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Diagnosing latent tuberculosis infection in haemodialysis patients: T-cell based assay (T-SPOT.*TB*) or tuberculin skin test?

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Abstract

Background. The international guidelines recommend screening haemodialysis (HD) patients for latent tuberculosis infection (LTBI). The aim of this study is to compare the diagnostic utility of tuberculin skin test (TST) with an interferon-γ-based assay (T-SPOT.*TB*) for the diagnosis of LTBI in HD patients.

Methods. A total of 411 patients [233 male (57%), mean age 56 ± 16 years] in five HD centres were prospectively tested by TST and T-SPOT.*TB* assays. A total of 302 patients (75%) had Bacillus Calmette-Guerin vaccination scar.

Results. LTBI was detected in 39 and 61% of patients by one-step TST and T-SPOT.*TB*, respectively. The booster phenomenon determined additional 60 (25%) LTBI among 243 patients. Overall, 218 (53%) patients showed a positive reaction to TST after performing the two-step TST. Among 250 one-step TST negative patients T-SPOT.*TB* assay was positive in 118 (47%). Of 158 patients with a positive one-step TST, T-SPOT.*TB* was negative in 34 (22%). On the other hand, T-SPOT.*TB* was negative in 16 (27%) of boosted patients. T-SPOT.*TB* was negative in 50 (23%) of overall TST-positive patients and positive in 71 (39%) of TST negative ones. Multivariate logistic regression analysis revealed that male gender was independently associated with positive T-SPOT.*TB*, and positive T-SPOT.*TB* was inversely associated with the presence of BCG vaccine scar, serum albumin level and HD duration. Annual conversion rates were 12 and 32% for TST and T-SPOT.*TB* tests, respectively.

Conclusion. Usage of T-SPOT.*TB* in HD patients with negative TST may enhance diagnosis of LTBI.

Keywords: haemodialysis; interferon-gamma release test; tuberculin skin test; tuberculosis; T-SPOT.*TB*

Introduction

Patients with end-stage renal disease (ESRD) who are on haemodialysis (HD) have a significantly higher incidence of *Mycobacterium tuberculosis* infection or disease than healthy individuals [1–6]. Most cases of active tuberculosis (TB) in patients with ESRD are due to the reactivation of a latent infection, and this patient group is at roughly 10- to 25-fold higher risk for reactivating TB infection than the general population [1–3]. Moreover, HD units have been shown to be important centres for the spread of infectious TB [7–9]. For these reasons, routine annual TB screening of HD patients has been recommended [7]. Until recently, the standard screening tool used to detect latent tuberculosis infection (LTBI) was the tuberculin skin test (TST); however, a large proportion of HD patients exhibit anergy and tuberculin non-reactivity, yielding high rates of false-negative results with this method [2, 8, 10, 11]. In addition, false-positive results can occur because of cross reactivity related to previous exposure to non-TB mycobacteria or prior immunization with Bacillus Calmette-Guerin (BCG). These are potential serious limitations of the TST for diagnosing LTBI and thus a better screening test is needed.

During the past decade, alternatives to TST have emerged in the form of a new category of *in vitro* T-cell-based assays, the interferon-gamma (IFN-γ)-release assays (IGRAs). T-SPOT.*TB* is an *ex vivo* enzyme-linked immunospot (ELISpot) assay that quantifies IFN-γ-secreting T-cells that are specific for early secreted antigenic target-6 kD (ESAT-6) and culture filtrate protein 10 kD (CFP-10). These proteins are encoded within region of difference 1 (RD1) of the *M. tuberculosis* genome, and RD1 is present in *M. tuberculosis* complex but absent from all strains of *Mycobacterium bovis* BCG and almost all environmental mycobacteria [12–14]. The pioneer study investigating the sensitivity and specificity of RD1-ELISpot assay were performed in 47 culture-confirmed TB patients and 47 controls who had diseases other than TB, and the sensitivity and specificity of the assay were found to be 96 and 92%, respectively [15]. The latest systematic review shows that the sensitivity and specificity of RD1-ELISpot-based IGRA (T-SPOT.*TB*) assay among active TB patients were 90 and 93%, respectively [16]. Moreover, among close contacts of active TB cases, the association between test positivity and extent of exposure is significantly stronger with RD1-ELISpot than with TST, suggesting that the RD1-ELISpot assay is more sensitive than TST for detecting

M. tuberculosis infection [17, 18]. Furthermore, unlike TST, the RD1-ELISpot is not confounded by previous BCG vaccination. It has also been shown that the ELISpot assay is superior to TST for diagnosing TB infection in the presence of many conditions, including human immunodeficiency virus infection and malnutrition, which can cause relative immunosuppression [19, 20]. However, T-SPOT.*TB* assay has not been evaluated extensively in ESRD patients undergoing HD.

The aims of this study were as follows: (i) to compare T-cell based TB test T-SPOT.*TB* assay with TST for diagnosing LTBI in ESRD patients on HD, (ii) identify risk factors for LTBI in HD patients and (iii) determine the annual incidence of LTBI in this patient group using both assays.

Materials and methods

Study population

Between May 2006 and May 2007, patients with ESRD who had been on HD >6 months and who did not have a diagnosis of active TB diseases or LTBI previously were recruited prospectively from five outpatient HD units in Istanbul, Turkey. The Marmara University School of Medicine Ethics Committee granted approval for the study. The characteristics of patients including gender, age, BCG vaccine scar status, serum albumin level, serum ferritin level, *Kt/V* were noted. Individuals with a history of prior TB or isoniazid prophylactic treatment were excluded. Patients who were positive on TST or T-SPOT.*TB* were evaluated for active TB by physical exam and chest x-ray. Isoniazid prophylaxis was recommended for all patients who were diagnosed with LTBI but no active disease based on T-SPOT.*TB* or TST.

Tuberculin skin test

The TST was done on the volar aspect of the forearm using the Mantoux method, with an injection of 0.1 mL (five tuberculin units) purified protein derivatives (PPD) tuberculin Tween 80 (BB-NCIPD Ltd, Sofia, Bulgaria) after withdrawal of blood samples for T-SPOT.*TB* assay on the same day. At 48–72 h after injection, the extent of induration was measured by one of the authors (D.T.) using the ballpoint pen method. TST response was scored as positive if induration diameter was ≥10 mm. To assess for the development of booster phenomenon, all patients with induration size <10 mm underwent two-step TST (TST-2), which involved re-testing at 1–3 weeks after the first TST (TST-1). An individual was considered to exhibit booster phenomenon if the extent of the induration after TST-2 was >10 mm and/or at least 6 mm larger than that observed after TST-1 [7].

*T-SPOT.*TB *assay*

According to the manufacturer's instructions (Oxford Immunotec, Oxford, UK), the peripheral blood mononuclear cells were separated off using the Ficoll-centrifuge method and plated (at 2.5×10^5 cells per well) with 50 μL of media, phytohaemagglutinin (PHA) or peptides from ESAT-6 and CFP-10 and the test was done as previously described [21].

Statistical analysis

Statistical analyses were performed using the STATA version 7.0 software package (STATA, College Station, TX). Student's *t*-test was used to compare group results for continuous variables. Group findings for categorical variables were compared using the chi-square test or Fisher's exact test as appropriate. Univariate and multivariate stepwise logistic regression analysis was performed to analyse the independent determinants of TST and T-SPOT.*TB* positivity. The following variables were included into the analysis: age, gender, presence of BCG vaccine scar, serum albumin and ferritin levels and *Kt/V*. Concordance between the two types of TB tests was assessed using the kappa coefficient: $K > 0.75$ indicating excellent agreement; $0.4 < K < 0.75$ indicating fair to good agreement; $K < 0.4$ indicating poor agreement. A P-value <0.05 was considered significant.

Results

A total of 411 patients with ESRD undergoing HD were enrolled and their ages ranged from 19 to 84 years. Patient characteristics are summarized in Table 1. Mean age, serum albumin and ferritin, presence of BCG vaccine scar and duration of HD were similar between females and males. However, only *Kt/V* was significantly higher in females ($P < 0.05$) (Table 1). No cases of active TB were detected during the study period. Of the 411 HD patients, 408 underwent TST-1 assay at time of enrolment and reactivity to this test was categorized as one of five ranges of induration size: 0–4 mm ($n = 190$, 46%); 5–9 mm ($n = 60$, 15%); 10–15 mm ($n = 99$, 24%); 16–20 mm ($n = 32$, 8%) and >20 mm ($n = 27$, 7%). Thus, in total, 250 (61%) of the 408 patients were negative on TST-1 and 158 (39%) were positive. All 411 patients underwent T-SPOT.*TB* at time of enrolment and 239 (61%) were positive (Figure 1).

Table 1. Characteristics of the study population

Characteristics	Whole study population ($N = 411$)	Female ($N = 178$)	Male ($N = 233$)
Age (years; mean ± SD; min–max)	56 ± 16 (19–84)	56 ± 15 (21–84)	56 ± 16 (19–84)
Sex (male)	233 (57)	–	–
Duration of dialysis (months; mean ± SD; min–max)	61 ± 51 (6–284)	65 ± 54 (6–264)	59 ± 49 (6–244)
BCG vaccine scar			
None (%)	109 (25)	49 (28)	51 (22)
≥1 (%)	302 (75)	129 (72)	182 (78)
Kt/V	1.4 ± 0.3 (0.7–2.9)	1.5 ± 0.3 (0.7–2.3)	1.3 ± 0.2 (0.8–2.9)*
Serum albumin (g/dL; mean ± SD; min–max)	4.0 ± 0.4 (2.5–5.3)	4.0 ± 0.3 (3.0–4.9)	4.0 ± 0.4 (2.5–5.3)
Serum ferritn (ng/mL; mean ± SD; min–max)	555 ± 496 (20–7209)	624 ± 635 (49–7209)	497 ± 347 (20–2496)
Aetiology of renal failure (%)			
Hypertensive nephropathy	94 (23)	42 (24)	52 (22)
Diabetic nephropathy	86 (21)	30 (17)	56 (24)
Glomerulonephritis	51 (12)	24 (13)	27 (12)
Polycystic kidney diseases	32 (8)	17 (10)	15 (6)
Urological diseases	35 (8)	21 (12)	14 (6)*
Other known conditions	33 (8)	13 (8)	20 (9)
Unknown	80 (19)	31 (17)	49 (21)

*$P < 0.01$ versus female group.

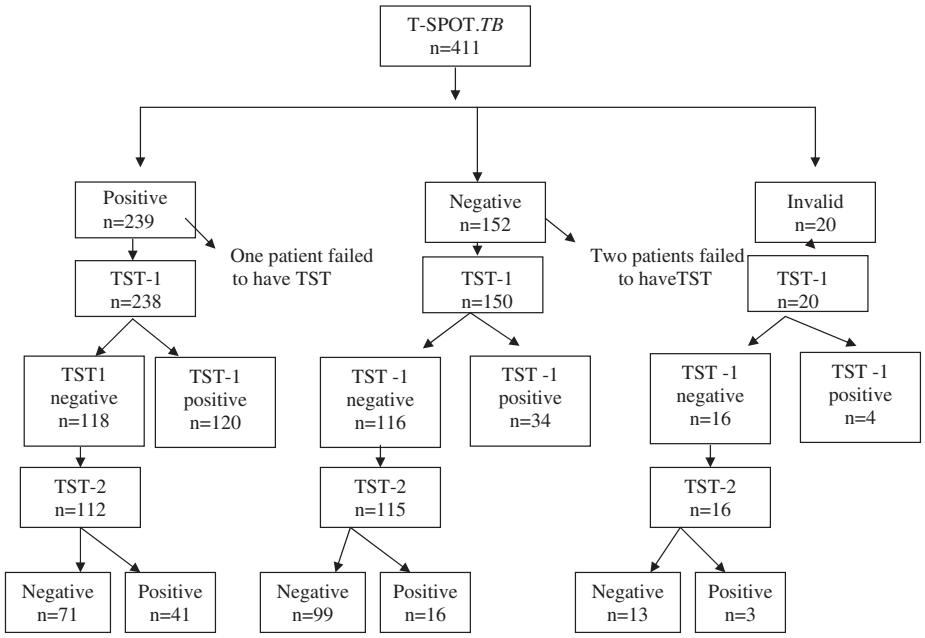

Fig. 1. Flow diagram of study results.

Table 2. TST-1 and T-SPOT.*TB* results[a]

	TST-1[b]		
T-SPOT.*TB*	Positive (*n*, %)	Negative (*n*, %)	Total (*n*, %)
Positive	120 (76)	118 (47)	238 (58)
Negative	34 (22)	116 (46)	150 (37)
Invalid	4 (2)	16 (7)	20 (5)
Total	158 (100)	250 (100)	408 (100)

[a]Agreement between TST-1 and T-SPOT.*TB* is 60%; $K = 0.25$.
[b]TST at the enrolment.

Table 3. Two-step TST and T-SPOT.*TB* results

	TST-2		
T-SPOT.*TB*	Positive (*n*, %)	Negative (*n*, %)	Total (*n*, %)
Positive	41 (68)	71 (39)	112 (46)
Negative	16 (27)	99 (54)	115 (47)
Invalid	3 (5)	13 (7)	16 (7)
Total	60 (100)	183 (100)	243 (100)

Of the 250 patients who were negative on TST-1, 118 (47%) were positive on T-SPOT.*TB*. Of the 158 patients who were positive on TST-1, 34 (22%) were negative on T-SPOT.*TB* (Figure 1, Table 2). It is noteworthy that 46 (36%) of the 127 patients who had 0 mm induration on TST-1 were positive on T-SPOT.*TB*.

When we compare the T-SPOT.*TB* assay results with TST results according to the three different cut-off values of TST (TST $\geq$ 5 mm, TST $\geq$ 10 mm and TST $\geq$ 15 mm) following results were found; T-SPOT.*TB* was found to be positive in 44, 47 and 56% of patients who had a negative TST-1 results according to the three different TST cut-off values, respectively. On the other hand, T-SPOT.*TB* was found to be negative in 25, 22 and 21% of patients who had a positive TST results according to the three different TST cut-off values, respectively.

TST-2 was performed on 243 of the 250 patients who were negative on TST-1. This second round of testing revealed booster phenomenon in 60 cases (25%) and a negative result in the remaining 183 (75%) cases. Overall, two-step TST increased the number (rate) of TST positives from 158 (39%) to 218 (53%). Overall, 53 and 61% of HD patients showed a positive reaction after a two-step TST and T-SPOT.*TB* tests, respectively. T-SPOT.*TB* was positive in 71 (39%) of the 183 patients who were negative on two-step TST (Figure 1, Table 3). Moreover, 16 (27%) of the 60 TST-boosted patients had a negative T-SPOT.*TB* result.

Finally, T-SPOT.*TB* was found to be negative on 50 (23%) of 218 overall TST-positive patients and positive on 71 (39%) of 183 overall TST-negative patients (Figure 1, Table 4).

Twenty patients (5%) had invalid T-SPOT-*TB* results. In four cases, there were >10 spots in the negative control well, and in 16 cases, there was no response to PHA.

Agreement between the T-SPOT.*TB* and TST-1 results was poor (60%; $K = 0.25$) and only improved slightly when the TST-2 results were incorporated (69%; $K = 0.36$) (Tables 2 and 4).

Patient characteristics relative to TST results for TB infection

Univariate analysis revealed no significant differences between overall (i.e. TST-1 and TST-2) TST-negative and overall TST-positive subjects with respect to mean age, mean serum ferritin, mean serum albumin and *Kt/V*. However, positive TST was significantly associated with male gender [odds ratio (OR) = 2.09, 95% confidence interval (CI): 1.37–3.19, $P = 0.0003$] and presence of BCG vaccine scar (OR = 1.58, 95% CI: 0.97–2.58, P = 0.046).

Table 4. Overall TST results and T-SPOT.*TB* results

	TST[a]		
T-SPOT.*TB*	Positive (*n*, %)	Negative (*n*, %)	Total (*n*, %)
Positive	161 (74)	71 (39)	232 (58)
Negative	50 (23)	99 (54)	149 (37)
Invalid	7 (3)	13 (7)	20 (5)
Total	218 (100)	183 (100)	401 (100)

[a]TST-1 and TST-2, together, Agreement between TST-1 and T-SPOT.*TB* is 69%; $K = 0.36$.

TST-positive patients had a significantly shorter mean HD duration than their TST-negative counterparts (57 ± 48 months versus 66 ± 55 months, respectively; P = 0.037). Multivariate logistic regression analysis revealed that male gender was the only independent factor associated with TST positivity (OR = 1.99; 95% CI: 1.3–3.0, P = 0.001).

*Patient characteristics relative to T-SPOT.*TB *results for TB infection*

Univariate logistic regression analysis showed no significant differences between T-SPOT.*TB*-negative and T-SPOT.*TB*-positive subjects with respect to mean age, mean serum ferritin level and *Kt/V*. However, T-SPOT.*TB* positivity was significantly associated with male gender (OR = 2.4; 95% CI: 1.54–3.73, P < 0.00001) and T-SPOT.*TB*-positive patients had lower mean serum albumin (3.99 ± 0.36 versus 4.08 ± 0.37 mg/dL, respectively; P = 0.01) and shorter mean HD duration (54 ± 47 versus 71 ± 57 months, respectively; P = 0.0008) than their T-SPOT.*TB*-negative counterparts. In addition, presence of BCG vaccine scar was inversely associated with T-SPOT.*TB* positivity (OR = 0.54, 95% CI: 0.31–0.92, P = 0.01),

Multivariate logistic regression revealed that male gender was independently associated with positive T-SPOT.*TB*, and positive T-SPOT.*TB* was inversely associated with presence of BCG vaccine scar, serum albumin level and HD duration (Table 5).

Findings at 1 year of follow-up

We were able to repeat TST on 139 of the total 183 HD patients who were negative on TST-1 and TsST-2 at enrolment (Figure 1). One year later, 16 (12%) of these 139 had converted to TST-positive status. We were able to repeat T-SPOT.*TB* on 111 of the total 172 HD patients whose T-SPOT.*TB* results at enrolment were negative or invalid. Among 111 T-SPOT.*TB*-negative HD patients, 35 (32%) had converted to TB-positive status according to T-SPOT.*TB* assay. There were no active TB during follow-up in any of the HD centres and the distribution of conversion rates was similar between the HD centres. However, there were four new cases of active TB diagnosed after the follow-up period (May 2007 to May 2011). For both types of testing, no significant associations were detected between annual conversion rate and age, presence of BCG vaccine scar, duration of HD, serum albumin level and serum ferritin level (P > 0.05 for all). Only male gender was associated with T-SPOT.*TB* annual conversion rate (OR = 2.66, 95% CI: 1.08–6.62, P = 0.01) but not TST conversion rate.

Table 5. Multivariate logistic regression analysis: factors associated with a positive T-SPOT.*TB* test

Variable	OR	95% CI	P-value
Sex (male)	2.72	1.65–4.84	0.0001
HD duration	0.99	0.98–0.99	0.007
Serum albumin level	0.47	0.25–0.91	0.025
BCG scar (≥1)	0.42	0.24–0.74	0.003

Discussion

As in the general population, active TB most often develops from the reactivation of LTBI in HD patients [22]. In this study, we found a high rate of one-step (39%) and two-step (53%) TST positivity in patients undergoing HD, which is similar to recent reports from Turkey [23–26]. The overall TST positivity was significantly higher in male patients than in female patients, as it was reported by Habesoglu *et al.* [25]. This gender-based difference was not evaluated in our study but it might be attributed to degree of environmental exposure in male versus female, and the dynamics of local spread [27]. It was also known that male gender predominates among TB patients in general population. In our study, we did not show any relationship between TST positivity and HD duration, age of patients, gender, serum albumin level, serum ferritin level and HD efficiency supporting the previous studies [25, 26,28–30].

The booster phenomenon that was detected in one-forth of our patients is an anamnestic reaction to diminished delayed type sensitivity reaction with time and it is usually associated with older age and BCG vaccination [31]. Although the most of our patients (75%) had a BCG vaccine scar, we were unable to show significant relationship between the booster phenomenon and the older age or the presence of BCG vaccine scar (data not shown). On the other hand, we found that presence of BCG vaccine scar was inversely associated with T-SPOT.*TB* positivity. We revealed that 66 of 92 (71%) BCG vaccine scar-negative HD patients were deemed to be infected compared with 173 of 299 (58%) BCG scar-positive HD patients on the basis of the T-SPOT.*TB* results; thus, presence of BCG vaccine scar associated with a protective effect in LTBI (Table 5). This finding is interesting since based on the nearly 100 years data after discovery of the BCG vaccine shows that the BCG vaccination reduced bacillary burden and dissemination after challenged with *M. tuberculosis* but does not protect infection [32]. However, our results suggest that at least part of BCG's protective effect may be attributable to protection against infection in adult HD patients. Similar to this finding, we previously have shown that presence of a BCG vaccine scar had a protective effect in TB infection diagnosed by IGRA in children with household contacts of active TB patients [33].

The annual TST conversion rate in our patients was 12%, which is similar to what it was reported (8%) from USA [34].

In this study, we also compared results of TST with T-SPOT.*TB* assays in a population that generally is considered to be at high risk for cutaneous anergy. We found that

61% of our study population was infected with TB bacilli detected by T-SPOT.*TB*. This rate is higher than the previously reported TB infection prevalence detected by TST [1, 2,23–26]. We also found that T-SPOT.*TB* detects TB infection more than initial TST (61 versus 39%). Consistent with our findings, Passalent *et al.* [35] reported that among 203 HD patients, the positivity rate of T-SPOT.*TB* (35%) was higher than that of TST (12.8%). Triverio *et al.* [36] also reported higher T-SPOT.*TB* (29%) positivity than TST positivity (19%) among 62 HD patients. On the other hand, Lee *et al.* [37] reported higher rate of TST positivity (62.5%) than IFN-γ-based assay (46.7%) among 32 ESRD patients. Chung *et al.* investigated the validity of IGRAs for diagnosing LTBIs in HD patients in an intermediate TB burden country (South Korea) with a high BCG vaccination rate, which is similar to our country. They enrolled 167 HD patients and found that positive rates for the TST, Quantiferon-TB Gold in tube, T-SPOT.*TB* tests were 23.5, 45.9 and 60.4%, respectively. They also revealed that previous BCG vaccination rate increased the TST positivity rate; however, it did not affect the rates for the Quantiferon-TB Gold in tube and T-SPOT.*TB* assays [38]. As far as we know, our study population is the largest patients group who underwent LTBI detection by IFN-γ based assays. Our study, Passalent's, Triverio's and Chung's studies have shown that T-SPOT.*TB* assay detected LTBI more frequent than TST. Among initial TST-negative subjects, we found that 47% of them had a positive T-SPOT.*TB*, which means that TST might miss nearly half of the TB-infected subjects in HD patients. After including the two-step TST results, the number of positive TST increased to 218 (53%), which is still lower than the positivity rate of T-SPOT.*TB.* Furthermore, among patients who had persistant negative two-step TST, T-SPOT.*TB* was positive in 39%, which may indicate that two-step TST might miss 39% of truly infected HD patients. Among initial TST-positive subjects, 22% had a negative T-SPOT.*TB.* Furthermore, among 60 TST-boosted patients, T-SPOT.*TB* was found to be negative in 27%. Overall, after adding the two-step TST results, T-SPOT.*TB* was negative in 23% of overall TST-positive subjects. Unfortunately, given our inability to determine definitively which patients did not have TB infection, we were unable to estimate the sensitivity or specificity of each diagnostic assay. Previous studies have shown IFN-γ-based assays to be more specific than the TST [39].

In this study, we also calculated annual incidence of TB infection by T-SPOT.*TB* and TST in our study population. We showed that annual T-SPOT.*TB* conversion rate is 32%, which is much higher than TST conversion (12%) rate. To our knowledge, this is the highest TB infection incidence in HD patients that has ever been published in the literature. The high rate of TB infection incidence in our population may be attributed to high prevalence of TB infection (40 per 100 000) in our country Turkey and higher risk of TB transmission in HD units [7–9]. On the other hand, repeating TST may boost T-SPOT.*TB* results. A systematic review done by van Zyl-Smit *et al.* [40] shows that TST administered 3 days prior to the IGRAs may boost IGRAs results. However, in our study, T-SPOT.*TB* assays were repeated in 111 patients with initial negative T-SPOT.*TB* results. Among them, 88 patients had a initial negative TST and underwent second TST, but only 30 of them had a T-SPOT.*TB* conversion. This means that 34% of TST repeated patients had a T-SPOT.*TB* conversion rate, which is similar to the overall T-SPOT.*TB* conversion rate (32%). Moreover, T-SPOT.*TB* were repeated in 22 patients with initial positive TST who did not get a second TST assay, T-SPOT.*TB* conversion rate is ~30% in this group of patients. All this results show that T-SPOT.*TB* conversion rate is not so much different among TST repeated, TST unrepeated and overall patient group. Also, when we reviewed the cited articles in the systematic review by van Zyl-Smit *et al.* [40], most of the studies investigated the boosting effect of TST in a one-month period, but in those studies investigating the boosting effect of TST on IGRAs over a longer time period (3–24 months after TST), the boosting effect of TST on IGRA results was not well proven. For example, in a study done by Richeldi *et al.* investigating TST effects on T-SPOT.*TB*, 44 BCG unvaccinated subjects with close contact to TB patients underwent repeated testing by TST and T-SPOT.*TB* at 9, 15 and 24 months after first TST testing and at 24 months, all 44 individuals remained T-SPOT.*TB* negative, although three had become positive with the TST, Thus, inoculation of three PPD skin tests over a 21-month period in 44 initially T-SPOT.*TB*-negative individuals did not induce any false-positive T-SPOT.*TB* results [41].

All those show that boosting effect of TST on T-SPOT.*TB* assay is not distinctive in our study.

Our study has certain limitations that warrant discussion. Foremost, it suffers from the same problem as previous studies that examined IFN-γ-based assays in TB infection. Since we were unable to determine the sensitivity and specificity of each diagnostic assay because of the lack of an established gold standard for TB infection.

In conclusion, our results suggest that screening for LTBI in HD patients with IGRAs in addition to TST would enhance the diagnosis. In HD patients, anergy is believed to play an important role in causing false-negative TST. Even a two-step TST is not sufficient to detect true LTBI in HD patients. We believe that the TST alone is not adequate to screen HD patients for LTBI because it is not sensitive enough in detecting LTBI. Furthermore, since the TST has been shown in previous studies to be less specific than T-SPOT.*TB*, we suggest the use of T-SPOT.*TB* in HD patients especially with negative TST assay.

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Conflict of interest statement. None declared.

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