Published in final edited form as:

Transpl Infect Dis. 2018 April ; 20(2): e12845. doi:10.1111/tid.12845.

Tuberculosis After Liver Transplantation in a Large Center in New York City: QuantiFERON®-TB Gold-Based Pre-transplant Screening Performance and Active TB Post-transplant

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Abstract

Introduction—Pre-transplant screening for LTBI is a complex consideration that varies by institution. Inconsistent performance of IGRA further complicates screening. Data regarding LTBI screening outcomes and test characteristics in large, foreign-born pre-transplant populations within the United States is limited.

Methods—In this retrospective study, patients who received QuantiFERON®-TB Gold (QFT) prior to LT were included. Characteristics of patients were compared by QFT result and predictors of indeterminate results were evaluated. Similar comparisons were performed between patients who developed active TB and those who did not.

Results—Of 148 patients screened the rate of positive, indeterminate, and negative testing was 13.5% (20/148), 27% (40/148), and 59% (88/148) respectively. An indeterminate QFT was more likely in patients with a MELD > 25 (OR 16.7, 95% CI: 2.1 – 132.0; p=0.008) and when performed internally compared to by commercial lab (OR 4.1, 95% CI: 1.34 – 12.44, p=0.013). The overall TB incidence was 1102/100,000 transplant cases. No patient who developed active TB

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J.H.: Concept/design, data collection, drafting article; S.H.,M.R.: Concept/design, critical revision of article; K.S.: Statistical analysis, data interpretation, drafting article; C.H.: Laboratory management, quality analysis, drafting article.

had a positive QFT. All were born outside of the US ($p=0.06$) and had pre-transplantation chest imaging demonstrating granulomatous disease ($p=0.006$).

Conclusion—Our experience further highlights the challenges of LTBI screening prior to LT and suggests QFT may be a poor predictor of active TB in higher risk pre-transplant populations. Candidates should be screened as early as possible to optimize QFT performance and local epidemiological data should be used to create institution-specific screening protocols in areas with large populations from TB-endemic regions. Management should consider TB risk factors, QFT, and imaging instead of reliance on QFT testing alone.

Keywords

latent tuberculosis infection; tuberculosis; QuantiFERON®-TB Gold; interferon gamma release assay; liver transplant; solid organ transplant

Introduction

The rising rates of liver transplantation (LT) coupled with enhanced immunosuppressive and procedural techniques have improved graft and patient survival with the unintended consequence of increased infection risk^{1,2}. Tuberculosis (TB) is reportedly 20–74 times more common in solid organ transplant recipients when compared to the general population³ and is associated with significant mortality^{4–8}. Though primary infection may be acquired after transplant and donor transmission has occurred, reactivation of prior disease is thought to be the most common mechanism of active disease³.

Screening for latent TB infection (LTBI) prior to liver transplantation is recommended although many complicating issues exist due to factors relating to both the host and the test characteristics in this complex population³. Evaluation of standard TB risk factors by medical, demographic, and social history combined with indirect TB testing using the tuberculin skin test (TST) or interferon gamma release assays (IGRA) and pulmonary imaging (typically chest x-ray) remains instrumental. However, LTBI screening protocols in solid organ transplant (SOT) candidates vary significantly between published guidelines and individual institutions in regard to modality, timing, frequency, interpretation and management⁶. Additionally TST and IGRAs, including QuantiFERON®-TB Gold (QFT, Cellestis) and T-SPOT®.TB (Oxford Immunotec Ltd, Abingdon, UK) are difficult to interpret in the setting of the immunosuppressive effects of medications and end-stage organ disease^{3,9}. Indeterminate QFT results may be observed more frequently in liver transplant candidates with high MELD scores^{3,9}. Further, negative testing does not completely exclude LTBI in this population³. While well-established historical TB risk factors are pertinent, specific pre-transplant risk factors for active TB in liver transplant recipients (LTR) are not well defined^{3,6}. Suboptimal performance of LTBI testing and a lack of clearly defined risk factors for active TB post-transplant makes pre-transplant risk stratification challenging.

In this retrospective study we evaluated LTBI screening with QFT in liver transplant candidates to define clinical characteristics that predict LTBI screening test results and risk for active TB after transplant.

Methods

Study population.

At the Mount Sinai Hospital in New York City from October 2010 – August 2014 a total of 149 patients who subsequently underwent liver transplantation were screened with QuantiFERON®- TB Gold pre-operatively. We retrospectively identified these patients and collected data on their clinical and demographic characteristics. Patients were excluded if QFT was not performed prior to transplant, was done after transplantation or if they were receiving immunosuppressive medications at the time of LTBI testing.

QuantiFERON®- TB Gold.

Prior to 10/31/13 QFT was sent out to a local reference lab. After 10/31/13 testing was performed in-house in accordance with the manufacturer's recommendations (QFT, Cellestis). One ml of blood taken from the patient was directly placed into three blood collection tubes. One tube contained heparin alone (negative control or nil tube), another contained heparin, dextrose and phytohemagglutinin (positive control or mitogen tube) and the third contained M. tuberculosis-specific antigens ESAT-6, CFP-10 and TB7.7 (the TB antigen tube). After filling the tubes they were shaken 10 times and transferred to an incubator within 16 hours. Tubes incubated at 37°C upright for 16 to 24 hours. Before centrifugation and after incubation tubes may have been held between 4°C and 27°C for up to 3 days. Samples underwent centrifugation and plasma was harvested and tested by enzyme-linked immunosorbent assay (ELISA) to assess the concentration IFN- γ . The difference in IFN- γ concentration from antigen stimulated plasma minus the IFN- γ concentration from plasma incubated without antigen was calculated to assess response. Tests were interpreted in accordance with the manufacturer's specifications.

Statistical analysis.

Baseline characteristics of this cohort were compared by QFT result (positive, negative or indeterminate) using the Wilcoxon test for continuous variables (age, absolute lymphocyte count) and the chi-square test. Similar comparisons were performed in the same cohort between patients who developed active TB to those who did not. Using significant predictors from our comparisons by QFT result, we then fit a multivariable logistic regression to evaluate predictors of an indeterminate result. The factors we included in this model were age, sex, MELD, non-United States born status, HCV, CKD, HCC, inpatient during test, test done at internal lab, absolute lymphocyte count and lung imaging. An incidence rate for TB among transplant recipients was calculated using the following formula: (number of TB cases 2010–2014 in recipients/number of transplants 2010–2014) * 100,000 to estimate a rate per 100,000 transplant cases. All statistical analyses were performed using STATA 13 (Stata Corporation, College Station, TX). The institutional review board of the Icahn School of Medicine permitted this research.

Results

Figure 1 shows the number of patients screened and included in the final evaluation stratified by test result (positive, negative, indeterminate), as well as patients who developed active TB

and those treated for LTBI. Overall, 215 patients were excluded: 167 had no QFT performed, 45 had QFT done after transplant and 3 had QFT done while taking immunosuppressive medications. Of the excluded patients, none developed active TB in the follow-up period. Of patients screened for LTBI the proportion of positive, indeterminate, and negative test results was 13.5% (20/148), 27% (40/148), and 59% (88/148) respectively. No patient with a positive QFT result developed active TB (30 month median follow-up) regardless of LTBI therapy. Patients with an indeterminate QFT result were more likely to be born outside of the United States (US) (Table 1; $p=0.002$), have a model for end-stage liver disease (MELD) score > 25 ($p < 0.001$), have chronic kidney disease ($p=0.004$), and be hospitalized during time of testing ($p < 0.001$). Indeterminate test results were also more common when testing was performed at our institution during that time period (not sent to a reference lab). Patients with a positive test had higher rates of hepatitis C ($p=0.003$) and hepatocellular carcinoma ($p=0.003$) as well as higher median absolute lymphocyte counts ($p = 0.03$) and chest imaging with evidence of prior granulomatous disease ($p < 0.001$). In the multivariate analysis (table 2) an indeterminate QFT result was more than 16 times more likely in patients with a MELD > 25 (OR 16.7, 95% CI: 2.11 – 132.04, $p=0.008$) and more than 4 times as likely to have been performed in our institution's lab (OR 4.1, 95% CI: 1.34 – 12.44, $p=0.013$). Patients with hepatitis C were less likely to have an indeterminate result (OR 0.33, 95% CI: 0.11–0.97, $p=0.043$).

Overall the active TB incidence rate among liver transplant recipients in our institution was 1102/100,000. No patient who developed post-transplant active TB had a positive QFT screening test prior to transplant (Table 2). Pulmonary disease was the most common manifestation of active TB. Patients who developed TB after transplant were all born outside of the US ($p=0.06$), had higher rates of non-alcoholic steatohepatitis ($p=0.01$), and chest imaging with evidence of prior granulomatous disease ($p=0.006$).

Discussion

Active tuberculosis is a feared complication of organ transplantation. In our retrospective cohort of liver transplant recipients who underwent pre-transplant LTBI testing we found high rates of indeterminate test results and QFT was a poor predictor of active TB. Our experience further highlights the challenges of LTBI assessment in liver transplant recipients.

Though the rate of active TB in the United States (US) is 3.0 cases per 100,000 persons (3.0/100,000) in certain New York City neighborhoods it is as high as 18.2/100,000 inhabitants with 82% of cases from 2015 occurring among foreign-born patients¹⁰. Rates of active TB in SOT recipients are significantly higher with estimates in US LTR of 85/100,000 and SOT recipients in other countries as high as 512/100,000^{7,11}. In our population of pre-transplant candidates screened we found a post-transplant, active TB rate of 1102/100,000. This is likely a result of the diverse patient population served by our institution and the TB rates in the surrounding communities. All four patients who developed active TB after transplant were from TB endemic regions outside of the continental US. However when compared to those patients who did not develop TB there was no significant difference in non-US status. Similar to reports from endemic areas¹², we found LTR with radiologic

features of prior TB were at higher risk of developing active TB after transplant. Though computed tomography (CT) of the chest was not routine in the screening of our candidates, this approach has been suggested to be more useful in LT candidates in an endemic area with CT abnormalities observed in patients with a negative chest x-ray¹³. Of the four patients who developed post-transplant TB three had negative QFT testing results and one had indeterminate results. Two other published series in transplant patients have described a total of three cases of TB in QFT-negative patients^{9,14}. Prevalence of indeterminate QFT testing in liver transplant candidates ranges from 0.8% – 19.6%^{15,16}. Our overall indeterminate rate in patients tested was 27% (40/148). Our high rate is likely a result of both a high MELD cohort and possible on-site lab testing performance. In multivariate analysis, indeterminate test results were nearly 17 times more likely in patients with a MELD score >25 (OR 16.7, 95% CI: 2.11 – 132.04, p=0.008). Manuel et al¹⁷ and Theodoropoulos et al⁹ similarly described that in patients with indeterminate QFT results, MELD scores were higher (MELD 17.8 and 20.3± 9.2 respectively) than those with no indeterminate test (MELD 13.3 and 14.3 ± 6.7 respectively). Conversely a report from Spain found QFT testing may be better at detecting LTBI than tuberculin skin testing (TST) in liver transplant candidates with more severe liver disease and higher MELD scores¹⁵ though QFT appears to be a poor predictor of progression to active TB¹⁸. Though no study has directly evaluated this mechanism, the multi-faceted impairment of cell-mediated immunity in patients with more severe liver disease, represented by the MELD score, likely leads to inadequate mitogen positive control response¹⁹. Patients with Hepatitis C virus (HCV) were less likely to have an indeterminate result and those with a positive test had higher rates of HCV (p=0.003) and hepatocellular carcinoma (p=0.003). Similar studies have also found lower rates of indeterminate results in patients with HCV^{9,17}. This observation may indicate that patients with HCV were evaluated earlier before transplant, at lower MELD scores, resulting from earlier diagnosis from national increases in HCV screening.

The effects of the lymphocyte count on the mitogen response have been well described²⁰. Previous studies have found lower lymphocyte counts to be associated with indeterminate results¹⁷ yet in our group of patients there was no significant association (p=0.7). However, patients with a positive QFT test had higher median absolute lymphocyte counts. Further, patients with prior granulomatous disease seen on chest imaging were also more likely to have a positive QFT result (p<0.001). These associations are consistent with previous literature in immunocompetent and immunocompromised hosts²¹.

When testing was done at our on-site lab there was a higher rate of indeterminate test results. The nuances of IGRA testing are significant and blood volume, tube shaking, prolonged incubation time and incubation delay have all been recognized to effect the result and its reproducibility²². Our lab has evaluated testing during this time and found that after bringing the testing in-house, there were prolonged incubation times up to 3 hours over the limit specified in the package insert. The overall indeterminate rate for non-immunosuppressed patients was low (below 5%) and comparable between the reference lab and the in-house lab. Nevertheless, the workflow was changed in 2015 to decrease incubation time and include more frequent specimen centrifugation. It is possible that

immunocompromised patient samples are more sensitive to changes in testing parameters than “healthy” non-immunosuppressed patients.

Of the twenty patients with positive QFT results only three were treated. None of the twenty patients with a positive result developed active TB within the median 30 month follow up period. Though treatment of these patients is recommended, there is commonly a fear of treatment related hepatotoxicity as well as an inadequate assessment of reactivation risk post-transplant. Occasional problems with false positive testing in the general population²³ as well as high conversion and reversion rates with serial testing in a low TB prevalence areas²⁴ extends the challenges of testing interpretation and subsequent therapy.

Our study has several limitations. As we did not screen all candidates prior to liver transplant we may have introduced selection bias into our analysis of test characteristics. Further the small numbers of TB cases in our patient cohort markedly limited our ability to identify unique characteristics of patients who developed active TB. Additionally, this study relied on retrospective clinical data, therefore there is a chance that some cases of active TB may have been missed, although transplant programs tend to retain their patients and are therefore likely to have good ascertainment of these outcomes.

Conclusion

In summary, our experience further demonstrates that IGRA testing may have poor sensitivity and limited specificity in patients with end-stage liver disease and may be a poor predictor of active TB after transplant. Providers should also be aware of the challenges related to reproducibility and lab variability. Careful protocols should be created for tuberculosis screening prior to liver transplant in centers with large populations of patients from TB-endemic areas to ensure appropriate screening, treatment and follow up. Additionally, candidates with liver disease should be screened for LTBI as early as possible in the evaluation process considering poor test performance in patients with high MELD scores. Given the difficulties of testing, diligent risk factor assessment and CT chest should be considered as part of the screening process in centers with large foreign-born populations. Additional testing for these patients (TST or T-SPOT) may also be warranted in this high risk population. Centers in the US with large foreign-born populations should carefully consider treating high risk patients with abnormal lung imaging regardless of LTBI testing result.

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Figure 1.
Latent tuberculosis infection screening and treatment prior to liver transplantation, results,
and active tuberculosis rate

Table 1.

Baseline characteristics of liver transplant candidates tested with QuantiFERON®-TB Gold prior to transplantation by test result

Characteristic	QFT-GIT Result			P-value
	Positive (n=20)	Indeterminate (n=40)	Negative (n=88)	
Age, median (IQR)	59 (53–64)	56 (41–62)	60 (55–64)	0.3
Male, n (%)	17 (85)	17 (42)	57 (65)	0.003
Non-US Birthplace, n(%)	3 (15)	26 (63)	40 (46)	0.002
Transplant Type, n(%)				0.05
Deceased Donor	19 (95)	40 (98)	74 (84)	
Living Donor	1 (5)	1 (2)	14 (16)	
Reason(s) for Transplant, n(%)				
Hepatitis C	14 (70)	12 (29)	49 (56)	0.003
Hepatitis B	2 (10)	5 (12)	5 (12)	
Alcoholic Cirrhosis	2 (10)	6 (15)	11 (13)	0.9
Hepatocellular Carcinoma	14 (70)	12 (29)	50 (57)	0.003
Primary Sclerosing Cholangitis	0 (0)	3 (7)	1 (1)	0.09
Non-Alcoholic Steatohepatitis	2 (10)	4 (10)	10 (10)	0.9
Other	1 (5)	7 (17)	9 (10)	0.3
MELD Score, n(%)				<0.001
<10	6 (30)	3 (7)	26 (30)	
11–18	9 (45)	9 (22)	37 (42)	
19–25	2 (10)	3 (7)	10 (11)	
>25	3 (15)	26 (63)	15 (17)	
Smoking Status, n(%)				.04
Current Smoker	2 (10)	0 (0)	12 (14)	
Former Smoker	13 (65)	18 (45)	40 (46)	
Never Smoker	5 (25)	22 (55)	36 (41)	
Diabetes, n(%)	12 (60)	62 (71)	33 (80)	0.2
Chronic Kidney Disease	3 (15)	18 (44)	16 (18)	0.004
Absolute Lymphocyte Count, median (IQR)	1.4 (1.0–1.7)	1 (0.7–1.4)	0.8 (0.5–1.3)	0.03
Inpatient at Time of Testing	0 (0)	24 (58)	22 (25)	<0.001
Follow up Time After Testing, months, median (IQR)	30 (22–41)	18 (13–26)	23 (17–30)	0.05
Chest Imaging with Granulomatous Disease	12 (63)	5 (12)	13 (15)	<0.001
QFT Testing Site				<0.001
External (before 10/31/13)	16%	19%	65%	
Internal (after 10/31/13)	1%	60%	37%	

MELD (natural) = model for end-stage liver disease; QFT = QuantiFERON®- TB Gold

Table 2.

Multivariate analysis of predictors for indeterminate QuantiFERON®-TB Gold

Variable	Multivariate analysis		
	OR	95% CI	P-value
Non-US Birthplace	2.4	0.91–6.4	0.08
MELD			
11–18	2.4	0.53–10.79	0.3
19–25	2.3	0.24–20.90	0.5
>25	16.7	2.11–132.04	0.008
HCC	2.0	0.56–7.19	0.3
HCV	0.3	0.11–0.97	0.04
CKD	1.2	0.33–4.13	0.8
Absolute Lymphocyte Count	1.1	0.71–1.91	0.7
On-site Lab	4.1	1.34–12.44	0.01

MELD (natural) = model for end-stage liver disease; QFT = QuantiFERON®- TB Gold; HCC = hepatocellular carcinoma; HCV = hepatitis C virus; CKD = chronic kidney disease

Table 3.

Clinical features of patients who developed TB after liver transplant

Demographics	Origin	Organ	Pre-SOT QFT	Pre-SOT Lung Imaging	MELD at QFT	Lymphocytes at QFT	Active TB Site	Time to Diagnosis Post-SOT (months)
69M	Puerto Rico	DD	Negative	Calcified LN, RUL scar, LUL calcified nodule	9	0.3	Pulm	5.5
63M	Bangladesh	DD	Indeterminate	Granuloma right base	26	1.1	Pulm	5
65M	China	DD	Negative	Nodule RML	51	0.2	Pulm	7.5
68F	Pakistan	LD	Negative	Clear	14	0.7	Lymph	3

TB = tuberculosis; SOT = solid organ transplant; DD = deceased donor; LD = living donor; MELD (natural) = model for end-stage liver disease, QFT = QuantiFERON®- TB Gold; LN = lymph node, RUL = right upper lobe; LUL = left upper lobe; Pulm = pulmonary; Lymph = lymphadenitis.