Peer reviewed article

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

Matthias Hoffmann[a], *Dimitrios Tsinalis*[b], *Pietro Vernazza*[a], *Walter Fierz*[c], *Isabelle Binet*[b]

[a] Division of Infectious Diseases, Cantonal Hospital St. Gallen, Switzerland
[b] Division of Nephrology, Dept. of Internal Medicine, Cantonal Hospital St. Gallen, Switzerland
[c] LogoLab, Kilchberg, Switzerland

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 "phythohaemagglutinine (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

Cellestis Europe Ltd., Germany, provided the QFT-GIT test kits free of charge. Cellestis had no influence on the study design, the interpretation of the results or the writing of this report.
Dr. Hoffmann received a travel grant from Cellestis to present the results of this study at the 2nd global symposium on IGRA 05/2009.

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 immuncompromising 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 immuncompromising 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 cut-off 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 enzyme-linked 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).

Results

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.).

Table 1

Patient characteristics.

	HD-patients (n = 39[1])	Healthy controls (n = 52)
Male [%] (absolute)	48.7 (19)	26.9 (14)
Median age [years] (range)	64 (30–87)	37 (19–64)
BCG-immunization [%] (absolute)		
– positive	46.1 (18)	not
– negative	23.1 (9)	assessed
– unknown	30.8 (12)	
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 = haemodialysis; nPCR = normalized protein catabolic rate; BCG = Bacille-Calmette-guerin; TB = tuberculosis; LTBI = latent tuberculosis infection

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).

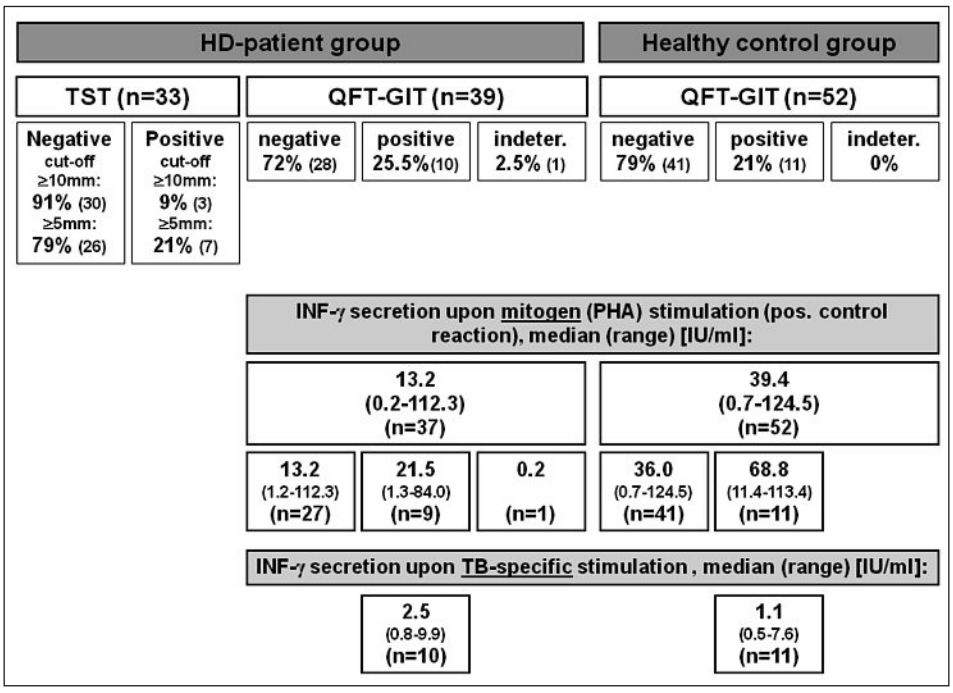

Figure 1

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 = phythohaemagglutinine; HD = haemodialysis; QFT-GIT = QuantiFERON Gold InTube; TST = tuberculin skin tets; TB = tuberculosis; indet. = indeterminate.

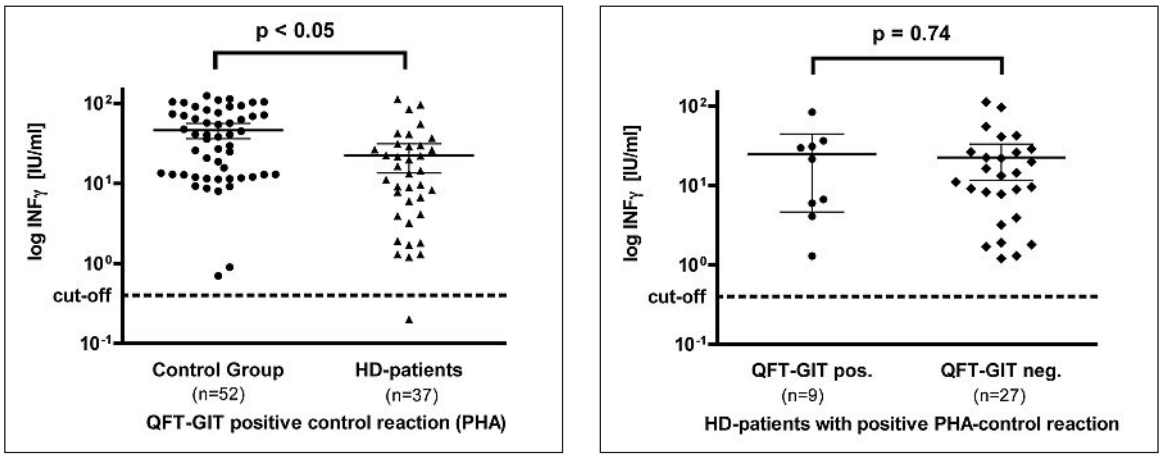

Figure 2 A & B

Comparison of INF-γ secretion upon mitogenic stimulation (A) in healthy controls (n = 52) vs HD-patients (n = 37), and (B) comparison within the HD-group in non-anergic patients who tested QFT-GIT negative (n = 26) or QFT-GIT positive (n = 10). The median INF-γ titre in the control group versus the HD-patient group was 46.2 IU/ml versus 22.4 IU/ml ($p < 0.05$). The median INF-γ secretion of the control reaction in HD-patients tested QFT-GIT positive or negative was 24.6 and 22.5 IU/ml, respectively ($p = 0.57$).
INF-γ = interferon γ; PHA = phythohaemagglutinine; HD = haemodialysis; QFT-GIT = QuantiFERON Gold InTube

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).

	Median (range)	Correlation with QFT-GIT PHA-control reaction	
		Spearman's rank correlation coefficient p	p-Value
Age [years]	64 (30–87)	–0.14	0.41
Time on HD [months]	31 (4–187)	0.01	0.94
spKt/v	1.59 (1.13–2.26)	0.27	0.10
Serum albumin [g/dl]	36.1 (19.2–43.9)	0.07	0.70
nPCR [g/kg/d]	0.86 (0.56–1.65)	–0.002	0.99
Leucocyte count [$\times 10^9$/l]	6.6 (3.9–12.0)	–0.30	0.07
PTH [ng/l][1]	244.9 (13.7–852.9)	0.15	0.37
Hb [g/l]	120.0 (98.0–153.0)	0.05	0.76

[1] n = 36
HD = haemodialysis; nPCR = normalized protein catabolic rate; QFT-GIT = QuantiFERON Gold InTube; INF-γ = interferon γ; PHA = phytohaemagglutinine, PTH = parathormone; Hb = haemoglobine

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).

	TST positive (≥10 mm)	TST negative (<10 mm)	Total
QFT-GIT positive	2 (6%)	7 (22%)	9 (28%)
QFT-GIT negative	1 (3%)[1]	22 (69%)	22 (72%)
Total	3 (9%)	29 (91%)	32 (100%)
Agreement	75%		

Table 3B

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

	TST positive (≥5 mm)	TST negative (<5 mm)	Total
QFT-GIT positive	5 (16%)	4 (12%)	9 (28%)
QFT-GIT negative	2 (6%)[1]	21 (63%)	23 (72%)
Total	7 (22%)	25 (78%)	32 (100%)
Agreement	81%		

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

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.

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.

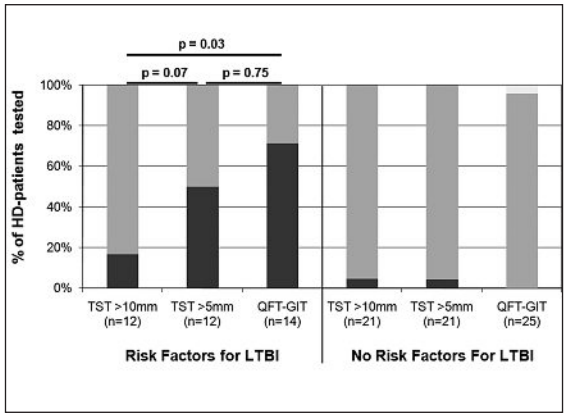

Figure 3

Association between TST (cut off 5 mm or 10 mm, respectively), QFT-GIT positivity and risk factors for LTBI. Dark grey: % positive test results, light grey: % negative (QFT-GIT) or negative and anergic (TST) test results; white: % indeterminate QFT-GIT results. INF-γ = interferon γ; PHA = phythohaemagglutinine; HD = haemodialysis; QFT-GIT = QuantiFERON Gold InTube; TST = tuberculin skin test; TB = tuberculosis; LTBI = latent tuberculosis infection.

ESRD itself compromises the cellular immune function and therefore possibly the IGRA test performance [39]. Reduced responsiveness of IGRAs 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 IGRAs 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 IGRAs because these tests eliminate both technical difficulties in performing a TST and subjectivity involved in TST-interpretation.

One explanation for the higher sensitivity of IGRAs 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 IGRAs are a more reliable and powerful diagnostic tool than TST. Although IGRA results are dependent on INF-γ production, the impaired INF-γ secretion in HD-patients does not affect the overall IGRA reliability. Furthermore, there are strong indications that IGRA sensitivity 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 IGRAs in immunocompromised dialysis and pre-dialysis patients.

We would like to thank Mrs. Brigitta Rusch for obtaining samples from healthy controls, PD Dr. Otto Schoch and Dr. Detlev Schultze for their helpful comments of the study protocol.

Correspondence:
Dr. med. Matthias Hoffmann
Fachbereich Infektiologie
Departement Innere Medizin
Kantonsspital St. Gallen
CH-9007 St. Gallen
E-Mail: matthias.hoffmann@kssg.ch

References

1 Laifer G, Widmer AF, Simcock M, Bassetti S, Trampuz A, Frei R, et al. TB in a low-incidence country: differences between new immigrants, foreign-born residents and native residents. Am J Med. 2007;120(4):350–6.
2 Horsburgh CR Jr. Priorities for the treatment of latent tuberculosis infection in the United States. N Engl J Med. 2004;350 (20):2060–7.
3 Moore DA, Lightstone L, Javid B, Friedland JS. High rates of tuberculosis in end-stage renal failure: the impact of international migration. Emerg Infect Dis. 2002;8(1):77–8.
4 Chia S, Karim M, Elwood RK, FitzGerald JM. Risk of tuberculosis in dialysis patients: a population-based study. Int J Tuberc Lung Dis. 1998;2(12):989–91.
5 Queipo JA, Broseta E, Santos M, Sánchez-Plumed J, Budía A, Jiménez-Cruz F. Mycobacterial infection in a series of 1261 renal transplant recipients. Clin Microbiol Infect. 2003;9(6):518–25.
6 Vandermarliere A, Van Audenhove A, Peetermans WE, Vanrenterghem Y, Maes B. Mycobacterial infection after renal transplantation in a Western population. Transpl Infect Dis. 2003;5 (1):9–15.
7 Shankar MS, Aravindan AN, Sohal PM, Kohli HS, Sud K, Gupta KL, et al. The prevalence of tuberculin sensitivity and anergy in chronic renal failure in an endemic area: tuberculin test and the risk of post-transplant tuberculosis. Nephrol Dial Transplant. 2005;20(12):2720–4.
8 Poduval RD, Hammes MD. Tuberculosis screening in dialysis patients – is the tuberculin test effective? Clin Nephrol. 2003;59 (6):436–40.
9 Fang HC, Chou KJ, Chen CL, Lee PT, Chiou YH, Hung SY, et al. Tuberculin skin test and anergy in dialysis patients of a tuberculosis-endemic area. Nephron. 2002;91(4):682–7.
10 Smirnoff M, Patt C, Seckler B, Adler JJ. Tuberculin and anergy skin testing of patients receiving long-term hemodialysis. Chest. 1998;113(1):25–7.
11 Woeltje KF, Mathew A, Rothstein M, Seiler S, Fraser VJ. Tuberculosis infection and anergy in hemodialysis patients. Am J Kidney Dis. 1998;31(5):848–52.
12 Horowitz HW, Luciano BB, Kadel JR, Wormser GP. Tuberculin skin test conversion in hospital employees vaccinated with bacille Calmette-Guerin: recent Mycobacterium tuberculosis infection or booster effect? Am J Infect Control. 1995; 23(3):181–7.
13 Fine PE, Sterne JA, Ponnighaus JM, Rees RJ. Delayed-type hypersensitivity, mycobacterial vaccines and protective immunity. Lancet. 1994;344(8932):1245–9.
14 Lalvani A. Diagnosing tuberculosis infection in the 21st century: new tools to tackle an old enemy. Chest. 2007;131(6):1898–906.
15 Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med. 2007;146 (5):340–54.
16 Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet. 2000;356(9235): 1099–104.
17 Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis. 2004;4(12):761–76.
18 Diel R, Loddenkemper R, Meywald-Walter K, Niemann S, Nienhaus A. Predictive value of a whole blood IFN-gamma assay for the development of active tuberculosis disease after recent infection with Mycobacterium tuberculosis. Am J Respir Crit Care Med. 2008;177(10):1164–70.
19 Doherty TM, Demissie A, Olobo J, Wolday D, Britton S, Eguale T, et al. Immune responses to the Mycobacterium tuberculosis-specific antigen ESAT-6 signal subclinical infection among contacts of tuberculosis patients. J Clin Microbiol. 2002; 40(2):704–6.
20 Sester U, Sester M, Hauk M, Kaul H, Köhler H, Girndt M. T-cell activation follows Th1 rather than Th2 pattern in haemodialysis patients. Nephrol Dial Transplant. 2000;15(8):1217–23.
21 Eleftheriadis T, Antoniadi G, Liakopoulos V, Kartsios C, Stefanidis I. Disturbances of acquired immunity in hemodialysis patients. Semin Dial. 2007;20(5):440–51.
22 Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. J Am Soc Nephrol. 1993;4(5):1205–13.
23 European Best Practice Guidelines Expert Group on Hemodialysis, European Renal Association. Section II. Haemodialysis adequacy. Nephrol Dial Transplant. 2002;17(Suppl 7):16–31.
24 Jindal KK, Goldstein MB. Urea kinetic modeling in chronic hemodialysis: Benefits, problems, and practical solutions. Semin Dial. 1988;1:82–5.
25 Anonymous. Targeted tuberculin testing and treatment of latent tuberculosis infection. American Thoracic Society. MMWR Recomm Rep. 2000;49(RR-6):1–51.
26 European Best Practice Guidelines Expert Group on Renal Transplantation. European best practice guidelines for renal transplantation. Section IV: Long-term management of the transplant recipient. IV.7.2. Late infections. Tuberculosis. Nephrol Dial Transplant. 2002;17(Suppl 4):39–43.
27 Wang L, Turner MO, Elwood RK, Schulzer M, FitzGerald JM. A meta-analysis of the effect of Bacille Camette Guérin vaccination on the tuberculin skin test measurements. Thorax. 2002;57:804–9.

28 Hoffmann M, Reichmuth M, Fantelli K, Schoch OD, Fierz W, Furrer H, et al. Conventional tuberculin skin testing versus T-cell-based assays in the diagnosis of latent tuberculosis infection in HIV-positive patients. AIDS. 2007;21(3):390–2.
29 Luetkemeyer AF, Charlebois ED, Flores LL, Bangsberg DR, Deeks SG, Martin JN, et al. Comparison of an interferon-gamma release assay with tuberculin skin testing in HIV-infected individuals. Am J Respir Crit Care Med. 2007;175(7): 737–42.
30 Brock I, Ruhwald M, Lundgren B, Westh H, Mathiesen LR, Ravn P. Latent tuberculosis in HIV positive, diagnosed by the M. tuberculosis specific interferon-gamma test. Respir Res. 2006;7:56.
31 Matulis G, Juni P, Villiger PM, Gadola SD. Detection of latent tuberculosis in immunosuppressed patients with autoimmune diseases: performance of a Mycobacterium tuberculosis antigen-specific interferon gamma assay. Ann Rheum Dis. 2008;67(1):84–90.
32 Kobashi Y, Mouri K, Obase Y, Fukuda M, Miyashita N, Oka M. Clinical evaluation of QuantiFERON TB-2G test for immunocompromised patients. Eur Respir J. 2007;30(5):945–50.
33 Piana F, Codecasa LR, Cavallerio P, Ferrarese M, Migliori GB, Barbarano L, et al. Use of a T-cell-based test for detection of tuberculosis infection among immunocompromised patients. Eur Respir J. 2006;28(1):31–4.
34 Passalent L, Khan K, Richardson R, Wang J, Dedier H, Gardam M. Detecting latent tuberculosis infection in hemodialysis patients: a head-to-head comparison of the T-SPOT.TB test, tuberculin skin test, and an expert physician panel. Clin J Am Soc Nephrol. 2007;2(1):68–73.
35 Winthrop KL, Nyendak M, Calvet H, Oh P, Lo M, Swarbrick G, et al. Interferon-gamma Release Assays for Diagnosing Mycobacterium tuberculosis Infection in Renal Dialysis Patients. Clin J Am Soc Nephrol. 2008;3(5):1357–63.
36 Lee SS, Chou KJ, Su IJ, Chen YS, Fang HC, Huang TS, et al. High Prevalence of Latent Tuberculosis Infection in Patients in End-Stage Renal Disease on Hemodialysis: Comparison of QuantiFERON-TB GOLD, ELISPOT, and Tuberculin Skin Test. Infection. 2009;37(2):96–102.
37 Triverio PA, Bridevaux PO, Roux-Lombard P, Niksic L, Rochat T, Martin PY, et al. Interferon-gamma release assays versus tuberculin skin testing for detection of latent tuberculosis in chronic haemodialysis patients. Nephrol Dial Transplant. 2009;24(6):1952–6.
38 Harada N, Higuchi K, Yoshiyama T, Kawabe Y, Fujita A, Sasaki Y, et al. Comparison of the sensitivity and specificity of two whole blood interferon-gamma assays for M. tuberculosis infection. J Infect. 2008;56(5):348–53.
39 Girndt M, Sester U, Sester M, Kaul H, Kohler H. Impaired cellular immune function in patients with end-stage renal failure. Nephrol Dial Transplant. 1999;14(12):2807–10.
40 Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet. 2004;364(9452):2196–203.
41 Inoue T, Nakamura T, Katsuma A, Masumoto S, Minami E, Katagiri D, et al. The value of QuantiFERON TB-Gold in the diagnosis of tuberculosis among dialysis patients. Nephrol Dial Transplant. 2009;24(7):2252–7.
42 Sester M, Sester U, Clauer P, Heine G, Mack U, Moll T, et al. Tuberculin skin testing underestimates a high prevalence of latent tuberculosis infection in hemodialysis patients. Kidney Int. 2004;65(5):1826–34.
43 Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med. 2007;357(25):2601–14.
44 Singh N, Paterson DL. Mycobacterium tuberculosis infection in solid-organ transplant recipients: impact and implications for management. Clin Infect Dis. 1998;27(5):1266–77.
45 World Health Organisation. Global tuberculosis control: surveillance, planning, financing. WHO report 2007. Geneva, World Health Organization (WHO/HTM/TB/2007.376). Available from: http://www.who.int/tb/publications/global_report/2007/en/index.html