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ORIGINAL RESEARCH ARTICLE

Diagnostic accuracy of different bronchoscopic specimens in sputum Xpert MBT/RIF- negative pulmonary TB patients

Omaima Ibrahim Badr,[1,2] Wael Aly Elrefaey,[2] Mohammed Shabrawishi,[2] Hamza M. Assaggaf,[3] Faisal Minshawi[3]

[1]Department of Chest Medicine, Mansoura University, Faculty of Medicine, Mansoura, Egypt
[2]Department of Pulmonary Medicine, Al-Noor Specialist Hospital, Mecca, Saudi Arabia
[3]Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Umm Al-Qura University, Mecca, Saudi Arabia

The study was conducted at the Al-Noor specialist tertiary hospital in Mecca, Saudi Arabia.

ABSTRACT

Background: Tuberculosis (TB) control remains a critical public health problem worldwide. Rapid diagnosis and proper treatment are beneficial for the effective control of tuberculosis transmission. Diagnostic challenges arise when a patient has a clinical and radiological suspicion of tuberculosis but cannot produce sputum, sputum acid-fast bacilli, or Xpert *Mycobacterium tuberculosis*/rifampicin (Xpert MTB/RIF) is negative, resulting in suboptimal management. As a result, more invasive techniques must be used on these patients to establish the diagnosis.

Methods: A retrospective study recruited 330 suspected pulmonary TB patients with negative sputum of Xpert MBT/RIF who underwent bronchoscopy between March 2018 and December 2021. The diagnostic yields of bronchoalveolar lavage fluid (acid-fast bacilli, Xpert MTB/RIF, and culture) and histopathologic examination (HPE) were calculated and compared to the final diagnosis and culture as a gold standard.

Results: Out of 330 suspected pulmonary TB patients, 181 had a final diagnosis of TB, and 149 had non-TB. The sensitivity of BALF (culture, Xpert, acid-fast bacilli) and trans-bronchial lung biopsy (HPE) was 80.7%, 72.9%, 21.1%, and 87.1%, respectively. Multiple nodules were associated with significantly higher BALF Xpert MTB, bronchoalveolar lavage fluid culture, and trans-bronchial lung biopsy (HPE) positivity.

Conclusions: Bronchoscopic specimens are essential for accurate and rapid diagnosis of sputum Xpert MBT/RIF negative patients with high clinical and radiological suspicion of tuberculosis.

Key words: Tuberculosis; bronchoscopy; bronchoalveolar lavage; Xpert MBT/RIF; trans-bronchial lung biopsy.

Correspondence: Omaima Ibrahim Badr, Department of Pulmonary Medicine, Al-Noor Specialist Hospital, 3rd Ring Rd, An Naseem, Mecca 24241, Saudi Arabia. Tel. +966.552109100. E-mail: omaimaibrahim@mans.edu.eg

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Ethics Approval: The study was approved by Saudi Arabia's Ministry of Health's Institutional Ethics Committee No. H-02-K-076-1021-589.

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Introduction

Despite significant efforts to control tuberculosis (TB), the disease remains one of the most common infectious diseases worldwide. According to a World Health Organization (WHO) report, estimated 10 million new cases of tuberculosis and 1.6 million deaths from tuberculosis occurred in 2018 [1]. According to the WHO, Saudi Arabia has a tuberculosis incidence rate of 12 (10-14) per 100,000 people, making the country a moderately burdened country for tuberculosis infection [2]. The Mecca region, on the other hand, has roughly twice the national TB incidence rate (24/100,000). This is most likely due to the region's annual influx of millions of Hajj and Umrah pilgrims [3,4].

Globally, tuberculosis control remains a critical public health issue, particularly in light of the emergence of drug-resistant disease. For effective TB transmission management, prompt diagnosis and treatment are essential [5].

The most commonly used test for TB detection is microscopic smear examination using Ziehl-Nelson (ZN) staining techniques. However, it is not efficient to identify TB in most patients. The sensitivity of sputum smear microscopy by the ZN method is low, ranging from 20% to 80%, and is dependent on the type of specimens collected, as well as the experience of preparing, staining, and examining the smear [6]. As a result, many TB patients are smear-negative and can spread the infection [7]. Smear-negative TB cases are typically 20-25% more contagious than smear-positive cases, and they account for 13-20% of transmissions [5-7]. However, after incorporating molecular (DNA) fingerprint clustering to conventional studies, Alsadi *et al*. found that the actual contribution of smear negative index case patients to tuberculosis transmission is 50% less than previously assumed. [8]. The gold standard for TB diagnosis and resistance is positive culture-based drug susceptibility testing [9]. Still, it is time-consuming (6-8 weeks), which has a detrimental influence on the spread of drug-resistant tuberculosis in the community and might result in increased mortality and morbidity [10]. Therefore, in 2011 the WHO recommended Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) as a quick, automated, cartridge-based molecular test for the early detection of probable tuberculosis cases. In less than 2 hours, the test detects *Mycobacterium tuberculosis* DNA and the primary mutations that induce rifampicin resistance [11]. Consequently, the WHO recommended the next-generation cartridge, Xpert MTB/RIF Ultra (Xpert Ultra), in March 2017. It is anticipated to improve the diagnosis of TB and rifampicin resistance [12]. Also, there have been some recent publications on the use of real time PCR, droplet digital PCR technology and Xpert MTB/MDR/XDR to identify MTB pathogens and drug resistance [13]. Therefore, WHO recognized the Xpert MTB/RIF assay in January 2020 [14]. It has demonstrated high diagnostic accuracy and improved patient outcomes when used as an initial test for identifying tuberculosis and rifampicin-resistant infections [5]. Patients with clinical, microbiological, and/or radiographic evidence of pulmonary tuberculosis are deemed to have active tuberculosis [15]. If the sputum acid-fast bacillus (AFB) and molecular test results are all negative, abnormal imaging results indicating pulmonary tuberculosis may be critical. In addition, computed tomography (CT) findings associated with sputum negative Xpert MTB/RIF might be helpful in TB diagnosis. A previous study evaluated CT results of pulmonary TB between sputum Xpert MTB/RIF negative and positive patients and found that chest CT finding interpretation could help with early identification and management of negative Xpert MTB/RIF- pulmonary TB [5].

Diagnostic challenges arise when a patient has a clinical and radiological suspicion of tuberculosis but cannot produce sputum, or has sputum (AFB and/or Xpert MTB/RIF) negative smears, resulting in suboptimal management. As a result, other non-invasive methods for detecting AFB in gastric wash or stool can be used. However, if sputum AFB and Xpert MTB/RIF smears are negative, more invasive techniques must be used to establish the diagnosis. For example, respiratory samples can be collected using a fibreoptic bronchoscope. Bronchoalveolar lavage (BAL), bronchial washing, and transbronchial lung biopsy (TBLB) are the three main used bronchoscopy-based procedures to obtain appropriate specimens [16].

There is little information and research on the role of bronchoscopy-based procedures in diagnosing sputum gene Xpert MBT/RIF negative probable TB patients. The purpose of this study is to evaluate the yield of bronchoscopy samples, such as bronchoalveolar lavage fluid (BALF) for AFB, Xpert MTB/RIF and culture, and transbronchial biopsies (histopathology examination, HPE) for diagnosis of TB patients with sputum Xpert MBT/RIF negative smear.

Methods

Study design

This is a single-centre, retrospective study. Between March 2018 and December 2021, we collected data for suspected pulmonary tuberculosis patients who were admitted to Al-Noor Specialist Hospital in Mecca with negative sputum AFB and Xpert MBT/RIF smears and underwent fibreoptic bronchoscopy to confirm the diagnosis. Al-Noor Specialist Hospital in Mecca is a specialist and teaching hospital with a 500-bed capacity located in the centre of holy city of Mecca. It is part of the Ministry of Health's services and provides tertiary care throughout Saudi Arabia's Mecca region [17,18]. The study was approved by Saudi Arabia's Ministry of Health's Institutional Ethics Committee (No. H-02-K-076-1021-589).

Study population

Three hundred and forty-seven patients (347) with suspected pulmonary tuberculosis were referred to the Bronchoscopy Unit from Inpatient Departments or Outpatient Clinics with three consecutive sputum AFB and Xpert MBT/RIF negative smears. The patients had at least two symptoms of TB and radiologic findings compatible with tuberculosis on their chest radiograph (CXR) or CT chest. In addition, an experienced chest physician clinically evaluated patients. Three hundred thirty patients were included. Patients under the age of 15, who had confirmed non-tuberculous mycobacteria results or who had started empirical anti-TB drugs for ≥2 weeks before bronchoscopy were excluded from this study.

The data were obtained from the medical database of the hospital including patients' demographics (age, gender, comorbidities, and previous TB history), clinical symptoms (fever, cough for at least weeks, haemoptysis, chest pain, shortness of breath, anorexia, weight loss, and night sweats), radiological finding (CXR and/or CT chest), laboratory results (BALF AFB, BALF Xpert MTB/RIF, and BALF Mycobacterial culture) and histopathology results of the biopsy samples (Figure 1).

Specimen collection

An Olympus electronic bronchoscopy system (1T260, Tokyo, Japan) was used for all patients under conscious sedation using midazolam/fentanyl. The clinical investigators performed the bronchoscope after giving each patient a brief explanation of the procedure and obtaining written consent. The bronchoscope was passed through the nose or mouth into the airway, followed by installing

sterile saline (0.9%) aliquots into the airway of the affected lung segment determined by CXR or CT chest. The volume of the saline was about 60-120 ml. The BALF was collected in sterile collection traps connected to a vacuum suction device. BALF samples were examined for gram stain, cultures (bacterial and fungal), cytology, and cell differential to exclude other alternative diagnoses. A trans-bronchial biopsy was obtained if technically possible and suitable for the clinical investigations of that patient. The number of biopsies was usually 4-6 biopsies using alligator forceps.

AFB smear microscopy

BALF smears were stained with fast Auramine O fluorescent dye for microscopy detection of AFB [19].

Mycobacterial TB culture and drug sensitivity

The BACTEC MGIT 960 rapid system (Becton Dickinson, Oxford, UK) was used to cultivate MTB from BALF specimens. The drug sensitivity was routinely evaluated using the BACTEC MGIT 960 SIRE kit (Becton Dickinson) if positive MTB culture. The specimens were tested for resistance to the first-line anti-TB medications, including RIF, isoniazid, ethambutol, and pyrazinamide [20].

Xpert MTB/RIF

According to the manufacturer's recommendations, the Gene Xpert device (Cepheid) was used to identify MTB in BALF smears and mutations that detect rifampicin resistance using separate primers and different molecular probes that attack the 81-bp core region of the Mycobacterial RNA polymerase (rpoB) gene [21].

Trans-bronchial lung biopsy

TBLB specimens were sent for histological examination. Histopathology was considered matched with TB if caseating or necrotizing granulomas were detected.

Case definition for final diagnosis of TB

Pulmonary tuberculosis cases are either: bacteriologically confirmed (by positive specimen for *M. tuberculosis* by Ziehl-Nelson staining, Xpert MTB/RIF, or culture) or clinically diagnosed (without bacteriologic confirmation for MTB) built on clinical and radiologic suspicion after excluding alternative diagnoses with clinical and radiological response to empirical anti-TB therapy (ATT). Patients were evaluated every two months for six months to assess their response to ATT. In addition, all sputum AFB and Xpert MBT/RIF negative cases with suspected pulmonary tuberculosis (clinical and radiological) underwent fibreoptic bronchoscopy to confirm the diagnosis.

Statistical analysis

Demographic and clinical data were expressed as the frequency with percentage and mean±SD. The sensitivity, specificity, pos-

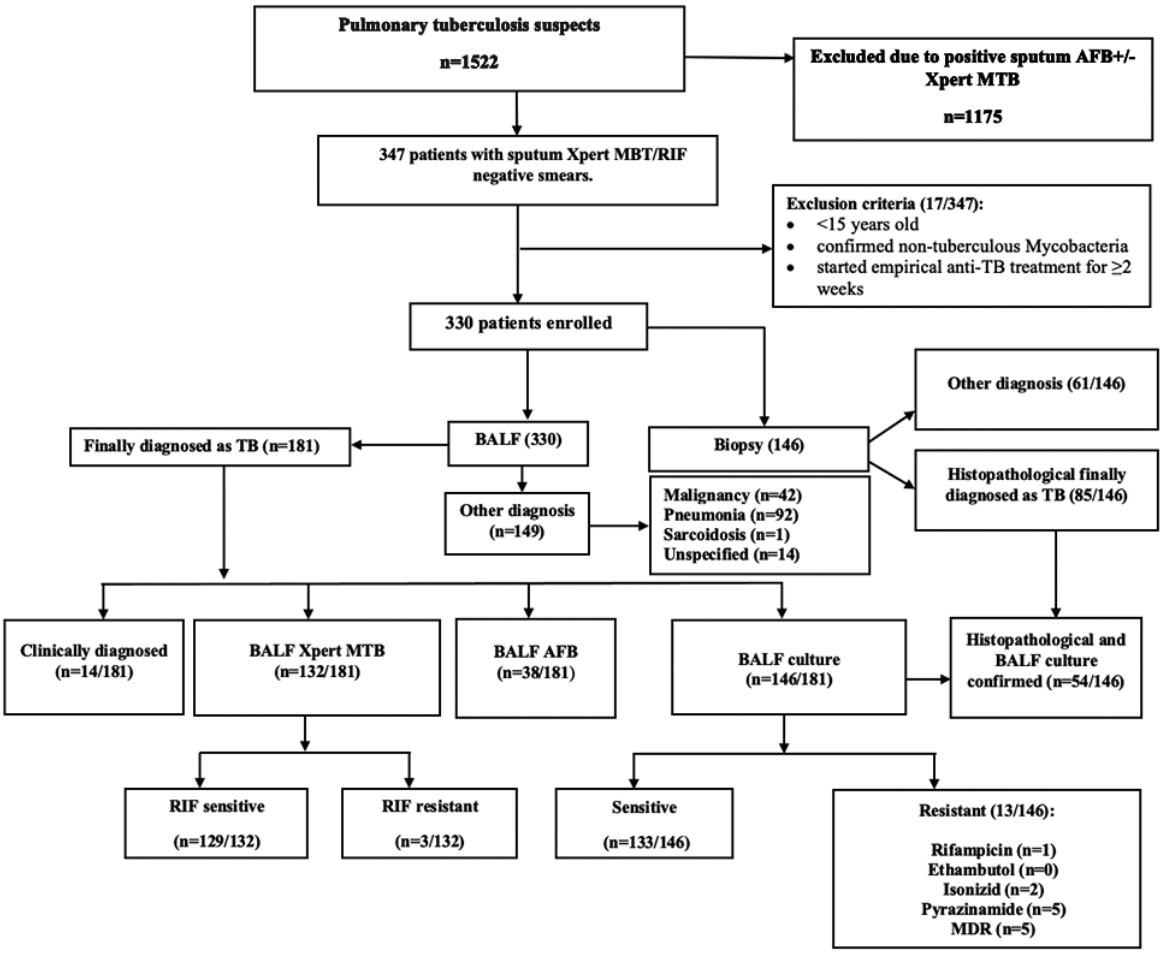

Figure 1. Flow chart of the studied patients.

itive predictive values (PPV), and negative predictive values (NPV) for BALF culture's diagnostic methods and performance were calculated for BALF AFB, Xpert MTB/RIF, and TBLB HPE and compared with chi-square. The IBM (SPSS v. 25 SPSS Inc. Chicago, IL, USA) software was utilized for the statistical analysis. A $p \leq 0.05$ was considered statistically significant.

Results

Clinical characteristics of patients with sputum Xpert MBT/RIF negative smears

Three hundred and thirty patients (330) were enrolled in this study. There were 229 males and 101 females, with a mean age of 51±18 years; 39.7% were smokers. The most common symptoms were cough 233 (70.6%) and fever 193 (58.5%). The distribution of other symptoms is summarized in Table 1. On the other hand, 49 (14.8%) patients had a previous history of TB, and 9 (2.7%) patients were HIV positive. The most common chest x-ray (CXR) finding was consolidation in 182 (55%) patients, while normal CXR was observed in 27 (8.2%). Consolidation was also the most common computed tomography (CT) chest finding in 141 (42%) patients, while mediastinal lymphadenopathy was the least common finding in 2 (0.6%) patients (Table 1).

Diagnostic efficacy of the BALF specimens in patients with sputum Xpert MBT/RIF negative smears

When culture was used as the gold standard, BALF Xpert MBT/RIF showed 89.7% sensitivity and 99.5% specificity. On the other hand, BALF AFB showed 26% sensitivity and 100% specificity. When the final diagnosis was used as the reference standard, BALF culture showed 80.7% sensitivity and 100% specificity. BALF Xpert MBT/RIF showed 72.9% sensitivity and 100% specificity. BALF AFB showed 21.1% sensitivity, 100% specificity (χ^2 = test 35.3, $p \leq 0.05$) (Table 2). The AFB and Xpert MTB/RIF assays in BALF were consistent with MTB culture in BALF. To analyse this indication of how the BALF AFB and BAFL Xpert MTB correspond with BALF Culture, we assessed the degrees of reliability. Our result showed the level of agreement of BALF AFB is 28%, and BAFL Xpert MTB/RIF is 91%. Overall, these results revealed that the BALF MTB culture and the Xpert MTB/RIF showed high reliability for TB patients' diagnostic performance (Table 3).

The diagnostic efficacy of TBLB HPE specimens in patients with sputum Xpert MBT/RIF negative smears

One hundred and forty-six (146) patients out of 330 underwent TBLB. We investigated the sensitivity and specificity of TBLB specimens to diagnose TB patients compared with culture as the gold standard and final diagnosis. TBLB HPE showed 84.4% sensitivity and 75.6% specificity compared with bacterial isolation from bronchial lavage. On the other hand, when TBLB is compared with the final diagnosis, the sensitivity and specificity increase (87.1% and 100%, respectively) (Table 4). Moreover, our data showed that the level of agreement between TBLB and BALF culture was 59% (Table 5).

Table 1. Baseline characteristics of the patients (n=330).

Variables	N	%
Demographics		
Age	51±18.4	
BMI	25.4±4.3	
Sex (male)	229	69.4
Smoking (yes)	131	39.7
Old tuberculosis history	49	14.8
Signs and symptoms		
Fever (yes)	193	58.5
Coughing (yes)	233	70.6
Haemoptysis (yes)	101	30.6
Loss of weight (yes)	155	46.9
Night sweating (yes)	100	30.3
Chest pain (yes)	67	20.3
Dyspnoea (yes)	80	24.2
Comorbidities		
Diabetes (yes)	61	18.5
Chronic pulmonary disease (yes)	16	4.8
HIV (yes)	9	2.7
CRF	14	4.2
CXR finding		
Consolidation	182	55.2
Cavitary lesions	49	14.8
Nodular	46	13.9
Effusion	26	7.9
Normal	27	8.2
CT finding		
Consolidation	141	42.7
Centrilobular nodules / tree in bud	67	20.3
Cavitary lesions	39	11.8
Multiple nodules	56	17
Pleural effusion	10	3
Mediastinal lymphadenopathy	2	0.6
Lobar collapse	15	4.5

BMI, body mass index; HIV, human immunodeficiency virus; CRF, chronic renal failure; CXR, chest X-ray; CT, computed tomography.

Table 2. Diagnostic efficacy of the BALF specimens in patients with sputum Xpert MBT/RIF negative smears.

	Sensitivity* % (n) 95% CI	Specificity % (n) 95% CI	PPV % (n) 95% CI	NPV* % (n) 95% CI	χ^2	p
Compared to culture						
BALF AFB	26 (38/146)	100 (184/184)	100 (38/38)	63 (184/292)	54.14	<0.001*
BALF Xpert MTB	89.7 (131/146)	99.5 (183/184)	99.2 (131/132)	92.4 (183/198)	270.7	<0.001*
Compared to final diagnosis						
BALF culture	80.7 (146/181)	100 (149/149)	100 (146/146)	81 (149/184)	215	<0.001*
BALF AFB	21.1 (38/181)	100 (149/149)	100 (38/38)	51 (149/292)	35.3	<0.001*
BALF Xpert MTB	72.9 (132/181)	100 (149/149)	100 (132/132)	75.3 (149/198)	181.1	<0.001*

BALF, bronchoalveolar lavage fluid; AFB, acid-fast bacilli; Xpert MTB, mycobacterial tuberculosis; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; *p≤0.05.

The relationships between various bronchoscopic specimens and radiological findings

Among 38 patients with positive BALF AFB, of whom 42.1% were diagnosed radiologically as consolidation, 28.9% were diagnosed as centrilobular nodules/tree-in-bud and cavitary lesions. However, none of the patients with positive BALF-AFB were diagnosed as multiple nodules, pleural effusion/mediastinal lymph node, or lobar collapse, with a statistically significant difference (p<0.001). Regarding BALF Xpert MTB: among 132 patients with positive BALF Xpert MTB, 38.6% were diagnosed radiologically as consolidation, 32.6% were diagnosed as centrilobular nodules/tree-in-bud, 18.9% as cavitary lesions, and 5.3% as multiple nodules, with a statistically significant difference (p<0.001). Regarding BALF culture: Among 146 patients with positive BALF culture, 39.0 % were diagnosed radiologically as consolidation, 32.9% were diagnosed as centrilobular nodules/tree-in-bud, 17.8% as cavitary lesions, and 5.5% as multiple nodules, with a statistically significant difference (p<0.001). Regarding TBLB: among 74 patients diagnosed with tuberculosis by TBLB, 29.7 % were diagnosed radiologically as consolidation, 45.9% were diagnosed as centrilobular nodules/tree-in-bud, 10.8% as cavitary lesions, and 9.5% as multiple nodules with a statistically significant difference (p<0.001) (Table 6).

Table 3. Diagnostic consistency of the BALF specimens compared to the culture as the reference in patients with sputum Xpert MBT/RIF negative smears.

		BALF culture				
		Positive	Negative	Total	Kappa	p
BALF AFB	Positive	38	0	38	0.28	<0.001*
	Negative	108	184	292		
	Total	146	184	330		
		Positive	Negative	Total	Kappa	p
BAFL Xpert MTB	Positive	131	1	132	0.91	<0.001*
	Negative	15	183	198		
	Total	146	184	330		

BALF, bronchoalveolar lavage fluid; AFB, acid-fast bacilli; Xpert MTB, mycobacterial tuberculosis; RIF, rifampin; *p≤0.05.

Table 4. The diagnostic efficacy of TBLB specimens in patients with sputum Xpert MBT/RIF negative smears.

	Sensitivity* % (n) 95% CI	Specificity % (n) 95% CI	PPV % (n) 95% CI	NPV* % (n) 95% CI	χ^2	p
Compared to culture TBLB (HPE)	84.4 (54/64)	75.6 (62/82)	73 (54/74)	86.1 (62/72)	51.8	<0.05*
Compared to final diagnosis TBLB (HPE)	87.1 (74/85)	100 (61/61)	100 (74/74)	84.7 (61/72)	107.7	<0.001*

TBLB, trans-bronchial lung biopsy; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 5. Diagnostic efficacy of the TBLB specimens compared to the culture as a reference standard in patients with sputum Xpert MBT/RIF negative smears.

		BALF culture				
		Positive	Negative	Total	Kappa	p
TBLB (HPE)	Positive	54	20	74	0.59	<0.001*
	Negative	10	62	72		
	Total	64	82	146		

TBLB, trans-bronchial lung biopsy; HPE, histopathological examination; *p≤0.05.

Table 6. Relation between various bronchoscopic specimens and radiological finding.

Radiological findings	BALF AFB Positive (n=38)	p	BALF Xpert MTB Positive (n=132)	p	BALF culture Positive (n=146)	p	TBLB (HPE) Tuberculosis (n=74)	p
Consolidation	16 (42.1%)		51 (38.6%)		57 (39.0%)		22 (29.7%)	
Centrilobular nodules / tree-in-bud	11 (28.9%)		43 (32.6%)		48 (32.9%)		34 (45.9%)	
Cavitary lesions	11 (28.9%)		25 (18.9%)		26 (17.8%)		8 (10.8%)	
Multiple nodules	0 (0%)		7 (5.3%)		8(5.5%)		7 (9.5%)	
Pleural effusion/mediastinal lymph node	0 (0%)		2 (1.5%)		3 (2.1%)		0 (0%)	
Lobar collapse	0 (0%)	<0.001	4 (3.0%)	<0.001	4 (2.7%)	<0.001	3 (4.1%)	<0.001

BALF, bronchoalveolar lavage fluid; AFB, acid-fast bacilli; Xpert MTB, mycobacterial tuberculosis; TBLB, trans-bronchial lung biopsy; HPE, histopathological examination.