Retrospective Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
48(10) 1–9

DOI: 10.1177/0300060520921640
journals.sagepub.com/home/imr
SAGE

Performance of the Xpert® MTB/RIF assay in the rapid diagnosis of tracheobronchial tuberculosis using bronchial washing fluid

Yan-Hua Song[1], Qiang Li[1], Li-Ping Ma[1], Rong-Mei Liu[1], Guang-Lu Jiang[2], Qi Li[1]* and Meng-Qiu Gao[1]*

Abstract

Objective: To assess the diagnostic value of the Xpert® MTB/RIF (GeneXpert) assay for tracheobronchial tuberculosis (TBTB) using bronchial washing fluid (BWF).

Methods: This retrospective study enrolled patients suspected of having TBTB and patients with non-TB pulmonary disease as controls. BWF were used to undertake acid-fast bacillus (AFB) smears, the GeneXpert assay and the Löwenstein–Jensen (LJ) culture method. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were compared among BWF AFB smears, BWF GeneXpert and the BWF LJ culture method.

Results: A total of 130 patients with TBTB and 102 patients with non-TB pulmonary disease were enrolled in the study. Sputum AFB smears were positive in 62 of 130 patients (47.7%) with TBTB. Using the clinical diagnosis of TBTB as the gold standard, the sensitivity, specificity, PPV and NPV of the three methods using BWF were as follows: 93.1%, 99.0%, 99.2% and 91.8% for BWF GeneXpert; 73.1%, 100.0%, 100.0% and 74.5% for BWF LJ cultures; 53.8%, 99.0%, 98.6% and 62.7% for BWF AFB smears. The diagnostic yield of BWF GeneXpert was significantly higher compared with BWF cultures for type III and IV TBTB.

Conclusion: The Xpert® MTB/RIF assay using BWF exhibited higher sensitivity than bacteriological diagnostic methods and was particularly useful for the early diagnosis of smear-negative TBTB.

[1]Department of Tuberculosis, Beijing Chest Hospital, Capital Medical University, Beijing, China
[2]Tuberculosis Clinical Laboratory, Beijing Chest Hospital, Capital Medical University, Beijing, China

*These authors contributed equally to this work.

Corresponding author:
Meng-Qiu Gao, Department of Tuberculosis, Beijing Chest Hospital, Capital Medical University, 9 North Guan Street, Tongzhou District, Beijing 10049, China.
Email: gaomq136520@163.com

Keywords
Tracheobronchial tuberculosis, Xpert® MTB/RIF, diagnosis

Date received: 25 April 2019; accepted: 31 March 2020

Introduction

Tuberculosis (TB) remains a major health concern though its prevalence has been reduced dramatically.[1] Tracheobronchial tuberculosis (TBTB) is a special clinical type of TB, which is caused by infection of the tracheobronchial tree by *Mycobacterium tuberculosis* (MTB).[2] The incidence of TBTB is 6–54% in pulmonary TB patients[3] and 5–10% in TBTB patients without tuberculous foci on the lungs.[2] TBTB is likely to be misdiagnosed as lung cancer, pneumonia and asthma due to few specific symptoms and bronchoscopic findings.[3–5] The delayed diagnosis of TBTB could lead to bronchial stenosis and severe complications.[6] Therefore, an early, fast diagnosis of TBTB and a reasonable anti-TB treatment are essential to reduce the incidence and spread of TB.

The sputum MTB test is an important method for diagnosing pulmonary TB. Although acid-fast staining is cheap and fast, its positivity rate in sputum is 0–53.3% for TBTB.[3,7–10] Furthermore, the results of a negative sputum acid-fast bacillus (AFB) smear do not exclude TBTB. Molecular biology techniques for MTB testing that can increase the early diagnosis rate of TBTB are faster and more sensitive than conventional bacteriological examinations.[11] Bronchoscopy is an important procedure in the diagnosis of TBTB, in which specimens are collected from the lesion in the lower respiratory tract and submitted for molecular biology detection of MTB. The Xpert® MTB/RIF (GeneXpert; Cepheid, Sunnyvale, CA USA) assay is a fully automated real-time semi-nested polymerase chain reaction (PCR) system.[12,13] Several studies have evaluated the diagnostic performance of this assay in the diagnosis of pulmonary TB with lower respiratory tract specimens.[14,15] However, there are limited data on the utility of GeneXpert for bronchial washing fluid (BWF) in the diagnosis of TBTB.[16–18] Therefore, the GeneXpert assay was performed using BWF to assess the efficacy of this method in diagnosing TBTB in a country with a high incidence of TB.

Patients and methods

Patient enrolment

This retrospective study enrolled consecutive patients suspected of having TBTB at the National Clinical Centre of TB, Beijing Chest Hospital, Beijing, China between March 2016 and May 2017. Patients were identified by searching the inpatient case registration system using the following diagnostic terms: tuberculosis of bronchus, tuberculosis of trachea, tracheobronchial tuberculosis and tuberculosis of bronchial mucosa. TBTB was defined as the presence of visible lesions at the tracheobronchial level affected by MTB to the segmental bronchus, with positive histological evidence from a bronchial biopsy or a positive AFB smear in sputum, BWF and bronchial brushing fluid (BBF), according to the Guidelines on the Diagnosis and Treatment of Tracheobronchial Tuberculosis (Trial).[2] The control group had bronchoscopic lesions but the final diagnosis was non-TB pulmonary disease. The BWF examination was undertaken using the GeneXpert assay, AFB staining and mycobacteria culture.

Two researchers (Y.S. & Q.L.) inputted the data from all of the study participants.

The following criteria were used to classify patients: (i) TBTB case, the patient had a positive AFB smear or mycobacteria culture (of sputum, BWF or BBF; species identified as non-tuberculous mycobacteria were excluded) or positive histological evidence + visible lesions; (ii) clinically diagnosed TBTB, the patient presented with clinical manifestations or a treatment response typical of TBTB, and had typical tracheobronchial lesions under the bronchoscope, but lacked bacteriological or pathological evidence.[2] For the clinically diagnosed TBTB after anti-TB treatment, the clinical symptoms were relieved, the lung image and the pathological changes were improved under the bronchoscope; (iii) newly diagnosed TBTB, patients that had not received anti-TB treatment; (iv) recurrent patients, those that had been successfully treated for pulmonary TB more than 1 year previously. Patients without positive bacteriological or pathological evidence of TB, or without a response after anti-TB treatment, but with a response to antibiotics, and those patients that presented with other pathological findings, were diagnosed with non-TB pulmonary diseases and assigned to the control group. Demographic characteristics, including age and sex, were collected from the medical records.

This study was approved by the Ethics Committee of Beijing Chest Hospital (no. 2018[60]). Informed consent was not required from the participants due to the retrospective design of this study. All data and information on the participants were fully anonymized.

Bronchoscopy procedures and microbiological examination

Morning sputum samples (3–5 ml) were collected from each patient enrolled in the present study. Immunofluorescence staining was performed and the smears were observed under a fluorescence microscope (ZEISS Primo Star iLED; Carl Zeiss Microscopy GmbH, Jena, Germany) as described previously.[19] All patients in this study were examined by bronchoscopy (Olympus® BF-P60 bronchoscope or Olympus® BF-XT40 fibreoptic bronchoscope; Olympus, Tokyo, Japan). After the bronchoscope reached the lesion, flushing was performed using 10–20 ml of sterile saline solution. Then, 6–12 ml of the BWF was collected and placed into a sterile container. Bronchial brushing of the lesion or the corresponding pulmonary segment was performed. The bronchoscope was withdrawn and AFB smears were prepared. BWF samples were used to undertake AFB smears, the GeneXpert assay and the Löwenstein–Jensen (LJ) culture method. Testing procedures, the preparation of AFB smears with bronchial brushings and BWF and interpretation of results were as described previously.[19]

For mycobacteria culture, all BWF specimens were cultured in LJ medium (Yinke, Zhuhai, China) according to a standard protocol.[20] The tubes were incubated at 37 °C and monitored for mycobacterial growth for 8 weeks. A positive result referred to a positive culture result and the strain was identified as *Mycobacterium tuberculosis* complex. Positive strains were further submitted for drug susceptibility testing and species identification using a microtitre assay (Yinke). The GeneXpert assay was performed according to the manufacturer's instructions (Cepheid) as follows. BWF (1 ml) was placed into a sterile tube with a screw cap and 2 ml of processing fluid was added. The tube was oscillated on a vortex mixer for 10 sec and left at room temperature for 15 min to achieve complete liquefaction. Then, 2 ml of the liquefied specimen was added to the GeneXpert cartridge and loaded into the

instrument. The result was interpreted based on the cycle threshold (Ct) value of the probe. A Ct value $\leq$38 for the internal control reference gene was considered a positive result.

Clinical classification of TBTB

Based on the Guidelines on the Diagnosis and Treatment of Tracheobronchial Tuberculosis (Trial),[2] TBTB was divided into the following subtypes: type I, inflammatory infiltration; type II, ulcerative necrosis; type III, proliferation of granulation tissues; type IV, cicatricial stenosis; type V, wall softening; type VI, lymphatic fistula.

Statistical analyses

All statistical analyses were performed using the SPSS® statistical package, version 17.0 (SPSS Inc., Chicago, IL, USA) for Windows®. The diagnostic sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the three methods using samples of BWF were determined. χ^2-test was used to compare categorical data. A P-value <0.05 was considered statistically significant.

Results

A total of 282 patients suspected of having TBTB were identified based on a search of the inpatient case registration system (Figure 1). Of these, 130 TBTB patients were included in this analysis. Among these 130 patients, 116 (89.2%) patients were microbiologically confirmed, nine (6.9%) patients were histologically confirmed, two (1.5%) patients were both microbiologically and histologically confirmed and three (2.3%) patients were clinically diagnosed with TBTB. All 130 patients had chest computed tomography

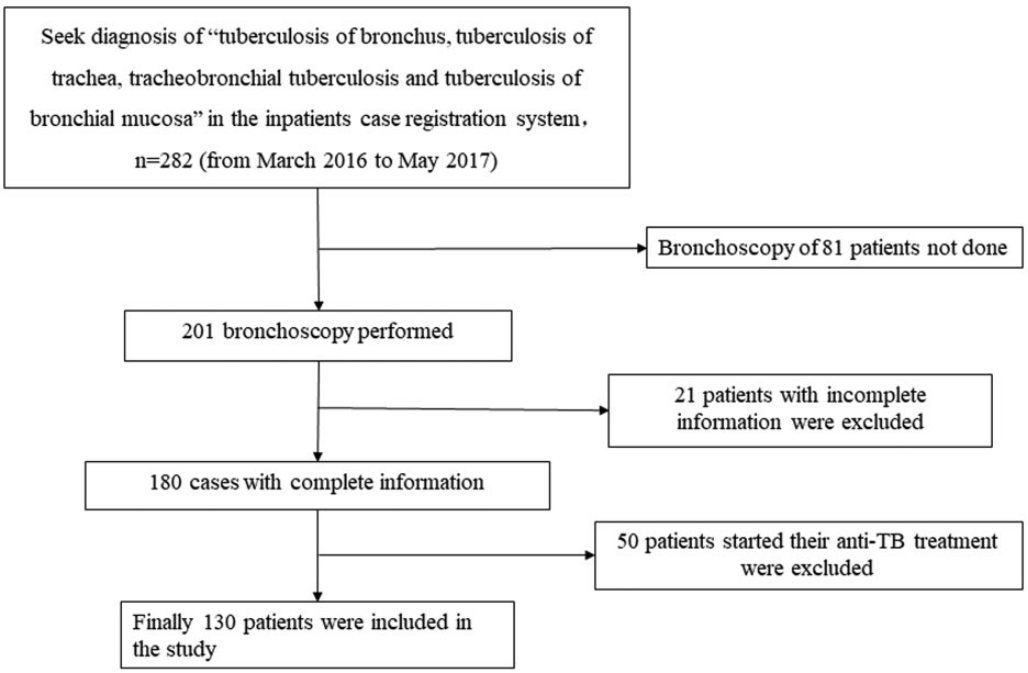

Figure 1. Flow diagram showing recruitment and analysis of patients with suspected tracheobronchial tuberculosis (TB).

and lung imaging changes. Among the 130 patients, 86 had stenosis or thickening of the bronchus and 57 had local consolidation or atelectasis. A total of 102 patients with non-TB pulmonary diseases were included and assigned to the control group. Of these, one control patient had a positive AFB smear using BWF and was found to be infected with non-tuberculous mycobacteria (NTM).

The demographic and clinical characteristics were compared between the TBTB and control groups (Table 1). The proportion of female patients in the TBTB group was significantly higher than that in the non-TB control group (odds ratio 8.53; 95% confidence interval 4.58, 15.86; $P < 0.001$). There was also a difference in the age distribution between the TBTB and control groups.

The diagnostic validity of BWF GeneXpert, BWF LJ culture and BWF AFB smears in the TBTB group are shown in Table 2. The sensitivity of BWF GeneXpert was significantly higher than that of the other two methods ($P < 0.001$ for all comparisons). Sputum AFB smears were positive in 62 of 130 patients (47.7%) with TBTB and negative in 68 of 130 patients (52.3%) with TBTB. The diagnostic yields of BWF GeneXpert and BWF LJ culture in patients with TBTB stratified according to a positive or negative sputum AFB smear result are shown in Table 3. The positive rates of BWF GeneXpert in either sputum smear-positive or smear-negative patients were higher than the BWF LJ culture method. The BWF LJ culture of 95 patients was positive and the rifampin (RIF) drug susceptibility test was performed. A total of nine patients were RIF-resistant. This was consistent with the results of the BWF GeneXpert rpoB gene detection, which yielded a sensitivity of 100%.

In addition, in 130 patients with TBTB, 75 patients (57.7%) had positive BBF specimens using the AFB smear method. There was no significant difference when compared with BWF AFB smear results (70 of 130; 53.8%).

The diagnostic yields of BWF GeneXpert and BWF LJ culture in patients

Table 1. Demographic and clinical characteristics of patients with tracheobronchial tuberculosis (TBTB) compared with control patients with non-TB pulmonary diseases.

Characteristics	TBTB group $n = 130$	Control group $n = 102$	Odds ratio (95% confidence interval)	Statistical analysis[a]	Total cohort $n = 232$
Sex					
Male	20 (15.4)	62 (60.8)	1	–	82 (35.3)
Female	110 (84.6)	40 (39.2)	8.53 (4.58, 15.86)	$P < 0.001$	150 (64.7)
Age, years					
<25	23 (17.7)	7 (6.9)	4.45 (1.55, 12.74)	$P = 0.004$	30 (12.9)
25–44	58 (44.6)	29 (28.4)	2.71 (1.25, 5.84)	$P = 0.01$	87 (37.5)
45–64	32 (24.6)	43 (42.2)	1.01 (0.46, 2.19)	NS	75 (32.3)
≥65	17 (13.1)	23 (22.5)	1	–	40 (17.2)
HIV infected	0 (0.0)	0 (0.0)			
Previous anti-TB treatment TBTB	8 (6.2)	–			
Newly diagnosed TBTB	122 (93.8)	–			

Data presented as *n* of patients (%).

[a]Categorical data were compared using χ^2-test.

HIV, human immunodeficiency virus; NS, no significant between-group difference ($P \geq 0.05$).

Table 2. Diagnostic validity of bronchial washing fluid (BWF) Xpert® MTB/RIF (GeneXpert), BWF Löwenstein–Jensen (LJ) culture and BWF acid-fact bacilli (AFB) smears in patients with tracheobronchial tuberculosis (TBTB).

Gold standard	Methods	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
BWF LJ culture	BWF GeneXpert	100.0% (95/95) (95.2, 100.0)	81.0% (111/137) (73.2, 87.0)	78.5% (69.9, 85.2)	100.0% (95.8, 100.0)
	BWF AFB smear	70.5% (67/95) (60.2, 79.2)	97.8% (134/137) (93.2, 99.4)	95.7% (87.2, 98.9)	82.7% (75.8, 88.0)
Clinical diagnosis[a]	BWF GeneXpert	93.1% (121/130) (86.9, 96.6)	99.0% (101/102) (93.9, 99.9)	99.2% (94.9-100)	91.8% (84.6, 96.0)
	BWF AFB smear	53.8% (70/130) (44.9, 62.6)	99.0% (101/102) (93.9, 100.0)	98.6% (91.4, 99.9)	62.7% (54.7, 70.1)
	BWF LJ culture	73.1% (95/130) (64.5, 80.3)	100.0% (102/102) (95.5, 100.0)	100.0% (95.2, 100.0)	74.5% (66.6, 81.0)

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[a]Clinical diagnosis includes definite TBTB cases and clinically diagnosed TBTB.
When LJ culture was set as the gold standard: χ^2-test = 32.84, $P < 0.001$, AFB smear sensitivity versus GeneXpert sensitivity.
When clinical diagnosis was set as the gold standard: χ^2-test = 51.31, $P < 0.001$, AFB smear sensitivity versus GeneXpert sensitivity; χ^2-test = 10.37, $P = 0.002$, AFB smear sensitivity versus LJ culture sensitivity; χ^2-test = 18.19, $P < 0.001$, LJ culture sensitivity versus GeneXpert sensitivity.

Table 3. Diagnostic yields of bronchial washing fluid (BWF) Xpert® MTB/RIF (GeneXpert) and BWF Löwenstein–Jensen (LJ) culture in patients with tracheobronchial tuberculosis (TBTB) stratified according to their sputum acid-fact bacilli (AFB) smear results (positive and negative).

	Diagnostic yield, %			
TBTB subgroups	BWF GeneXpert	BWF LJ culture	χ^2	Statistical analysis
Sputum AFB smear positive, $n = 62$	100.0 (62/62)	87.1 (54/62)	–	$P = 0.006$[a]
Sputum AFB smear negative, $n = 68$	86.8 (59/68)	60.3 (41/68)	12.24	$P < 0.001$

[a]Fisher exact probability method.

with TBTB stratified according their bronchial TBTB subtype are shown in Table 4. There was no significant difference in diagnostic yield between these two methods for type I and II TBTB. However, the diagnostic yield of BWF GeneXpert was significantly higher than that of BWF LJ culture for types III and IV TBTB ($P = 0.004$ and $P = 0.002$, respectively).

Discussion

In this current study, all patients with TBTB were diagnosed by lung imaging, sputum bacteriology, clinical symptoms, serum immunology and differential diagnosis to exclude lung tumours, pneumonia and other diseases, and they were all followed up after treatment. TBTB is considered an

Table 4. Diagnostic yields of bronchial washing fluid (BWF) Xpert® MTB/RIF (GeneXpert) and BWF Löwenstein–Jensen (LJ) culture in patients with tracheobronchial tuberculosis (TBTB) stratified according to their bronchial TBTB subtype.

TBTB subtype	Diagnostic yield, %		χ^2	Statistical analysis
	BWF GeneXpert	BWF LJ culture		
I, $n = 51$	96.1 (49/51)	86.3 (44/51)	1.95	NS[a]
II, $n = 18$	100.0 (18/18)	100.0 (18/18)		NS[a]
III, $n = 35$	91.4 (32/35)	62.9 (22/35)	8.012	$P = 0.004$
IV, $n = 26$	84.6 (22/26)	42.3 (11/26)	10.035	$P = 0.002$

[a]Fisher exact probability method.
NS, not significant ($P \geq 0.05$).

important complication associated with pulmonary TB.[6]There is an urgent need to develop a sensitive and rapid diagnostic tool for TBTB. The sputum AFB smear is a commonly used method, but the positivity rates fluctuate considerably (0–53.3%).[3,7–10] In the present study, the rate of sputum AFB smear positivity was 47.7%, which was consistent with existing reports.[3] The reasons for low positive rates in the bacteriological testing of sputum include insufficient bronchial drainage, proximal granulation tissues enveloping the mucus, lesions undergoing natural recovery and bronchial fibrosis.[3]

It has been reported that the range of positive rates of BWF AFB smears is 10–85% in TBTB and the range of positive rates of pathological biopsies is 30–84%.[8,10] Both ranges are higher than that of the sputum smear. In the present study, the positive rate of BWF AFB smears (53.8%) was higher than that of sputum smears (47.7%), but lower than that of BWF LJ cultures (73.1%). The bacteriological testing of BWF is easier and safer than biopsies and has a lower risk of complications. However, the positive rate of bacteriological testing could be affected by the type of local lesion. The positivity rate is usually higher in active lesions, such as ulcers and caseous necrosis, but lower in cicatricial lesions.[10]In addition, BWF LJ cultures usually take 6–8 weeks and this could easily lead to delayed diagnosis.

Currently, the GeneXpert assay is an excellent method for detecting MTB and is highly suitable for the rapid diagnosis of TBTB using BWF specimens. Specimens used for the GeneXpert assay are less likely to be contaminated when compared with conventional cultures and other PCR techniques.[10] Furthermore, there is no need for repeated sample disposal, which prevents the loss of MTB in the specimens. The GeneXpert assay can identify MTB and NTM;[21] and it can reduce the false-positive risk of NTM in AFB smears or mycobacteria culture for tuberculosis diagnosis. It has been reported that the sensitivity and specificity of GeneXpert are higher than that of conventional smears and cultures for tuberculosis diagnosis.[22,23] In the present study, the sensitivity of the BWF GeneXpert was 93.1%, which was significantly higher than that of the BWF LJ cultures and BWF AFB smears ($P < 0.001$). Sputum AFB smear-negative TBTB needs to be differentiated from pneumonia and lung cancer to avoid missed diagnoses and misdiagnoses. In the present study, for 68 sputum AFB smear-negative TBTB patients, the diagnostic yield of BWF GeneXpert was 86.8%, which was significantly higher than that for the BWF LJ cultures (60.3%). According to the present

results, compared with conventional BWF LJ cultures, the diagnostic yield of BWF GeneXpert was significantly higher. The result of the BWF GeneXpert rpoB gene detection was highly consistent with the RIF drug sensitivity test.[22] Therefore, the GeneXpert assay could be a rapid and accurate ancillary tool for bacteriological testing and could contribute to the early diagnosis and treatment of TBTB.

All of the patients in this current study were classified as having type I–IV TBTB.[2,24] Type IV TBTB is the healing stage of the course of bronchial tuberculosis with less bacterial load than types I–III.[2,9] The diagnostic yield results of the present study for GeneXpert were >90% for types I, II and III TBTB. The GeneXpert assay exhibited better sensitivity in the diagnosis of MTB from BWF in patients with type IV TBTB when a lower mycobacterial load might be expected, than BWF cultures, which is conducive to the diagnosis of TBTB.

This current study had several limitations. First, it was a single-centre study with a small sample size and a bronchoscopy examination was not performed for all patients suspected of having TBTB. This might have affected the results. Secondly, this study was a retrospective analysis of clinical data and biopsies were only collected from some of the patients. The majority of patients with TBTB had bacteriological or pathological evidence, but a small number of patients only had tracheoscopic manifestations (such as scar stenosis), which could only be used to achieve a clinical diagnosis. Therefore, some cases could only obtain a clinical diagnosis though the treatment process. Nevertheless, the current study has demonstrated the diagnostic value of the GeneXpert assay for TBTB based on the analysis of real clinical data.

In conclusion, this current study demonstrated that the GeneXpert assay of BWF could provide a faster and more sensitive detection of MTB when compared with conventional bacteriological tests, especially for sputum smear-negative patients or patients who were negative for bacteriological testing with BWF. The GeneXpert assay was an appropriate complementary tool to use with conventional culture methods, which could facilitate the early diagnosis and treatment of TBTB.

Author contributions

Conception and design: Yan-Hua Song, Qi Li, Meng-Qiu Gao; administrative support: Qi Li, Meng-Qiu Gao; provision of study materials or patients: Yan-Hua Song, Qiang Li, Li-Ping Ma, Rong-Mei Liu, Guang-Lu Jiang; collection and assembly of data: Yan-Hua Song, Qiang Li, Li-Ping Ma, Rong-Mei Liu, Guang-Lu Jiang; data analysis and interpretation: Yan-Hua Song, Qi Li, Meng-Qiu Gao; manuscript writing: all authors; final approval of manuscript: all authors.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ORCID iD

Meng-Qiu Gao https://orcid.org/0000-0001-5899-1845

References

1. World Health Organization. Global tuberculosis report 2019. Geneva. Switzerland. (https: //www.who.int/tb/publications/global_report/en/.).
2. Chinese Medical Association for tuberculosis. Guidelines on the Diagnosis and Treatment of Tracheobronchial Tuberculosis (Trial). *Chinese Journal of Tuberculosis and Respiratory Diseases* 2012; 35: 581–587.

3. Shahzad T and Irfan M. Endobronchial tuberculosis – a review. *J Thorac Dis* 2016; 8: 3797–3802.
4. Aneja A, Krishnaswamy UM, Thyagaraj V, et al. Endobronchial tuberculosis: two case reports and review of the literature. *Case Rep Pulmonol* 2014; 2014: 283972.
5. Argun Baris S, Onyilmaz T, Basyigit I, et al. Endobronchial Tuberculosis Mimicking Asthma. *Tuberc Res Treat* 2015; 2015: 781842.
6. Jung SS, Park HS, Kim JO, et al. Incidence and clinical predictors of endobronchial tuberculosis in patients with pulmonary tuberculosis. *Respirology* 2015; 20: 488–495.
7. Şimşek A, Yapiciİ, Babalik M, et al. Bronchoscopic diagnostic procedures and microbiological examinations in proving endobronchial tuberculosis. *J Bras Pneumol* 2016; 42: 191–195.
8. Yu W and Rong Z. Clinical analysis of 90 cases with endobronchial tuberculosis. *Zhonghua Jie He He Hu Xi Za Zhi*1999; 22: 396–398 [Article in Chinese, English abstract].
9. Ozkaya S, Bilgin S, Findik S, et al. Endobronchial tuberculosis: histopathological subsets and microbiological results. *Multidiscip Respir Med* 2012; 7: 34.
10. Hou G, Zhang T, Kang DH, et al. Efficacy of real-time polymerase chain reaction for rapid diagnosis of endobronchial tuberculosis. *Int J Infect Dis* 2014; 27: 13–17.
11. World Health Organization. *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update*. Geneva, Switzerland: WHO/HTM/TB/, 2013 (https://apps.who.int/iris/handle/10665/112472).
12. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010; 363: 1005–1015.
13. Marlowe EM, Novak-Weekley SM, Cumpio J, et al. Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of Mycobacterium tuberculosis complex in respiratory specimens. *J Clin Microbiol* 2011; 49: 1621–1623.
14. Ko Y, Lee HK, Lee YS, et al. Accuracy of Xpert(®) MTB/RIF assay compared with AdvanSure^{TM} TB/NTM real-time PCR using bronchoscopy specimens. *Int J Tuberc Lung Dis* 2016; 20: 115–120.
15. Park KS, Kim JY, Lee JW, et al. Comparison of the Xpert MTB/RIF and Cobas TaqMan MTB assays for detection of Mycobacterium tuberculosis in respiratory specimens. *J Clin Microbiol* 2013; 51: 3225–3227.
16. Stevens WS, Scott L, Noble L, et al. Impact of the GeneXpert MTB/RIF technology on tuberculosis control. *Microbiol Spectr*2017; 5.
17. Yoon C, Cattamanchi A, Davis JL, et al. Impact of Xpert MTB/RIF testing on tuberculosis management and outcomes in hospitalized patients in Uganda. *PLoS One* 2012; 7: e48599.
18. Creswell J, Rai B, Wali R, et al. Introducing new tuberculosis diagnostics: the impact of Xpert(®) MTB/RIF testing on case notifications in Nepal. *Int J Tuberc Lung Dis* 2015; 19: 545–551.
19. Xia H, Song YY, Zhao B, et al. Multicentre evaluation of Ziehl-Neelsen and light-emitting diode fluorescence microscopy in China. *Int J Tuberc Lung Dis* 2013; 17: 107–112.
20. Fundamental Professional Committee of Chinese Antituberculosis Association. *Laboratory test protocol for the diagnosis of tuberculosis*. Beijing, China. 2006.
21. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol* 2010; 48: 229–237.
22. Lawn SD and Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiol* 2011; 6: 1067–1082.
23. Gao CJ and Zhu SY. Diagnostic value of Xpert MTB/RIF assay in bronchial washing fluid for smear-negative pulmonary tuberculosis. *J Clin Pulm Med*2016; 21: 2192–2196.
24. Guo X, Wang C, Wang X, et al. Characteristics and risk factor analysis of 410 cases of tracheobronchial tuberculosis. *Exp Ther Med* 2014; 8: 781–784.