


CASE REPORT

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Negative conversion of T-SPOT results after hemodialysis: case series and literature review

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Abstract

Background Latent tuberculosis infection (LTBI) affects 11.9% of outpatients under maintenance hemodialysis in Japan. In addition, the risk of reactivation of LTBI in hemodialysis patients is up to 10–25 times higher than that in the general population. Therefore, the accurate diagnosis and treatment for LTBI are extremely important. The interferon- γ release assays are widely used for screening LTBI; however, the impact of hemodialysis on the assay results has been uncertain.

Case presentation Twenty-seven hemodialysis patients (17 males, 69.9 ± 10.1 year old) were performed T-SPOT[®]. TB test (T-SPOT) both before and after hemodialysis. In cases where T-SPOT results were different before and after hemodialysis, T-SPOT was re-evaluated in a same manner. As a result, two cases showed consistent T-SPOT diagnostic discrepancy before and after hemodialysis in two measurements. In the first case, T-SPOT was indeterminate/positive before hemodialysis, but changed to negative after hemodialysis. In the second case, T-SPOT was positive before hemodialysis, but changed to indeterminate after hemodialysis.

Conclusions We experienced the negative conversion of T-SPOT after hemodialysis in cases of LTBI. T-SPOT may show false-negative when measured after hemodialysis due to immunomodulation caused by hemodialysis.

Keywords Hemodialysis, IGRAs, T-SPOT, *Mycobacterium tuberculosis*, LTBI

Background

Tuberculosis (TB) remains a significant global health concern despite the availability of effective treatments. After the infection with *Mycobacterium tuberculosis*, approximately 3–4% of patients develop TB within the first year, but in the rest patients, the organism is not eradicated and remains a state of persistent immune

response to stimulation by antigens without developing TB, referred to latent tuberculosis infection (LTBI) [1]. Patients with LTBI are at risk of developing active TB when their immune system is compromised.

The prevalence of LTBI in outpatients under maintenance hemodialysis (HD) in Japan was reported to be 11.9% (14/118 patients) [2]. The risk of reactivation is 10–25 times higher in HD patients than in the general population due to immune dysfunction associated with uremia [3]. Furthermore, when HD patients develop TB, the prognosis is often poor and treatment options are limited. Additionally, the risk of transmission to other HD patients sharing the same dialysis unit is high as *Mycobacterium tuberculosis* is airborne. Therefore, accurate diagnosis of LTBI among HD patients is extremely

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important and treating LTBI in HD patients is strongly recommended [3, 4].

The interferon- γ release assays (IGRAs); QuantiFERON[®]-TB Gold (QFT-G) and T-SPOT[®].TB test (T-SPOT) are thought to have greater diagnostic accuracy in determining LTBI in HD patients compared to other TB tests, such as the tuberculin skin test [3]. Therefore, IGRAs are widely used for screening LTBI in HD patients; however, the impact of HD on IGRA results has yet to be fully investigated.

Case presentation

We measured T-SPOT before and after HD in 27 patients (17 males, 10 females, 69.9 \pm 10.1 year-old). The backgrounds are shown in Table 1. Twenty-one patients were T-SPOT negative before and after HD, but five patients (18.5%) were T-SPOT positive and were diagnosed as LTBI and one patient (3.7%) was diagnosed as undetermined as T-SPOT was indeterminate. In addition, four patients of six patients had different T-SPOT results before and after HD, then T-SPOT was measured again before and after HD. As a result, two out of four patients showed consistent T-SPOT diagnostic change before and after HD. The other two patients showed the different results before and after HD in the second assay (positive or negative, respectively).

The first case was a 63-year-old man with chronic glomerulonephritis on HD for 5 years with quantity of blood flow (QB) 200 ml/min for 4 h. The T-SPOT diagnosis was indeterminate (ESAT-6: 5, CFP-10: 2) before HD, but changed to be negative (ESAT-6: 1, CFP-10: 4) after HD. The second T-SPOT measurement also showed consistent result being positive (ESAT-6: 0, CFP-10: 9) before HD and negative (ESAT-6: 2, CFP-10: 4) after HD. The second case was a 77-year-old man with chronic glomerulonephritis on HD for 17 years with QB 300 ml/min for 4 h. He was diagnosed as positive (ESAT-6: 8, CFP-10: 15) before HD, but changed to indeterminate (ESAT-6: 6,

CFP-10: 7) after HD. The second assay was also reproducible, with positive (ESAT-6: 7, CFP-10: 10) before HD, but indeterminate (ESAT-6: 7, CFP-10: 2) after HD (Table 2).

Mini-review

Our study showed that 18.5% of HD patients were diagnosed as LTBI. Among these LTBI patients, we found negative conversion of T-SPOT results after HD in two patients.

Table 2 Consistent negative conversion in T-SPOT after HD

	1	2
<i>Background</i>		
Age (year-old)	63	77
Sex	M	M
Causes of CKD	HNS	HNS
Dialyzer	FB-210Ub	NVF-21H
QB (ml/min)	200	300
Dialysis vintage (years)	5	17
Dialysis time (hours)	4	4
Anticoagulation	Heparin	Heparin
<i>T-SPOT</i>		
1		
Pre-HD		
Negative-control	0	0
Positive-control	446	718
ESAT-6	5	8
CFP-10	2	15
Diagnosis	Indeterminate	Positive
Post-HD		
Negative-control	0	2
Positive-control	226	710
ESAT-6	1	6
CFP-10	4	7
Diagnosis	Negative	Indeterminate
2		
Pre-HD		
Negative-control	0	1
Positive-control	371	989
ESAT-6	0	7
CFP-10	9	10
Diagnosis	Positive	Positive
Post-HD		
Negative-control	0	0
Positive-control	426	749
ESAT-6	2	7
CFP-10	4	2
Diagnosis	Negative	Indeterminate

T-SPOT T-SPOT[®].TB test, *HD* hemodialysis, *ESAT-6* early secretory antigenic target 6, *CFP-10* culture filtrate protein 10, *CKD* chronic kidney disease, *QB* quantity of blood flow, *HNS* hypertensive nephrosclerosis

Table 1 Backgrounds

Age (year-old, mean \pm SD)	69.9 \pm 10.1
Sex (male, %)	63.0
Causes of CKD (n)	
HNS	11
DM	12
CGN	1
Other	3
Dialysis vintage (years, mean \pm SD)	8.8 \pm 6.9
Dialysis time (hours, mean \pm SD)	3.9 \pm 0.29

CKD chronic kidney disease, *HNS* hypertensive nephrosclerosis, *DM* diabetes mellitus, *CGN* chronic glomerulonephritis, *SD* standard deviation

The risk of TB reactivation is 10–25 times higher in HD patients than in the general population. Therefore, proactive LTBI screening for HD patients especially aged 60 and above, who represent the most prominent risk group for LTBI, is strongly recommended [2]. The elevated risk of TB reactivation in HD patients is due to immunosuppression caused by uremia. Decreased function of neutrophil function, macrophage Fc receptor function, and cellular immunity have been observed, especially within 1 year after initiation of HD, warranting caution. Those immunosuppression recovers with the improvement of uremia through continued HD; these parameters may decrease again in long-term cases exceeding 15 years of HD vintage. As the duration of dialysis prolongs, the risk of HD reactivation is presumed to increase again [5]. Furthermore, chronic glomerulonephritis, a common cause of end-stage renal disease, often requires long-term administration of immunosuppressive drugs, including corticosteroids. Patients who initiated HD while using corticosteroids, the higher the dose and the longer the period of use, the higher risk of TB reactivation [6]. Additionally, it is known that there is a two- to fourfold higher risk of active TB in patients with diabetes mellitus, including those with diabetic kidney disease, compared to the general population [7].

The tuberculin skin test has long been used as a method for LTBI screening, but in recent years, it has been replaced by the IGRAs such as T-SPOT and QFT-G. T-SPOT is an enzyme-linked immunosorbent spot test that detects and quantifies the number of peripheral blood-derived IFN- γ -secreting T cells under stimulation with mycobacteria-specific peptide antigens such as ESAT-6 and CFP-10. The low molecular mass antigen ESAT-6 was identified from culture filtrate because of its strong recognition in animals infected with *Mycobacterium tuberculosis*. In addition, the promoter region of the ESAT-6 gene was cloned, and another antigen as CFP-10 with the same species distribution as ESAT-6 was identified. ESAT-6 and CFP-10 are absent in all strains of BCG and in environmental isolates with the exception of *Mycobacterium kansasii*, *Mycobacterium marinum*, and *Mycobacterium szulgai*. Therefore, T-SPOT has a higher specificity for TB diagnosis compared to the tuberculin skin test [8].

There are several reports of LTBI screening of HD patients using the IGRAs in Japan. Ogawa et al. and Inoue et al. reported that the prevalence of LTBI in maintenance HD patients was 11.9% (14/118) and 12.3% (20/162) by using QFT-3G QuantiFERON[®] TB Gold IGRA (QIAGEN, Germantown, MD, USA) and QuantiFERON[®] (Cellestis Ltd, Carnegie, Victoria, Australia), respectively. However, there are limited reports on T-SPOT in Japan. The only report to our knowledge is

Kimura et al. used T-SPOT as a screening at initiation of dialysis introduction, finding the prevalence of LTBI was 17.9% (27/151). We demonstrated T-SPOT was measured for maintenance HD patients with a mean dialysis vintage of 8.8 years, which revealed that 18.5% (5/27) of them were diagnosed as LTBI.

HD is known to affect both innate and adaptive immune responses [9]; therefore, the immune response in IGRAs may be different before and after HD, and it is interesting to know whether it is more appropriate as a screening test to perform pre- or post-HD. When comparing immune responses to TB antigens before and after HD, minimizing the individual differences in lymphocyte count changes before and after HD is essential. A single HD session develops T-cell lymphopenia due to the induction of apoptosis [9]. In addition, hemoconcentration due to body fluid removal during HD varies significantly between patients. Hursitoglu et al. [10] recently reported that 16% of patients had demonstrated negative conversion in QFT-G results after HD. However, the QFT-G test measures IFN- γ concentration when 1 ml of peripheral blood is stimulated with TB antigens [11], making it susceptible to individual differences in lymphocyte count change before and after HD. T-SPOT adjusts cell counts after monocyte separation from peripheral blood to ensure consistency and measure the number of IFN- γ -producing cells in response to TB antigen stimulation. T-SPOT may be more appropriate when minimizing the effects of the individual differences in lymphocyte count change before and after HD.

We observed a negative conversion of T-SPOT after HD in two patients (7.4%). Although reports measuring T-SPOT before and after HD are few, Putri et al. measured T-SPOT before and after HD in 45 HD patients and found negative conversion of T-SPOT after HD in three patients (6.7%). However, the report measured only once, so it is still unclear that negative conversions are reproducible findings. In our study, four patients demonstrated conversion of T-SPOT in the first measurement but only two of them (50%) showed consistent T-SPOT conversion in the second measurement.

Previous studies have shown that the number of IFN- γ positive cells significantly decreased after HD compared to before [12]. In addition, patients complicated with active TB showed significantly low IFN- γ levels after HD than before [13]. Therefore, we assume that our patients had a negative conversion of T-SPOT results due to a decrease in TB antigen-specific IFN- γ producing T cells after HD. The detail mechanisms for this decrease remain unclear; however, inflammation associated with HD may be a contributing factor. HD increase the production of interleukin-12 (IL-12) from peripheral blood mononuclear cells (PBMCs) through complement activation, and

IL-12 promotes INF- γ production by stimulating Th1 cells. However, as a result of chronic stimulation by IL-12 production during HD, it is supposed that an expression of IL-12 in response to new stimuli by post-HD PBMCs has been downregulated, together with decrease in IL-12 receptors on targeting-T cells [12, 13]. Therefore, chronic IL-12 stimulation by HD may have caused IL-12 tolerance and reduced the number of IFN-producing T cells after HD.

As a limitation, we measured T-SPOT in a small number of patients. Therefore, a study of LTBI screening in maintenance HD patients using T-SPOT is required with a larger number of patients.

Conclusion

In conclusion, we experienced a negative conversion in T-SPOT after HD in cases of LTBI. T-SPOT may be false-negative when measured after HD due to chronic inflammation by HD, suggesting the IGRAs need to be done before HD.

Abbreviations

TB	Tuberculosis
LTBI	Latent tuberculosis infection
HD	Hemodialysis
IGRAs	Interferon- γ release assays
QFT-G	QuantiFERON [®] -TB Gold
T-SPOT	T-SPOT [®] .TB test
ESAT-6	Early secretory antigenic target 6
CFP-10	Culture filtrate protein 10
QB	Quantity of blood flow
IL-12	Interleukin-12
PBMCs	Peripheral blood mononuclear cells

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Author contributions

AY, NH, and MR designed for this study. MT and AY drafted the manuscript. All authors revised the paper, and all authors approved the final version of the manuscript.

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Declarations

Ethics approval and consent to participate

The Ethics Committee of Tokyo Saiseikai Central Hospital approved this study (Approval Number 28-10). Informed Consent Statement: All participants provided consent in this study.

Consent for publication

We obtained consent for publication from the patients.

Competing interests

The authors declare that they have no competing interests.

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