Egyptian Journal of Medical Microbiology Volume 26 / No.4 / October 2017 55-60

ORIGINAL ARTICLE

Screening for Latent Tuberculosis Infection in Patients with Hematologic Malignancies using Interferon-Gamma Release Assays (IGRAs)

[1]Hadir El-Mahallawy, [1]Safaa S. Hassan*, [1]Safaa El-Gohary, [2]Amany El-Kholy, [3]Magdy Saber
[1]Clinical Pathology Department- National Cancer Institute-Cairo University
[2]Clinical Pathology Department -Kasr El-Ainy- Cairo University
[3]Medical Oncology Department- National Cancer Institute-Cairo University

Key words:
Latent Tuberculosis- Quantiferone (QFT) - IGRAs- Cancer patients

Corresponding Author:
Safaa Shawky Hassan
Assistant Professor -Clinical Pathology Department- National Cancer Institute-Cairo University
safaa_shawky@hotmail.com
Tel: 01224267183

ABSTRACT

Background: *The development of interferon-gamma release assays (IGRAs) is important in the diagnosis- of latent tuberculosis infection (LTBI).* ***Objectives:*** *The aim of this study was to evaluate the role of QuantiFERON®-TB Gold In-Tube test (QFT-GIT) in detection of LTBI in patient with hematological malignancy and to identify individuals who are at increased risk for the development of tuberculosis (TB).* ***Methodology:*** *the study was conducted on 52 newly diagnosed patients with hematologic malignancies at National Cancer Institute, Cairo University. The interferone- gamma secreted by T-lymphocytes following stimulation by antigens unique to Mycobacterium tuberculosis was measured. The QFT results were evaluated in relation to different clinical and hematologic parameters and outcome after induction therapy.* ***Results:*** *Nine out of 52 cases (17.5%) were positive for QFT. The patients included were 30 males (58%) and 22 females (42%), with a mean age of 42.75±15.39 years. Out of the 52 patients, 21 (40%) were Leukemic, while 31 patient (60%) had lymphoma. Among the QFT positive cases, 6/9 were lymphoma (67%) and 3/9 were leukemic cases (33%). Manifestations of infection were observed in 13/52 (25%) of cases and 55.5% of positive QFT cases (5/9) while positive chest radiological findings were reported in 44.4% of positive QFT cases (4/9). Lymph node enlargement were reported in 6/9 (66.7%) of QFT positive cases. After induction chemotherapy, 8 patients had an unfavorable outcome, 4 of whom were QFT positive.* ***Conclusion:*** *Interferon-Gamma Release Assays (IGRAs) can aid in diagnosing latent tuberculosis infection in patients with hematologic malignancies. This may prevent the spread of infection and identify who would benefit from treatment of latent TB infection.*

INTRODUCTION

Worldwide, TB disease is still considered an important health problem and is one of the leading causes of mortality for a single infectious disease, with an estimated 10.4 million new cases in 2015 and 1.4 million deaths attributable to the disease [1]. Although the number of TB deaths fell by 22% between 2000 and 2015, TB remained one of the top 10 causes of death worldwide in 2015. Thus, early diagnosis of new cases and prevention of reactivation of latent infection (LTBI) are crucial strategic elements in the aim to control the TB epidemic [2]. In Egypt, the surveillance data in 2011 revealed that the prevalence rate of TB is 26/100,000 population and its incidence rate is 17/100,000 population [3].

Latent tuberculosis infection (LTBI) is the state in which humans are infected with M. tuberculosis without any clinical symptoms, radiological abnormality, or microbiological evidence.[4]. About one- third of the world population is latently infected with mycobacterium tuberculosis, especially in developing countries [5]. The QuantiFERON-TB Gold In-Tube test (QFT-GIT) assay (Cellestis, Carniege, Victoria, Australia) measures the IFN- concentration in whole blood after stimulation by specific tuberculosis antigens (e.g., early secreted antigenic target-6 (ESAT6), culture filtrate protein-10 (CFP10)), and TB7.7 antigen [6,7]. Mycobacterial TB is a communicable disease with a high probability of spread; in addition to the high possibility of reactivation of latent TB among immune-suppressed patients.

In a previous study at National Cancer Institute, Cairo University, it was concluded that *M. tuberculosis* is an important pathogen in patients with malignant diseases and should be promptly searched for in this population of patients in case of clinical suspicion; with a rate of 0.5/1000 new cancer cases [8].

The delay in TB diagnosis in patients with leukemia and active TB results in transmission of *Mycobacterium tuberculosis* to other

immunosuppressed patients. Thus, we aimed to investigate the possibility of occurrence of latent tuberculosis infection in immunosuppressed patients with hematological malignancies as a pilot study in order to decide accordingly necessary further actions to treat latently infected patients and avoid spread of infection.

METHODOLOGY

Study population:

This was a cross sectional study to investigate latent TB. The cases included in the study were newly diagnosed adult patients with hematological malignancies presenting to the outpatient clinic of National Cancer Institute, Cairo University. Patients were examined for latent TB prior to hospitalization and receiving chemotherapy.

Clinical assessment of the patients:

All patients were subjected to full history taking for the presence of clinical manifestations of infection in the form of fever, cough, and dyspnea. They were examined for lower respiratory tract involvement and lymph node enlargement.

Laboratory work-up:

Peripheral blood and bone marrow aspirate studies were done for the diagnosis of the hematological malignancies. Total Leukocytic count, absolute neutrophilic count and absolute lymphocytic count were done as indicators for immunosuppression and/or infection.

The QuantiFERON-TB Gold IT test The QuantiFERON® - in TB Gold IT system specialized blood collection tubes were used to collect whole venous blood. The included tubes were a Nil (negative) control tube, TB Antigen tube and a Mitogen tube (positive control). The QFT-GIT test was conducted on 1 mL of venous blood in antigen tube that was incubated at 37ºC for 16 to 24 hours. Software (version 2.17.2) USA version, was used to analyze raw data and calculate results. The software performed a Quality Control assessment of the assay, generated a standard curve and provided a test result for each subject. The software is available at www.quantiferon.com.The test is considered positive when the TB Antigen tube is significantly above the Nil IFN-γ. [9,10].

Statistical analysis

Data were analyzed using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, ILs). Numerical data were expressed as mean and standard deviation. Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (nonparametric t-test). All tests were two-tailed. A p-value < 0.05 was considered significant.

RESULTS

This was a cross sectional study conducted on 52 newly diagnosed patients with hematological malignancies at National Cancer Institute (NCI), Cairo University within 6 months duration between August 2014 and February 2015. We measured the interferon-gamma (IFN-γ) secreted by the T-lymphocytes sensitized to Mycobacterium tuberculosis proteins when whole blood was incubated with the Mycobacterium tuberculosis specific antigens used in the test. We evaluated the QFT results in relation to the different clinical and hematological parameters and the outcome after induction therapy.

The patients included were 30 males (58%) and 22 females (42%). Their age ranged from 18 to 81 years with a median age of 43 years and a mean ±SD of 42.75±15.394 years. Twenty one patients (40.4%) were diagnosed as acute leukemia, while 31 patient (59.6%) were histologically proven as lymphoma (table 1).

Table 1: Demographic and laboratory findings in the 52 newly diagnosed cases of hematological malignancies investigated for latent TB using The QuantifER0N-TB Gold IT test

Parameter	Mean ±SD	Median (range)
Age, years	42.75 ± 15.394	43 (16-81)
Sex, male Female	(30) 58% (22) 42%	
Hematological parameters PB TLC ×109/L	(47.48±62.48)	23 (1 to 251)
PB Hb gm/dl	(8.31±2.34)	8 (4.5 to 14.6)
PB Platelets ×109/L	(191.1±251)	145 (7 to 1170)
BM Blast%	(57± 34.5)	73 (3 to 97)

PB: peripheral blood, BM: bone marrow, TLC: total leucocytic count, Hb: hemoglobin, SD: standard deviation

Regarding the QFT results, 9/52 (17.3%) were positive while 43/52 (82.7%) were negative as shown in figure 1. Positive QFT cases were 77.8% (7/9) males, and 22.2% females. Out of 9 positive QFT cases, 3/9 were leukemic patients (33.3%), while 6/9 were diagnosed as lymphomas (66.6%). Manifestations of chest infection OR suspicious TB infection in the form of fever, persistent cough, dyspnea, night sweat, weight loss observed in 13/52 (25%) of cases and reported in 5/9 (55.5%) of Quantiferone positive cases. Positive lower respiratory tract radiological findings in the form of patchy area of consolidation, nodular shadows and hilar lymphadenopathy reported in 14/52 (27%) of all cases and 4/9 (44.4%) of positive QFT cases. Lymph node enlargement was detected in 15/52 cases (29%)

and detected in 6/9 (67%) of positive QFT cases 6/9 at presentation. Figure 2 illustrates the positivity of QFT in relation to the type of hematological malignancy.

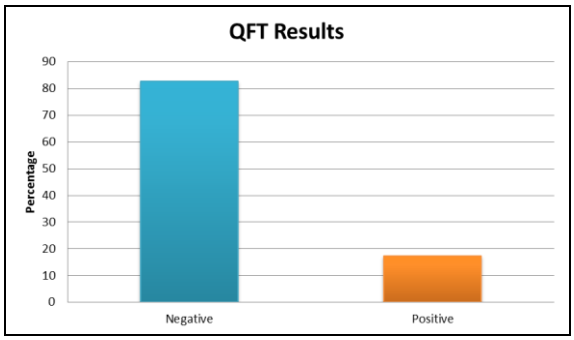

Fig. 1: of the QFT done for the 52 newly diagnosed cases of haematological malignancies investigated for latent TB

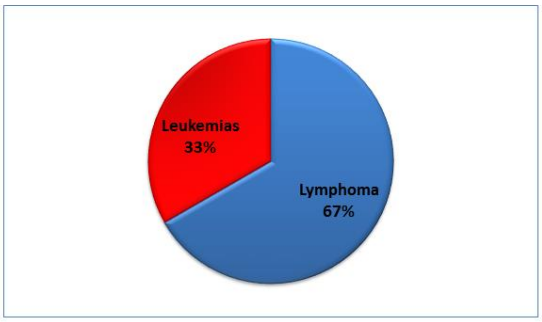

Fig. 2: Positive QFT results in relation to the type of hematological malignancy

As regards outcome after induction chemotherapy, 44 of the 52 patients studied were alive while 8 patients died within six months after induction therapy. Of the patients who had unfavorable outcome, 4 patients were QFT test positive, and 4 patients were QFT negative as shown in figure 3. Demographic and laboratory findings of patients are demonstrated in table 1. Correlation between QFT results and different patients' criteria including diagnosis, age, gender and laboratory findings demonstrated in table 2.

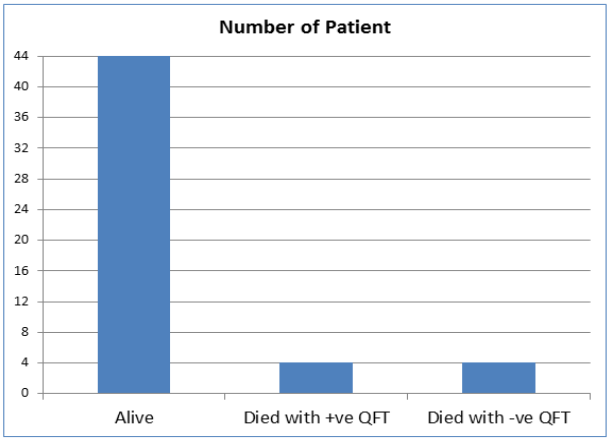

Fig. 3: Outcome of cases After Induction Therapy

Table 2: Correlation between QFT results of the 52 newly diagnosed cases of haematological malignancies investigated for latent TB and different patients' criteria including diagnosis, age, gender, manifestations of infection and laboratory findings

Patients' criteria		**Positive QFT**	**Negative QFT**	**p-value**
Diagnosis	Leukemias	3 (33.3%)	18 (41.9%)	0.98235
	Lymphoma	6 (66.7%)	25 (58.1%)	
Age	<40	3 (33.3%)	21 (48.8%)	0.6307
	>40	6 (66.7%)	22 (51.2%)	
Gender	Males	7 (77.8%)	23 (53.5%)	0.3319
	Females	2 (22.2%)	20 (46.5%)	
***Hematological parameters** TLC ×109/L Hb gm/dl platelets×109/L BM blast%		62±41.43 6.86±1.05 20.33±11.67 82.67±15.69	45.33±65.34 8.514±2.4 214.32±259.37 50.83±35.376	0.6267 0.2634 0.2162 0.1603

*Values are in mean± SD
p-value >0.05 is statistically not significant

DISCUSSION

One third of the world population is infected with M. tuberculosis in a latent state, which involves an eventual risk of progressing to active TB disease. Patients with immune-suppression, such as those suffering from hematological malignancies, have a greater risk of progressing to TB disease once infected. It is estimated that the Relative Risk of TB disease in patients with hematologic malignancies is 2–40 times that of the general population (11). The diagnosis of TB in immune-suppressed patients- with impaired cell mediated immunity- is often challenging as they usually present with clinical characteristics that differ from those without any underlying disease, so early diagnosis of latent tuberculosis can prevent spread of infection, promote early management of T.B and so can reduce the higher rate of mortality due to TB in such patients. Therefore, it is recommended to diagnose latent TB infection and consider preventive therapy that could avoid the progression from a latent state to active TB disease. There are currently two methods for diagnosing latent TB infection: the Tuberculin Skin Test (TST) and the Interferon-Gamma Release Assays (IGRA). Due to the lack of sensitivity in patients with immune-deficient conditions, a combined TST-IGRA testing is probably the best way for latent TB diagnosis in order to gain sensitivity [11].

In the current study, the interferone- gamma secreted by T-lymphocytes following stimulation by antigens unique to Mycobacterium tuberculosis was measured. Nine out of 52 newly diagnosed patients with hematological malignancies demonstrated QFT positive response. Of the QFT positive cases, more positivity was observed in the lymphoma patients than those diagnosed with acute leukemia, 6 versus 3, respectively. The association between lymphoma and QFT positivity was not significantly different, mostly due to small numbers. Other studies observed that patients with non-Hodgkin lymphoma had a higher incidence rate of M. tuberculosis than patients with leukemia [12]. Kamboj and Sepkowitz reported that the highest rate of TB was among allogeneic hematopoietic stem cell transplant recipients, followed by patients with non-Hodgkin"s lymphoma and patients with Hodgkin"s lymphoma [13]. Another study reported that the association between HL and TB must be considered, especially in countries where TB is endemic [14].

In most individuals, Mycobacterium tuberculosis infection is contained initially by host defence, and infection remains latent with the potential to develop into active disease at any time [15]. Screening for latent TB is recommended for high risk patients to experience disease activation

Identification and treatment of latent tuberculosis infection can reduce the risk of development of disease by as much as 90% [16]. Therefore early detection and treatment of latent TB in immune-compromised cases has the potential to avoid the spread of TB by reducing the number of potential sources of infection.

Regarding the age groups in the present study, 67% of QFT positive patients were ≥40 years old, while 33.3%% of our studied positive patients for TB were <40 years old. Global tuberculosis report, (2015) reported that highest incidence of notified cases of TB in Egypt was in the age groups 45-64 years old in males and 35-44 years old in females while the lowest incidence of notified cases was in the age groups 0-4 years old [17]. Wu et al., reported that 68% of the studied tuberculous cancer patients were >60 years old [12]. Alwani et al. reported that the incidence of TB was higher in age groups 15-45 years old while the lowest incidence occurred in the extremes of age; below 15 years old and over 60 years old [18]. Generally, in developing countries the majority of cases infected with TB are younger than 50 years, and about half of them may be younger than 15 years. In developed countries of Western Europe (having an aging population) the majority of cases are older than 50 years. These findings could also be explained by the increased incidence of smoking in this active age group. Poverty, malnutrition, and physical, mental, and occupational stress are well known to be associated with this age group [18].

In the current study, the majority of positive QFT cases were males 78% (7/9) and only 22% were females. Chen and his coworkers supported our observation with 70% of their studied patients with hematological malignancies infected with TB were males while 30% were females[19]. Manifestations of chest infection OR suspicious TB infection in the form of fever, persistent cough, dyspnea, night sweat, weight loss were reported in 13/52 (25%) and 5/9 (55.5%) of positive Quantiferone cases. Silva et al., found that multivariate analysis identified only cough and shortness of breath as the statistically significant symptoms associated with tuberculosis. Therefore, no specific clues to the diagnosis of tuberculosis seem to arise from the analysis of symptoms [20]. The most frequent symptoms of tuberculosis are persistent cough, fever, night sweats, weight loss, shortness of breath, hemoptysis and chest pain. Since these findings are also frequent in patients with hematologic malignancies, the diagnosis of tuberculosis in these patients may be difficult. Furthermore, in addition to the overlap in clinical symptoms, the two diseases may occur simultaneously. TB patients with different types of cancer, including those with HM, often present clinical characteristics that are distinct to those of patients without any other underlying disease. [21]. According to our study, clinicians should remain alert to the

differential diagnosis of tuberculosis in hematological patients with prolonged fever of unknown etiology.

Lymph node enlargement were detected in 6/9(66.7%) of QFT positive cases. Lymph node enlargement in the current study might be due to patient's underlying disease or might be considered as an extra-pulmonary manifestation of TB. Lymph node enlargement in our population of patients is difficult to interpret their significance in relation to latent TB, so it further investigations are recommended. Extra-pulmonary manifestations was previously reported to account for 28% of TB disease in patients with hematological malignancies [21].

Positive CT chest radiological findings were detected in 44.4% of positive QFT cases (4/9) in the present study. Another study also reported that 44.4% of positive patients for latent TB demonstrated positive CT radiological [21]. Reactivation of tuberculosis in patients with hematological malignancies could result from impaired immunity by underlying hematological malignancies and/or chemotherapy induced immunosuppression. The definite timing of tuberculosis reactivation in patients with hematological malignancies is still not clear. Previous studies revealed that immunodeficiency contributes to a significantly increased TB reactivation rate [22]. High risk population with impaired immunity had a TB incidence of 20 times higher than that of the general population [23]. High-risk factors (HIV/AIDs, transplantation, malignancy) contribute to a significantly increased TB reactivation rate. [22]

The crude mortality in the current study was 15%. Of the 8 patients who had an unfavourable outcome after induction therapy, 50% were QFT test positive. Though it is difficult to attribute unfavourable outcome in our QFT positive patients to TB disease activation after receiving induction chemotherapy as no further testing was done, still this possibility should be investigated in a follow up study. Silva et al. reported that the mortality attributable to tuberculosis is high in patients with hematologic malignancies; with a crude mortality of 75% for patients with tuberculosis, and an attributable mortality of 62.5% [20].

This study had the following limitations. First, only hematological malignancy patients were enrolled. Second, tissue biopsies, cultures and other investigations for TB were not available for all the patients and concurrent infection in patients with febrile neutropenia cannot be completely excluded. Third, this study was a cross sectional study on a limited number of patients. Further large scale study should be conducted to confirm the findings based on our limited case numbers. Also further follow up is required of the patients with QFT positive results to evaluate predictive value of the positive QFT test.

On the basis of our findings, we can conclude that latent TB is common in adult patients with hematological malignancies including acute leukemias and lymphomas. Thus, patients with hematological malignancies in our institute are at increased risk of developing TB disease. Diagnosing or excluding tuberculosis disease, and assessing the probability of LTBI, requires a combination of epidemiological, history, medical, and diagnostic findings that should be taken into account when interpreting QFT results, raising the importance of larger and more developed studies to evaluate the exact relation between latent TB and hematological malignant disorders. Screening for LTBI in order to identify individuals who are at increased risk for the development of tuberculosis and therefore who would benefit from treatment of TB is of crucial importance in countries with a moderate prevalence of TB.

Acknowledgment

This study was supported by the National Cancer Institute- Cairo University.The authors acknowledge the microbiology staff in Clinical Pathology Department who helped in accomplishing this work and acknowledge the staff of medical oncology department who helped us in data collection and patients" examination.

Conflict of Interest

No conflict of interest is considered for any of the authors of this study.

REFERENCES

1. WHO. Global Tuberculosis Report 2016. Available online: http://www.who.int/tb/publications/global_report/en/ (accessed on 2 January 2017).
2. World Health Organization. Global Tuberculosis Report 2013. Geneva, Switzerland: World Health Organization; 2013.
3. World Health Organization (2012). Global tuberculosis: WHO report 2012, WHO, Geneva, Switzerland.
4. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. Lancet. 2003;362:887–899
5. Houben, R.M.; Dodd, P.J. The Global Burden of Latent Tuberculosis Infection: A Re-Estimation Using Mathematical Modelling. PLoS Med. 2016, 25, e1002152. [CrossRef] [PubMed]
6. Carvalho AC, Pezzoli MC, El-Hamad I, Arce P, Bigoni S, Scarcella C, Indelicato AM, Scolari, C, Carosi G, Matteelli A. QuantiFERONTB Gold test in the identification of latent tuberculosis infection in immigrants. J. Infect. 2007, 55, 164–168. [CrossRef] [PubMed]
7. Dheda K, Pooran A, Pai M, Miller RF, Lesley K, Booth HL, Scott GM, Akbar AN, Zumla A, Rook GA. Interpretation of Mycobacterium tuberculosis antigen-specific IFN- release assays (T-SPOT.TB) and factors that may modulate test results. J. Infect. 2007, 55, 169–173.

8. El-Mahallawy HA, Eissa SA, Tuberculosis in cancer patients: Role of newer techniques in relation to conventional diagnostic methods,Journal of advanced research. 2010,1,157-162.
9. CDC. Laboratory Procedure Manual. Available from: http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/tuberculosis-tb_qft_met.pdf. 2013 [Accessed 3 May 2016].
10. Cellestis. QuantiFERON®-TB Gold (QFT®) Package Insert. Available from: http://www.quantiferon.com/irm/content/PI/QFT/2PK/CA.pdf. 2012.
11. Anibarro L, Pena A. Tuberculosis in Patients with Haematological Malignancies. Meditter J Hematol Infect Dis 2014 Apr 7;6(1): e2014026.
12. Wu CY, Hu HY, Pu CY, Huang N, Shen HC, Li CP, Chou YJ. Aerodigestive tract, lung and haematological cancers are risk factors for tuberculosis: an 8-year population-based study. Int J Tuberc Lung Dis. 2011; 15(1): 125–30.
13. Kamboj M, Sepkowitz KA. The risk of tuberculosis in patients with cancer. Clin Infect Dis. 2006;1;42(11): 1592–5.
14. Piotr Centkowski, Joanna Sawczuk-Chabin, Monika Prochorec & Krzysztof Warzocha Hodgkin's lymphoma and tuberculosis coexistence in cervical lymph nodes Leukemia & Lymphoma 2005; 46 (3)471-475.
15. Pai M, Behr MA, Dowdy D, et al. Tuberculosis. Nat Rev Dis Primers 2016; 2:16076.
16. Blumberg HM, Ernst JD. The challenge of latent TB infection JAMA.2016;316(9):931-933.
17. WHO. Global tuberculosis report, 2015. Available from: http://www.who.int/tb/publications/global_report/gtbr15_annex04.pdf?ua=1. 2015
18. Alwani AK, Al-Aarag AH, Omar MM, Abdelghaffar Hibah NA. Incidence of tuberculosis before and after DOTS (direct observed therapy short course strategy) implementation in El-Behira Governorate, Egypt. Egypt J Bronchol 2015;9:101-8.
19. Chen CY, Sheng WH, Lai CC, Liao CH, Huang YT, Tsay W, Huang SY, Tang JL, Tien HF, Hsueh PR. Mycobacterial infections in adult patients with hematological malignancy. European Journal of Clinical Microbiology & Infectious Diseases. 2012; 31(6):1059-66.
20. Silva FA, Matos JO, de Q Mello FC, Nucci M. Risk factors for and attributable mortality from tuberculosis in patients with hematologic malignances. Haematologica. 2005 Aug;90(8):1110-5.
21. Chen CY, Sheng WH, Cheng A, Tsay W, Huang SY, Tang JL, Chen YC, Wang JY, Tien HF, Chang SC. Clinical characteristics and outcomes of Mycobacterium tuberculosis disease in adult patients with hematological malignancies. BMC Infect Dis. 2011; 11: 324.
22. Jing-Wen Ai, Qiao-Ling Ruan, Qi-Hui Liu and Wen-Hong Zhang: Updates on the risk factors for latent tuberculosis reactivation and their management. Emerging Microbes & Infections (2016) 5, e10; doi:10.1038/emi.2016.10.
23. Aguado JM, Herrero JA, Gavalda J et al. Clinical presentation and outcome of tuberculosis in kidney, liver and heart transplant recipients in Spain. Spanish Transplantation Infection Study Group, GESITRA. Transplantation 1997; 63: 1278–1286.