/ml on both antigen wells and if the IFN- γ level of the positive Control well (after subtraction of the value for the nil well) was >0.5 IU/ml. The test result was considered indeterminate and impossible to interpret if the IFN- γ level was <0.35 IU/ml in both antigen wells and <0.5 IU/ml in the positive control well, or if the IFN- γ level was below half of the negative control well in both antigen wells and >0.7 IU/ml in the negative control well.

Tuberculin skin testing (TST)

The 5 TU (tuberculin unit) of purified protein derivative (PPD) RT23 (Statens Serum Institute, Copenhagen, Denmark) in 0.1 ml was injected intradermally by Mantoux method and the induration was measured between 48 and 72 h after PPD injection by a physician. Induration of 5 mm was considered a positive TST result.

Status of TB infection

We classified TB infection into three categories: active TB, previous TB and non-TB. For all patients, we collected demographic, clinical, radiologic and microbiologic data. Physicians routinely carried out complete blood counts, blood chemistries including CRP, chest radiographs, history taking, general physical examinations and epidemiologic surveys (self reported history of active TB, self-reported contact with an active case of TB, occupational history). When we suspected

active TB based on these routine examinations, we added sputum smear, culture, PCR, enhanced chest CT scans and enhanced abdominal CT scans. Depending on the situation, we also performed pleural effusion examinations (including smear, culture, adenosine deaminase), lymph node biopsy. Patients were finally classified as having active TB if either culture was positive for *Mycobacterium tuberculosis* or the experienced physician decided that the patients suffered from TB based on clinical findings, patient history, imaging study, histology indicating mycobacterial infection (i.e. granulomatous necrosis or identification of acid-fast bacilli) and the patients responded clinically and radiologically to anti-TB treatment. We classified patients who had a history of TB treatment (recorded in charts or history taking) or who had some radiological evidence of TB as previous TB patients. Patients with no current or previous active TB or treatment were classified as non-TB patients.

Primary renal disease

Primary renal diseases were classified according to aetiological diagnosis into six categories: diabetic nephropathy, nephrosclerosis, chronic glomerulonephritis, collagen disease, vasculitis, and other.

Statistical analysis

We calculated proportions of positive, negative and indeterminate results across active TB, non-TB and previous TB patients. All analyses were performed using computer software (SPSS, version 10.0; SPSS, Inc; Chicago, IL).

Results

Fifty patients (35 men and 15 women; mean age 65.4 years) underwent QFT tests. The mean duration of dialysis was 45.4 months. Characteristics of the patients are summarized in (Table 1). Twenty-seven patients had highly suspected active TB with symptoms including persistent fever of unknown origin or inflammation, unilateral pleural effusion, pneumonia or abnormal lung shadow. Among them, six cases (22%) of active TB were diagnosed after admission (Table 2).

Among the 50 dialysis patients, QFT results were positive in 10 (20%), negative in 25 (50%) and indeterminate in 15 (30%). Six (60%) of 10 patients with a positive result had active TB. Two (20%) of these 10 patients had a history of TB disease and 2 (20%) did not have active or previous TB. None of the 25 patients with a negative result had active TB disease.

Table 1 Demographics of study subjects.

Patients (N)	50
Age (years; mean \pm SD)	65.4 \pm 12
Sex (M/F)	35/15
Duration of dialysis (months)	45.4
<i>Cause of renal disease</i>	
Diabetic nephropathy	25
Nephrosclerosis	8
Chronic glomerulonephritis	7
Collagen disease	5
Vasculitis	3
Unknown	2

Table 2 Reasons for hospital admission (No. of patients).

Fever or inflammation of unknown origin	10
Unilateral pleural effusion	6
Pneumonia	5
Abnormal lung shadow	6
Heart failure (including ischaemic heart disease)	6
Infection (except pneumonia)	6
Initiation of dialysis	6
Bone fracture	3
Vascular access problem	2

Table 3 Results of QFT in dialysis patients.

	Active TB	Non-TB	Previous TB	Total
Positive results	6	2	2	10
Negative results	0	22	3	25
Indeterminate results	0	13	2	15
Total	6	37	7	50

Twenty-two (88%) of 25 patients with negative results did not have a history of active or previous TB, and the remaining 3 patients (12%) had a history of TB. All 15 patients (30%) with indeterminate results showed a positive control failure on QFT-TB results (IFN- γ < 0.5 IU/ml). None of them had active TB. Thirteen (86.7%) of 15 patients did not have any history of active or previous TB, and 2 patients (13.3%) had a history of previous TB (Table 3). Of the 10 patients with positive QFT results, 6 had active TB. Among these 6 patients, two had tuberculous lymphadenitis, two had tuberculous pleuritis and two had pulmonary TB. In both pulmonary TB patients, active TB was easily diagnosed using sputum smear, PCR and culture. On the other hand, a detailed investigation was needed to diagnose extra pulmonary TB in four patients with active TB. Two of the four extrapulmonary TB cases presented as fever of unknown origin, and two showed pleural effusion, although the cause was difficult to determine. In one patient, tuberculous lymphadenitis, this case was presented with fever of unknown origin and was diagnosed as mediastinal tuberculous lymphadenitis by enhanced chest CT and response to TB treatment. Only one of six was TST positive. This is consistent with the recent report that showed the lack of utility of TST in diagnosing active TB [7]. Among the 7 patients with a history of active TB, 2 (28.6%) had a positive result, 3 (42.8%) had a negative result and 2 (28.6%) had an indeterminate result on the QFT test. It is difficult to interpret QFT results in patients with previous TB or indeterminate QFT results. So, when we excluded indeterminate results and/or previous TB, we were left with 30 cases. Among the 30 cases after excluding the patients with previous TB or indeterminate results, the sensitivity of the QFT is 100% (6 of 6) and the specificity is 91.7% (22 of 24 cases).

Discussion

Our findings indicate that QFT is a useful test to diagnose active TB in dialysis patients, as all active TB patients showed positive QFT results, and no patient with a negative or indeterminate result had active TB. These results are consistent with studies done by Inoue et al., [8] and Chung et al., [9]. This

study focused on dialysis patients, who are likely to develop active TB as well as extrapulmonary TB. Among the six active TB patients in this study: four (67%) had extrapulmonary TB (two had tuberculous lymphadenitis and two had tuberculous pleuritis) and two (33%) had pulmonary TB. The regular occurrence of extrapulmonary TB among TB cases in this study supports the findings of Hussein et al., [1].

Except for six active TB cases, no active TB cases were found among subjects with positive QFT results, even after detailed investigations. However, one case who had positive QFT results but who was initially ruled out from having active TB developed active TB during the follow-up period. This indicates that we need to rule out the existence of active TB when QFT results are positive. Moreover, even if we cannot confirm active TB after a positive QFT test result, we should carefully follow up these cases. On the other hand, none of the 25 patients with negative QFT results had active TB, allowing us to use negative results of this test to rule out active TB. In addition, cases with indeterminate results might be regarded as negative because no cases with indeterminate results had active TB. Considering that indeterminate results can be treated the same as negative results, we suggest that the sensitivity of QFT is 100% (6 of 6) and its specificity is 91.6% (22 of 24 cases). Winthrop et al., [10] tested 100 patients with ESRD who received hemodialysis and they reported that QFT-G and T-SPOT-TB might offer a better method for detecting TB infection in ESRD patients. Inoue et al., [8] reported that the sensitivity of the QFT was 100% and the specificity was 89.7% in hemodialysis patients. In our study, the positivity of QFT-G was similar to these studies.

Our study estimated the sensitivity of QFT-IT as being higher than the reported range in the earlier studies from high endemic countries. The probable reasons for obtaining lower sensitivity for interferon gamma release assay (IGRA) include HIV coinfection, advanced disease, malnutrition, host immune response and variation in the *M. tuberculosis* strain. In most of the studies conducted, HIV positive subjects were included [11]. HIV is the one of the major influencing factors of performance of QFT-IT [12]. Exclusion of HIV patients in our study may be one of the reasons for obtaining higher sensitivity for QFT-IT in contrast to other studies.

In terms of previous TB, the percentage of positive QFT results in patients with previously treated TB but without active TB has been reported to be 36.6% in the general population ($n = 134$) [13]. The present study showed a rate of 28.5% (2/7) in dialysis patients. There has been no previous report that suggests that QFT can be used to differentiate between previous and current TB. Therefore, the high percentage of positive QFT results makes it difficult to determine whether these patients have active or previous TB.

Only a few studies on indeterminate results among dialysis patients have been reported. The incidence of indeterminate results ranged from 2% to 40%, and appeared to depend on whether they were outpatients or inpatients, had primary renal diseases and whether complications were present. Our study showed that 30% of patients had indeterminate results, which is consistent with previous reports [14].

In conclusion, our data suggest that the QFT test is a useful supplementary tool for the diagnosis of active TB, and that negative results may be used to exclude active TB in dialysis patients. Results also suggest that the QFT test is valuable in detecting extrapulmonary TB, which can be difficult to diagnose. In spite of the high rate of indeterminate QFT results in our study, no patient with indeterminate results had active TB. This might mean that we can consider indeterminate results among dialysis patients as negative results and rule out active TB in these patients.

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