In-Depth Review

Diagnosis of Tuberculosis in Dialysis Patients: Current Strategy

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Patients with ESRD undergoing chronic dialysis are much more prone to develop tuberculosis (TB) than the general population. In these patients, the diagnosis of TB disease is often difficult because of prevailing extrapulmonary involvement and nonspecific symptoms. The prevalence of latent TB infection (LTBI) in ESRD patients is elevated, and those who become infected are at high risk of developing active disease. Therefore, screening for LTBI in this population is recommended, aiming to prevent progression to active TB and secondary contamination of others. The tuberculin skin test (TST), the classic diagnostic tool for LTBI, has several major drawbacks, including poor sensitivity (because of a high prevalence of anergy in dialysis patients) and specificity [with false-positive tests in those vaccinated with bacille Calmette–Guérin (BCG)]. In the past 10 years or so, new immunological tests using IFN-γ release assays (IGRAs) have become available and have shown superior sensitivity and specificity for the diagnosis of TB compared with the TST in several studies, some very recent ones including ESRD patients. Therefore, current strategy in dialysis patients should use these tests instead of TST for LTBI screening and as an aid for the diagnosis of active TB.


Patients with ESRD undergoing chronic dialysis are 6 to 25 times more likely to develop tuberculosis (TB) than the general population, mainly because of the impaired cellular immunity characteristic of this condition (1–3). Nosocomial transmission of TB has also been reported in hemodialysis (HD) centers (4). In a U.S. Renal Data System (USRDS) retrospective analysis, age, unemployment, Asian and Native American race, smoking, reduced body mass index, low serum albumin, ischemic heart disease, and anemia were all significant risk factors for HD patients to develop TB (5).

The mortality rate of TB in dialysis patients is high, ranging from 17% to 75% (6). In the USRDS analysis mentioned above, TB was independently associated with a 42% increased mortality (5).

Active TB in ESRD Patients: Atypical Clinical Presentation

In ESRD, the diagnosis of TB disease is often difficult because of prevailing extrapulmonary involvement and nonspecific symptoms. Extrapulmonary TB has been reported in as many as 60% to 80% of cases, either alone or associated with pulmonary TB. The most common forms of presentation are lymphadenitis, gastrointestinal, bone, genitourinary, peritonitis, pleural effusion, pericardial effusion, miliary TB, and pyrexia of unknown origin (7–10). On the other hand, uremia is commonly associated with fatigue, malnutrition, and other nonspecific complaints, possibly concealing the course of an underlying TB disease (7,11,12).

This atypical presentation may often lead to a delay in accurate diagnosis and therapy, sometimes resulting in patients’ death. Therefore, nephrologists should always have a high degree of suspicion and consider the possibility of TB whenever confronted with an ESRD patient presenting with general symptoms such as fever, weight loss, and/or lymphadenopathy (10). The diagnosis would then require the isolation of acid-fast bacilli, the finding of typical caseating granuloma on biopsy, or the recovery of tubercle bacilli from the culture of the biopsy material (7).

Difficulties in Diagnosing Latent TB Infection: The Limitations of the TST

According to several studies using different diagnostic approaches, the prevalence of latent TB infection (LTBI) in chronic dialysis patients is high, ranging between 20% and 70% (2,13,14), and those who become infected are at high risk of developing active disease (15,16). Therefore, screening for LTBI in this population is recommended, aiming to prevent progression to active TB and secondary contamination of other patients and healthcare workers (15). It has been shown that preventive treatment of latently infected people diminishes the risk of subsequent development of active TB by approximately 90% (17).

In persons with LTBI, the very low bacterial burden makes it impossible to directly detect Mycobacterium tuberculosis: acid-fast bacilli are rarely seen on sputum smear examination and tubercle bacilli are not found in cultures of respiratory speci-
mens. Serologic testing is unreliable and the chest radiograph is usually normal (15,18).

The classic diagnostic tool for LTBI is the tuberculin skin test (TST), which is based on the strong cell-mediated immune response induced by LTBI. The TST measures the delayed-type hypersensitivity response to intradermal inoculation of tuberculin purified protein derivative (PPD), a crude mixture of >200 M. tuberculosis proteins (18). In 1995, the Centers for Disease Control and Prevention (CDC) recommended screening for LTBI with TST in persons with a medical condition that increases the risk of TB, including those with ESRD. A skin induration over 10 mm in diameter is considered positive (19).

However, the high-risk groups that are targeted for screening and preventive therapy are also those in which the TST most often fails to detect LTBI (15,20). The prevalence of anergy to TST in the ESRD population is significantly higher than in the general population (44% versus 16%) (21). This same limitation of the skin test applies to its use as a diagnostic aid in the evaluation of patients with suspected active TB (when microbiological confirmation is awaited or not possible). In one study in HD patients diagnosed with active TB, anergy to TST was found in over 50% of patients (7). Thus, because of its poor sensitivity, a negative TST in these patients cannot be used to eliminate the possibility of latent or active TB (15).

Various strategies to improve the sensitivity of the TST in dialysis patients have been advocated, including two-step testing to induce a “booster phenomenon.” In a study by Dogan et al. (22), 11.3% of 124 chronic HD patients showed a positive reaction with the first test, whereas the second test added 12.1% more. Multiple (>2) testing does not seem to add significant benefit. Wauters et al. administered four consecutive TSTs (at 7-day intervals) to 224 HD patients (1): after the first test, 14.7% of the patients showed a positive reaction; the second test added 13.1% more TST-positive patients; and the third and the fourth TST only resulted in an additional 4.2% and 4.4% new positive patients, respectively. However, repeated testing most likely increases sensitivity at the expense of specificity. Also, repeated testing has operational limitations linked to the need of many return visits. Setting a lower TST threshold (e.g., 5-mm induration instead of 10) would also increase the sensitivity of the test, but again it would do so at the cost of a larger number of false-positive TST reactions, meaning many individuals possibly exposed to unnecessary preventive treatment (18).

The second major drawback of TST is its low specificity. Because PPD is a culture filtrate of tubercle bacilli containing many antigens shared with the bacille Calmette–Guérin (BCG) vaccine and most nontuberculous mycobacteria (20), individuals vaccinated with BCG but not infected with M. tuberculosis can test falsely positive using the TST. However, in the study of Wauters et al., a history of BCG vaccination did not correlate with skin testing. Whereas 85.7% of control patients with PPD reactivity in vitro were skin-test positive, the respective percentage among HD patients was significantly lower—51.4%. The authors concluded that, unlike the skin test, measurement of PPD reactivity by in vitro quantitation of PPD-specific T cells was not affected by uremia-associated immunosuppression, and they suggested that this assay might thus be a valuable alternative to TST. However, the method did not receive further attention from researchers because other more specific blood assays for the diagnosis of LTBI have subsequently been developed and introduced in clinical practice.

The IFN-γ Release Assays: Advantages over the TST

These new tests use two proteins encoded by a unique genomic segment (stretch of DNA) termed “Region of Difference 1,” which is absent from all strains of Mycobacterium bovis, BCG, and most nontuberculous mycobacteria but is present in M. tuberculosis (25). These proteins, early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10), are major targets of T-helper type 1 cells in patients with M. tuberculosis infection (26,27). Therefore, a T cell response to these antigens could serve as a specific marker of M. tuberculosis infection, avoiding the antigenic crossreactivity of PPD, the main cause of poor specificity of the TST.

The T-SPOT.TB test (Oxford Immunotec, Abingdon, United Kingdom) was developed by A. Lalvani and co-workers in the late 1990s (28). T cells from individuals with M. tuberculosis infection become sensitized to ESAT-6 or CFP-10 in vivo; when the T cells re-encounter these antigens ex vivo in the overnight T-SPOT.TB assay, they release a cytokine, IFN-γ. By the next morning, each such T cell gives rise to a dark spot, which is the “footprint” of an individual M. tuberculosis-specific T cell (26). The readout is thus the number of spots that are counted using a magnifying lens or automated reader. The QuantiFERON-TB Gold (QFT-G) test (Cellestis, Carnegie, Australia) is a whole-blood ELISA that measures the IFN-γ concentration in the supernatant of a sample of diluted whole blood after 24 hours of incubation with ESAT-6 and CFP-10 (29). Originally devel-
The QuantiFERON-TB Gold assay, developed in the 1980s for detecting TB in cattle, was adapted for human use in the 1990s. The U.S. Food and Drug Administration has approved it for *in vitro* diagnostics, and a guideline from the CDC has been published (30). A newer version of the QuantiFERON-TB Gold assay, the so-called “In-Tube” test (QFT-GIT), uses tubes prefilled with antigens; this simplifies the laboratory procedures, making it more suitable for “on-field” usage in settings with limited resources (18). The three of these assay platforms are sometimes collectively referred to as “T cell-based IFN-γ-release assays” (IGRAs). A summary of the characteristics of the IGRAs in comparison with the TST is presented in Table 1.

In theory, the T-SPOT.TB-based test should be more sensitive because it detects IFN-γ in the immediate vicinity of the T cell from which it is released (where it is still at a locally high concentration), whereas the ELISA detects IFN-γ after it has diffused into the supernatant and become diluted in the total volume of the test sample (18).

Reviews of several studies in various populations (18,31) suggested similar levels of specificity for the two IGRAs and these were statistically higher than those for TST, particularly in subjects vaccinated by BCG. Four case-control studies on 127 BCG-vaccinated low-risk individuals in aggregate showed that T-SPOT.TB has a specificity of 100% (26,27,32–34). A case-control study on 216 Japanese BCG-vaccinated adults showed a 98% specificity of QFT-G, much higher than for TST (35%) (29).

On the other hand, establishing the diagnostic accuracy for LTBI of the IGRAs is a major challenge because a gold standard is lacking. However, when testing patients with active TB, T-SPOT.TB showed sensitivity ranging from 83% to 97% in five studies on 266 cumulative patients, whereas in another five studies on 330 cumulative patients the sensitivity of QFT-G ranged from 70% to 89%. When these results were pooled, the sensitivity of T-SPOT.TB seemed to be significantly higher compared with QFT-G (18). The sensitivity of the IGRAs is at least equivalent to that of TST and, in certain studies, superior with T-SPOT.TB. Finally, several studies in contacts have been undertaken with the aim of measuring the concordance between these biologic tests and the TST. The essential finding was a very good correlation between positivity of the blood tests and the degree of exposure of the contacts (31).

Studies in immunocompromised populations confirmed the superiority of IGRAs for the diagnosis of LTBI in comparison with TST, which has notoriously low sensitivity in these settings (35). In HIV-seropositive persons, it has been demonstrated that T-SPOT.TB has a higher sensitivity than the TST (27,36–39). However, the results with QFT-G seem to

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the TST and of the IGRAs (18)</th>
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<tbody>
<tr>
<td><strong>T-SPOT.TB</strong></td>
</tr>
<tr>
<td>Antigens</td>
</tr>
<tr>
<td>Positive internal control</td>
</tr>
<tr>
<td>Uniformity of methods and reagents</td>
</tr>
<tr>
<td>Potential for boosting effect in repeated tests</td>
</tr>
<tr>
<td>Need for return visit</td>
</tr>
<tr>
<td>Time required for results</td>
</tr>
<tr>
<td>Setting of test</td>
</tr>
<tr>
<td>Interpretation of test</td>
</tr>
<tr>
<td>Readout units</td>
</tr>
<tr>
<td>Technological platform</td>
</tr>
<tr>
<td>Test’s substrate</td>
</tr>
<tr>
<td>Outcome measure</td>
</tr>
<tr>
<td>Readout system</td>
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\(^a\)Mantoux test versus Heaf test, induration versus erythema, PPD-S versus PPD RT-23.
be less convincing. For example, Raby et al. (40) evaluated the QFT-G in a cross-sectional study of Zambian adults with smear-positive TB and found that the sensitivity of the QFT-G was significantly lower in HIV-positive than in HIV-negative individuals. Subsequent studies have also confirmed that with decreasing CD4 count, false-negative and indeterminate results are seen more frequently, which may affect the diagnostic utility of the QFT-G in this population (37,41,42). In patients with autoimmune diseases treated with immunosuppressive agents, the experience with IGRAs in diagnosing LTBI is rather limited and is mainly drawn from small-scale cross-sectional studies (35). In general, these studies have found that agreement between the TST and IGRAs is, at best, fair (43–45) and that, although false-negative IGRA results may occur, the diagnostic sensitivity of IGRAs is more robustly maintained than that of the TST (35).

The IGRAs have an internal positive control (i.e., a sample well stimulated with a potent nonspecific stimulator of IFN-γ production by T cells). Although a negative TST in immunosuppressed individuals can be a false negative, the failure of the positive control in the blood tests provides the important information that the test’s result cannot be reliably interpreted because it may reflect an underlying in vivo immunosuppression, negatively affecting T cell function in the in vitro stimulation (18). Studies suggest that indeterminate results are relatively common with the ELISA, occurring in 5% to 40% of patients with QFT-G but less frequently with the newer QFT-GIT (46–48). Indeterminate QFT results seem to be associated with extremes of age (<5 and >80 years) and immunosuppression, especially HIV infection (41). In contrast, indeterminate results are more rare with the T-SPOT.TB, occurring in 0% to 5.4% (49).

Other important advantages of the IGRAs compared with the TST include lack of boosting effect in repeated tests, no need for return visit, shorter time required for results (16 to 24 hours versus 48 to 72 hours), and objective (instrument-based) interpretation of the test (18).

It is generally thought that the higher diagnostic accuracy together with the operational advantages of the IGRAs should improve the effectiveness of TB control programs. Higher specificity will reduce false-positive test results, thus avoiding unnecessary chemoprophylaxis. On the other hand, higher sensitivity would identify more infected persons among those with a false-negative TST result. More true-positive results in infected people would increase the rate of diagnosis and treatment of LTBI before progression to active TB (18).

Nevertheless, it is of paramount importance to also understand the limitations of the IGRAs: lower sensitivity in severe disease, unknown predictive value for future development of active TB (because no studies have been performed on this subject), and lack of distinction between LTBI and active TB (31). Limited availability of IGRAs (in contrast with the worldwide readily accessible TST) is another important issue. A 2008 survey of the Infectious Disease Society of America’s Emerging Infections Network (IDSA EIN) showed that QFT-G was available for use in only 63% of U.S. regions, with the lowest availability reported in the southern and eastern states (50). Most likely, the availability of these tests is even lower in low-income countries. However, the single major disadvantage of IGRAs is their high cost, which is approximately $30 for QFT-G, $25 for QFT-GIT, and $57 for T-SPOT.TB in the United States. Most of this cost is related to the laboratory, but other procedures, such as phlebotomy, specimen transport, and processing also count (51). In comparison, the PPD used in the TST is only about $10; however, additional costs of the follow-up visit for reading the TST reaction must be considered (52). The introduction of blood tests in clinical practice is expected to initially increase the cost of TB control. However, the fact that most of the costs for TB control are incurred during diagnosis and treatment of patients with active TB suggests that higher diagnostic sensitivity and higher specificity could induce cost savings in the medium and long term by reducing the future burden of cases of active TB and decreasing the number of uninfected BCG-vaccinated people inappropriately treated for LTBI (18). Studies in Switzerland and Germany have shown that the most cost-effective method for LTBI screening is the two-step strategy (TST followed by IGRA in TST-positive patients) (53,54). The cost-effectiveness of this strategy is probably related to low prevalence of LTBI and high prevalence of BCG history in these countries. IGRA testing alone is only cost-effective at LTBI rates >40% (55). Furthermore, the costs of IGRAs are likely to decline with increasing usage of these tests.

**Table 2. Summary of studies on the value of IGRAs for the diagnosis of TB in ESRD patients**

<table>
<thead>
<tr>
<th>Study (Reference)</th>
<th>n</th>
<th>Objective</th>
<th>IGRA</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Indeterminate Results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoue et al. (56)</td>
<td>162</td>
<td>Diagnosis of active TB</td>
<td>QFT-G</td>
<td>100</td>
<td>89.7</td>
<td>24.1</td>
</tr>
<tr>
<td>Zoccali et al. (57)</td>
<td>29</td>
<td>Diagnosis of active TB</td>
<td>T-SPOT.TB</td>
<td>91.7</td>
<td>64.7</td>
<td>NA</td>
</tr>
<tr>
<td>Passalent et al. (3)</td>
<td>203</td>
<td>Diagnosis of LTBI</td>
<td>T-SPOT.TB</td>
<td>73.1 to 78.6</td>
<td>NA</td>
<td>5.1</td>
</tr>
<tr>
<td>Triverio et al. (14)</td>
<td>62</td>
<td>Diagnosis of LTBI</td>
<td>T-SPOT.TB</td>
<td>22</td>
<td>61.2</td>
<td>11.0</td>
</tr>
<tr>
<td>Chung et al. (58)</td>
<td>167</td>
<td>Diagnosis of LTBI</td>
<td>T-SPOT.TB</td>
<td>65.7</td>
<td>41.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Hoffmann et al. (59)</td>
<td>39</td>
<td>Diagnosis of LTBI</td>
<td>QFT-G</td>
<td>62.5</td>
<td>63.5</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>QFT-GIT</td>
<td>71.4</td>
<td>100</td>
<td>2.6</td>
</tr>
</tbody>
</table>
In recent years, only a few studies have been published that tried to assess the value of the blood assays for the diagnosis of TB in HD patients. A summary of these studies is presented in Table 2.

For the diagnosis of active TB, the sensitivity and the specificity of the IGRAs were found to be 100% and 89.7%, respectively, for QFT-G (56) and 91.7% and 64.7%, respectively, for T-SPOT.TB (57).

On the other hand, the value of the IGRAs for the diagnosis...
of LTBI in dialysis patients, as well as in the general population, is difficult to estimate in the absence of a gold standard. In a study by Passalent et al. (3), LTBI was diagnosed by an expert panel of physicians on the basis of three elements: patient self-reported history of active TB, radiographic findings consistent with previous TB infection, and a TST of induration >10 mm. The T-SPOT.TB was positive in 78.6%, 72.7%, and 73.1% of patients, respectively, whereas TST was positive in only 21.4% of participants with positive history and in 18.2% of those with suggestive x-rays. Defining “probable LTBI” as chest radiography suggestive of prior infection and/or established “at risk” contact with a patient with contagious TB, Triverio et al. (14) found a higher sensitivity (46%) for QFT-G than for T-SPOT.TB (22%) and TST (25%). Similarly, Chung et al. (58) defined patients as being at “high-risk for LTBI” if they had a history of close contact with TB patients, old TB lesions on chest x-rays, or a history of TB infection. They showed that QFT was independently associated with the high-risk group (adjusted odds ratio 2.58; P = 0.036), whereas TST and T-SPOT.TB were not. Finally, Hoffmann et al. (59) found that, in comparison with TST of induration ≥10 mm, QFT-GIT was more closely related to “TB exposure” (i.e., previous TB-disease, chest x-ray suggestive for LTBI, or origin from a medium TB-prevalence region) as a proxy for LTBI.

Winthrop et al. (60) reported that patients in a dialysis center with TB case contact were likely to have a positive QFT-G (P = 0.02) and T-SPOT.TB (P = 0.04), but not a positive TST (P = 0.7).

Furthermore, Lee et al. (13) performed TST, T-SPOT.TB, and QFT-G in 32 ESRD patients that were subsequently followed up for 2 years. They found that only QFT-G but neither T-SPOT.TB nor TST predicted the development of active TB in these patients.

When QFT-G and T-SPOT.TB were used, in three of the previously mentioned studies in HD patients the agreement between the two tests has been found to be fair or moderate (κ = 0.27 to 0.60), whereas the agreement between TST and IGRA was only poor or fair (κ = 0.16 to 0.27 for QFT-G and 0.16 to 0.32 for T-SPOT.TB) (13,14,58). On the other hand, in the study of Winthrop et al. (60), the concordance between results was better, ranging from 71% (TST versus T-SPOT.TB) to 79% (TST versus QFT-G) to 87% (QFT-G versus T-SPOT.TB).

The rate of indeterminate results in HD patients varies between 2% and 24% for QFT-G and 3% and 11% for T-SPOT.TB according to different studies (Table 2).

In the study of Chung et al. (58), previous BCG vaccination significantly increased the rate of positive TST results, but did not affect the QFT and T-SPOT.TB results.

Screening for LTBI in Dialysis Patients: Current Strategy

With all of their limitations, the superiority of the new blood assays over the TST is indisputable and their introduction in clinical practice is certainly a major progress in the diagnosis of LTBI. Many countries (at least 17 so far) have developed new national guidelines for the detection of LTBI, including the place of IGRA in this process (30,61–70) (Table 3). In immunocompetent persons, the vast majority of these guidelines recommend a two-step approach (i.e., TST followed by IGRA to confirm TST-positive cases), which seems to be the most cost-effective strategy. On the other hand, in immunocompromised individuals in whom the TST may be falsely negative, almost all existing guidelines recommend using IGRA, if available, as the sole test for LTBI.

Summarizing the findings in ESRD patients described above, we may point out that (1) these patients are at high risk for M. tuberculosis infection, and, once infected, they are very prone to develop active disease, which may be severe and difficult to diagnose; (2) screening for LTBI is therefore recommended, but the anergy rate to TST is high; (3) the IGRA are more sensitive and specific than the TST for detecting LTBI; (4) the IGRA also have operational advantages over the TST; and (5) the rate of indeterminate results of IGRA tests is rather low.

Therefore, we suggest that chronic dialysis patients should be screened for LTBI by using an IGRA test, if available, instead of TST. Any of the three types of assays may be used, but QFTs are less expensive and probably more sensitive in these patients. However, cost-effective studies are desirable to support the implementation of this strategy in dialysis units.

A positive IGRA test requires excluding active disease before affirming LTBI, and this should include checking for specific symptoms and signs, a chest radiograph, and sometimes examination of sputum or other clinical samples for the presence of M. tuberculosis. After TB has been excluded, treatment of LTBI should be considered. On the other hand, a negative test cannot exclude TB when suggestive history, symptoms, or physical signs are present. The high susceptibility to TB infection of dialysis patients must always be kept in mind, particularly in endemic areas. In symptomatic patients, further evaluation (including chest radiographs and microbiologic or pathologic exams) is necessary, and IGRA may also be used in difficult situations as an aid for diagnosis. Indeterminate IGRA results cannot be interpreted; they may entail repeated testing in patients with a high probability of having TB, but not in those with a low probability (30).

Disclosures

None.

References


4. Centers for Disease Control and Prevention: Tuberculosis
Diagnosis of Tuberculosis in Dialysis Patients


