Assessment of an Interferon-γ release assay for the diagnosis of latent tuberculosis infection in haemodialysis patients

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Summary

Background: The accurate diagnosis of latent tuberculosis infection (LTBI) in haemodialysis patients remains elusive. Impaired immune function associated with chronic kidney failure causes a high number of anergic tuberculin skin tests (TST). Interferon-γ (INF-γ) release assays (IGRAs) measuring the INF-γ secretion of tuberculosis specific T-cells have several advantages over the TST but their significance in dialysis patients is currently uncertain.

Methods: This study examines the test-performances of the QuantiFERON Gold InTube (QFT-GIT) in a cohort of 39 haemodialysis (HD) patients and 52 healthy individuals.

Results: INF-γ secretion in HD patients was significantly lower than in healthy controls, however, mitogen-anergic QFT-GIT results were only found in 2.5% of HD-patients. INF-γ secretion was independent of duration of HD treatment, dialysis quality and nutritional status. The QFT-GIT showed a closer association with TB risk factors as a proxy for past exposure to TB than the TST.

Conclusions: We conclude that the QFT-GIT is a valid alternative to the TST. Together with the survey of TB risk factors, it may help to diagnose LTBI more accurately in HD-patients.

Key words: haemodialysis; INF-γ release assay; LTBI; tuberculosis

Introduction

There is a growing need for increased awareness of latent tuberculosis infection (LTBI) in low TB endemic countries due to increased migration from higher endemic regions [1] and increased use of immunosuppression and organ transplantation [2]. Patients with chronic renal failure requiring haemodialysis (HD) are at increased risk of developing TB-disease due to their systemic immunosuppression [3, 4] and the subsequent need for immunosuppressive treatment after kidney transplantation [5, 6].

Until recently, the accurate diagnosis of LTBI in HD-patients remained elusive. The tuberculin skin test (TST), used so far, has two major disadvantages: a high percentage of the HD-patients show no reliable skin reaction and remain anergic [7–11], and the test lacks specificity due to cross-reactivity with the Bacille-Calmette-Guerin (BCG)-immunization [12, 13].

Newly developed interferon-γ (INF-γ) release assays (IGRAs) measure the INF-γ secretion of T-cells upon stimulation with Mycobacterium tuberculosis specific antigens [14–17]. Their main advantages – especially in a population of immunocompromised patients in a TB low-endemic region – are the lack of cross-reactivity towards BCG-immunization and the availability of a positive control to exclude anergy. In the positive control (in this paper referred to as "phythohaemagglutinin (PHA)-control reaction"), the patient's lymphocytes are stimulated by the mitogen PHA. Failing mitogenic stimulation indicates anergy and results in an inconclusive IGRA-result.

IGRAs allow a more accurate diagnosis of LTBI in immune-competent patients [14, 15, 17] and their prognostic value is presumably equal or superior to the TST [18, 19]. However, IGRAs are dependent on INF-γ secretion and HD-patients show a reduced ability to secrete INF-γ due to impaired T-cell activation upon mitogenic stimulation [20]. Furthermore, several factors associated with end stage renal disease (ESRD) and / or HD
treatment influence the cellular immune response in general, although not INF-γ secretion in particular [21]. This may negatively influence the IGRA test-reliability resulting in an increase of inconclusive IGRA-results and might decrease the diagnostic test performance.

The primary goal of this study is to determine the test-reliability of the QuantiFERON Gold InTube (QFT-GIT) (Cellestis Ltd., Australia) in HD-patients compared with healthy controls. Secondly, we assessed the association of the QFT-GIT PHA-control reaction with dialysis quality, nutritional status, and further factors in ESRD patients influencing their cellular immunity. Thirdly, we addressed the diagnostic performance by assessing the correlation between TB-exposure and test-positivity in a head to head comparison of the QFT-GIT to the TST test results in HD-patients.

Subjects and methods

Study population

39/42 of all eligible patients at the HD centre at the Kantonsspital, St.Gallen, agreed to take part in the study. All samples from HD-patients were collected within a period of one week after initial screening for study eligibility. Exclusion criteria were immunosuppressive treatment and/or immunocompromising diseases other than ESRD or diabetes mellitus (DM), age <18 years, and acute infection. All patients underwent HD treatment three times a week for chronic renal failure for >3 months. A detailed review was conducted of all available data on TB-risk factors and BCG-immunization.

52 healthy volunteers without evidence of renal insufficiency or immunocompromising condition by medical history were recruited among hospital personnel and served as control group. Samples were collected consecutively in all consenting health care workers (HCW) over a two month period at the medical services for HCWs at our hospital.

The study was approved by the local Ethical Review-Board. All patients gave written consent prior to study enrolment. QFT-GIT positive HD-patients received secondary prophylactic treatment if they were candidates for kidney transplantation. If QFT GIT positive patients were not transplant candidates, treatment was offered on an individual basis according to the risk factors for TB reactivation.

Dialysis efficacy: spKt/v, serum albumin and nPCR

We used standard criteria to assess the efficacy of the HD by single-pool (sp)Kt/v [22, 23] and the nutritional status of the patients by normalised protein catabolic rate (nPCR) [24] and serum albumin concentrations at the time (±10 days) of QFT-GIT / TST testing in HD-patients. spKt/v is a formula developed for the approximate measurement of HD adequacy describing the clearance (K) of total body water (v) during the time of HD treatment (t). The nPCR is a function of protein catabolism and is determined by measuring the inter-dialytic appearance of urea in body fluids, reflecting HD adequacy. Additionally parathormone (PTH) and haemoglobin (Hb) levels were recorded.

Tuberculin Skin Test (TST)

The TST consisted of 2 units of PPD (Tuberculin PPD 23 SSI, Statens Serum Institute, Denmark) equivalent to the recommended dose of 5 tuberculin units Seibert [25–27] applied using the Mantoux-technique by a single investigator for all HD-patients. The diameter of the induration was assessed after 48 hours. A positive TST test result was defined by the current recommended cutoff value ≥10 mm [25, 26]. In addition, the TST-positivity rates were also reported, defined by a lower cut-off value of ≥5 mm which is used in different immunocompromised patients [2]. TST and QFT-IT were performed on the same day; first blood was drawn for QFT-IT, then TST was performed.

Interferon-γ release assay (IGRA)

QFT-GIT assays were conducted according to the manufacturer’s instructions with a cut-off for a positive test result of 0.35 IU/ml. In brief, a total of 3 ml of whole blood was collected (for HD-patients at the beginning of a HD session and before the TST was performed) using the three antigen-precoated tubes supplied by the manufacturer. One tube contained no antigen (negative control), one tube contained the mitogen PHA (positive control) and the last tube peptides of the M. tuberculosis antigens Early Secretory Antigenic Target 6 (ESAT-6), Culture Filtrate Protein 10 (CFP-10) and TB7.7(p4). The tubes were incubated for 24 hours at 37 °C, centrifuged at 3000 rpm for 15’ and stored at 4 °C until further processing. The INF-γ release was measured batch-wise by enzymelinked immunosorbent assay (ELISA)-technique according to the manufacturer’s instructions. Additionally, the leukocyte count was recorded.

Statistical analysis

All variables were tested for normal distribution. Mann-Whitney U-test was used to determine differences between groups, and Spearman R correlation was applied. Frequency of categorical variables was compared using a two-tailed Fisher’s exact p. All statistical calculations were performed on STATISTICA version 7 (StatSoft Inc., Tulsa, USA).
Detection of latent tuberculosis infection in HD-patients

The median INF-γ secretion in the PHA-control reaction was similar in HD patients with a positive or negative QFT-GIT against the TB antigens (fig. 2B, \( p = 0.74 \)).

**QFT-GIT test performance is not dependent on HD efficacy**

Next, we evaluated in HD-patients the influence of age, leukocyte count, time on HD-treatment, HD quantity measured by Kt/V, nutritional status measured by serum albumin levels and nPCR, PTH- and Hb-levels on the INF-γ secretion in the PHA-control reaction. In a univariate analysis, no correlation was found between INF-γ expression in the PHA-control reaction and any of the evaluated parameters (table 2).

**Table 1**

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>HD-patients (n = 39)</th>
<th>Healthy controls (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male [%] (absolute)</td>
<td>48.7 (19)</td>
<td>26.9 (14)</td>
</tr>
<tr>
<td>Median age [years] (range)</td>
<td>64 (30-87)</td>
<td>37 (19-64)</td>
</tr>
<tr>
<td>BCG-immunization [%] (absolute)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– positive</td>
<td>46.1 (18)</td>
<td>not</td>
</tr>
<tr>
<td>– negative</td>
<td>23.1 (9)</td>
<td>assessed</td>
</tr>
<tr>
<td>– unknown</td>
<td>30.8 (12)</td>
<td></td>
</tr>
</tbody>
</table>

TB-exposure as proxy for LTBI and consecutive TB-reactivation [%] (absolute)

| – origin medium TB-prevalence region \(^2\) | 18.0 (7)            | 0                         |
| – history of TB-disease / LTBI               | 10.3 (4)            | 0                         |
| – chest X-ray consistent with old TB         | 10.3 (4)            | 0                         |

\(^1\) 37/39 patients were included in the evaluation of the QFT-GIT test-performance using the INF-γ titre of the control (mitogen) reaction. In two HD-patients, the quantitative PHA-control was not assessable. The TST was obtained in 33/39 HD-patients: 5 patients refused TST testing and one patient failed to return after 48 hrs for the assessment of the reaction.

\(^2\) Medium TB-prevalence countries with an estimated TB-prevalence of 25–100 cases/100 000 people (Albania and Serbo-Croatia) (45).

**HD patients show reliable QFT-GIT test results despite a significantly reduced cellular immune response**

In 37/39 HD-patients and 52/52 healthy controls the PHA-control reaction could be used to assess the QFT-GIT performance (fig. 1 and table 1).

HD-patients showed a significantly reduced immune response measured by INF-γ secretion upon mitogenic stimulation in the QFT-GIT PHA-control reaction (fig. 2A, \( p < 0.05 \)). Nevertheless the diminished INF-γ secretion had a limited impact on the test reliability: Only one HD-patient showed an inconclusive GIT-QFT result.

The median INF-γ secretion in the PHA-control reaction was similar in HD patients with a positive or negative QFT-GIT against the TB antigens (fig. 2B, \( p = 0.74 \)).

**QFT-GIT test performance is not dependent on HD efficacy**

Next, we evaluated in HD-patients the influence of age, leukocyte count, time on HD-treatment, HD quantity measured by Kt/V, nutritional status measured by serum albumin levels and nPCR, PTH- and Hb-levels on the INF-γ secretion in the PHA-control reaction. In a univariate analysis, no correlation was found between INF-γ expression in the PHA-control reaction and any of the evaluated parameters (table 2).

**QFT-GIT is more accurate than TST in HD patients in diagnosing LTBI**

A head to head comparison of the QFT-GIT with the TST test results was performed in HD-patients to determine the diagnostic performance. Due to the lack of a “gold standard”, TB-exposure of HD-patients (previous TB-disease, chest X-ray suggestive for LTBI, or origin from a medium TB-prevalence region; table 1) was taken as a proxy for LTBI (25).

Positive QFT-GIT results were found in 10/39 HD-patients (25.5%). One patient showed an inconclusive result (2.5%) (fig. 1). A TST was conducted in 33/39 HD patients (85%). The recommended cut-off for a positive TST is ≥10 mm in HD-patients [25, 26]. Three patients showed a positive TST ≥10 mm. An additional four patients showed a TST between ≥5 and <10 mm (fig. 1).

**Figure 1**

Rate % (absolute number) of positive, negative and indeterminate (QFT-GIT only) results for the TST and QFT-GIT, and QFT-GIT median (range) INF-γ titre upon mitogen (PHA, positive control reaction) and TB-specific stimulation (IU/ml) in HD-patients and healthy controls. INF-γ = interferon γ; PHA = phytohagglutinin; HD = haemodialysis; QFT-GIT = QuantiFERON Gold InTube; TST = tuberculin skin tests; TB = tuberculosis; LTBI = latent tuberculosis infection
32 patients had a valid QFT-GIT and TST result and could therefore be used for a head-to-head comparison of the test performances. Of the 9 patients with a positive QFT-GIT, two also had a positive TST ≥10 mm (table 3A). Another three patients with a positive QFT-GIT could have been recognised by a TST cut-off of ≥5 mm (table 3B). Nevertheless 8 and 5 patients, respectively, were detected by QFT-GIT only.

All positive QFT-GIT results were found in patients with risk factors associated with probable LTBI. A positive IGRA result was closely associated with previous TB-exposure and subsequent probable LTBI, as was the TST using a lower cut-off of ≥5 mm (for both p< 0.05). In contrast, a TST ≥10 mm was not associated with previous TB exposure (p = 0.21) (fig. 3). The INF-γ secretion upon TB-specific stimulation was not different in QFT-GIT positive HD-patients (n = 10) compared with QFT-GIT positive healthy controls (n = 11) (median INF-γ secretion 2.5 (range 0.8–9.9) vs 1.1 (range 0.5–7.6) IU/ml) (fig. 1).

QFT-GIT positivity was independent of the immunization status. Two patients with a TST ≥5 mm with known BCG-immunization and no risk factors for LTBI remained QFT-GIT negative. Most patients (85.7%) with a history of prior BCG immunization were TST negative.

Table 2
Correlation of possible factors influencing QFT-GIT test performance (INF-γ concentration of the PHA-control reaction) in HD-patients (n = 37, Spearman R correlation).

<table>
<thead>
<tr>
<th>Median (range)</th>
<th>Correlation with QFT-GIT PHA-control reaction</th>
<th>Spearman’s rank correlation coefficient r</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>64 (30–87)</td>
<td>-0.14</td>
<td>0.41</td>
</tr>
<tr>
<td>Time on HD [months]</td>
<td>31 (4–187)</td>
<td>0.01</td>
<td>0.94</td>
</tr>
<tr>
<td>spKt/v</td>
<td>1.59 (1.13–2.26)</td>
<td>0.27</td>
<td>0.10</td>
</tr>
<tr>
<td>Serum albumin [g/dl]</td>
<td>36.1 (19.2–43.9)</td>
<td>0.07</td>
<td>0.70</td>
</tr>
<tr>
<td>nPCR [g/kg/d]</td>
<td>0.86 (0.56–1.65)</td>
<td>-0.002</td>
<td>0.99</td>
</tr>
<tr>
<td>Leucocyte count [×10⁹/l]</td>
<td>6.6 (3.9–12.0)</td>
<td>-0.30</td>
<td>0.07</td>
</tr>
<tr>
<td>PTH [ng/l]</td>
<td>244.9 (13.7–852.9)</td>
<td>0.15</td>
<td>0.37</td>
</tr>
<tr>
<td>Hb [g/l]</td>
<td>120.0 (98.0–153.0)</td>
<td>0.05</td>
<td>0.76</td>
</tr>
</tbody>
</table>

1 n = 36
HD = haemodialysis; nPCR = normalized protein catabolic rate; QFT-GIT = QuantiFERON Gold InTube; INF-γ = interferon-γ; PHA = phytohaemagglutinin; PTH = parathormone; Hb = haemoglobin

Table 3A
Association between TST (cut-off ≥10 mm) and QFT-GIT results in HD-patients, excluding patients without TST or inconclusive QFT-GIT result (n = 32).

<table>
<thead>
<tr>
<th></th>
<th>TST positive (≥10 mm)</th>
<th>TST negative (&lt;10 mm)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT-GIT positive</td>
<td>2 (6%)</td>
<td>7 (22%)</td>
<td>9 (28%)</td>
</tr>
<tr>
<td>QFT-GIT negative</td>
<td>1 (3%)</td>
<td>22 (69%)</td>
<td>22 (72%)</td>
</tr>
<tr>
<td>Total</td>
<td>3 (9%)</td>
<td>29 (91%)</td>
<td>32 (100%)</td>
</tr>
<tr>
<td>Agreement</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3B
Same as 3A with lower TST cut-off (≥5 mm).

<table>
<thead>
<tr>
<th></th>
<th>TST positive (≥5 mm)</th>
<th>TST negative (&lt;5 mm)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT-GIT positive</td>
<td>5 (16%)</td>
<td>4 (12%)</td>
<td>9 (28%)</td>
</tr>
<tr>
<td>QFT-GIT negative</td>
<td>2 (6%)</td>
<td>21 (63%)</td>
<td>23 (72%)</td>
</tr>
<tr>
<td>Total</td>
<td>7 (22%)</td>
<td>25 (78%)</td>
<td>32 (100%)</td>
</tr>
<tr>
<td>Agreement</td>
<td>81%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 due to known BCG-immunization
TST = tuberculin skin test; QFT-GIT = QuantiFERON Gold InTube; HD = haemodialysis
Discussion

Overall, QFT-GIT test reliability was confirmed in the chronic HD-patients evaluated in this cross-sectional study. Despite a significantly compromised capability of HD-patients’ T-cells to produce INF-γ upon mitogenic stimulation compared to immunocompetent controls, only one HD-patient (2.5%) showed an inconclusive QFT-GIT result in this study. A higher proportion of inconclusive IGRA-results was reported in other immunocompromised patients [28–33]. Other studies in ESRD patients found indeterminate results varying from 2.1 to 11% using different IGRA platforms [34–37]. In this study, we used the improved 3rd generation test format (QFT-GIT) with a superior sensitivity compared with the QFT-Gold (QFT-G) [38], partly explaining the low rate of inconclusive results. Possible explanations for the high inter-study variability might be the different study settings – especially HD-quality- and the pre-analytical sample handling which may play a crucial role.

ESRD itself compromises the cellular immune function and therefore possibly the IGRA test performance [39]. Reduced responsiveness of IGRA due to poor nutritional status has been described [40]. Therefore the nutritional status and dialysis quantity may influence the IGRA-performance. In this HD-patient group, the test-performance was independent of the nutritional status (serum albumin, nPCR) and of the amount of dialysis (spKt/v). However, this could be a consequence of the sample size. Further studies should investigate the test performances in patients with lower Kt/v or nPCR. A recent study found a significant increase of indeterminate results with the time since HD initiation [41], This study aimed to detect active TB in HD-patients but was performed with a 2nd generation IGRA independently prone to indeterminate test results [38] and did not account for dialysis efficacy. To our knowledge, none of the published studies to date have examined this parameter [34–38, 41].

Although several additional factors might influence the cellular immune responses in ESRD-patients (e.g., secondary hyperparathyreoidism, Hb-level, 1,25-(OH)2-D3 deficiency), the exact influence of these factors on the T-cell response remains controversial and none have been shown to influence INF-γ secretion directly, to date (reviewed in [21]). In this study, the QFT-GIT test performance was not influenced by these parameters suggesting that the use of IGRA in HD-patients is not affected by ESRD and its associated co-morbidities. However, the overall homogeneous PTH- and Hb-levels and the routine vitamin D substitution, when needed, could represent limitations of this study group.

In the absence of a “gold standard” for the diagnosis of LTBI, it is impossible to determine the sensitivity or specificity directly within a given patient’s collective. Nevertheless a positive result in the QFT-GIT appeared to be more closely correlated with previous TB-exposure (defined by suggestive chest X-ray findings, history of previous TB-disease, or immigration from a medium TB-prevalence region [25]) as a proxy for the test sensitivity. The association found in this small study has to be confirmed in larger studies. However, Triverio et al. found the same association in a similar cohort of Swiss HD patients [37]. The current findings are furthermore supported by a cohort study in TB-exposed HD-patients; Winthrop et al. showed a significant association of the QFT-G-positivity with TB-case contact whereas the TST-positivity lacked this association [35]. Lee et al. were recently able to show that QFT-G-positivity is associated with an increased rate of developing active TB in ESRD patients, although their observation did not reach statistical significance [36].

When the cut-off for a positive TST-result was set at 5 mm nearly as many patients were positive in the TST as in the QFT-GIT, but the lower TST cut-off is likely to result in a considerable loss of TST-specificity as it has been shown in a recent study [18], especially in populations with high BCG-immunization rate. Therefore we would not recommend the use of a lower TST cut-off. A striking finding in the current study was the high number of negative TST results that exceeded the rate reported previously in HD-patients [7–11]. This observation emphasises the probable superiority of IGRA because these tests eliminate both technical difficulties in performing a TST and subjectivity involved in TST-interpretation.

One explanation for the higher sensitivity of IGRA in HD-patients is that in vitro assays are able to detect the very first steps in the immune activation cascade. They may therefore be less influenced by the uraemic immune suppression than the TST. In contrast, the TST-read out depends on the integrity of the whole immune activation cascade resulting in a cutaneous induration in vivo. Indeed, Sester et al. showed previously that HD-patients were still able to produce INF-γ ex vivo.
upon PPD-stimulation, although they were TST-negative [42]. This suggests an immune dysfunction at a later stage of the activation cascade resulting in cutaneous anergy. This hypothesis is emphasised by our finding that the TB-specific INF-γ secretion is not compromised in HD patients compared with healthy controls who tested QFT-GIT positive in contrast to the significantly diminished PHA-specific (mitogenic) secretion that is stimulated through a different pathway.

An accurate diagnosis of LTBI is essential in HD-patients, especially in those awaiting transplantation, as immunosuppression dramatically increases the risk to develop TB-disease [26, 43, 44]. In this setting IGRA results are dependent on INF-γ production, diminished PHA-specific (mitogenic) secretion is not compromised in HD patients compared with healthy controls who tested negative [42]. This suggests an immune dysfunction and specificity in HD-patients are higher compared to TST. Nevertheless, the IGRA results have to be interpreted carefully and their assessment has to take into consideration individual TB-risk factors and the overall TB prevalence of the population. Further studies are needed to address the prognostic value of the IGRA’s in immunocompromised dialysis and pre-dialysis patients.

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References


