

Pathological and DNA-based Detection of Tracheobronchial Tuberculosis in China

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ABSTRACT

Objectives: To compare the DNA-based sputum evaluation with histopathology for the diagnosis of tracheo-bronchial tuberculosis (TBTB).

Study Design: Case series.

Place and Duration of Study: Tianjin Haihe Hospital, Tianjin Institute of Respiratory Diseases, Tianjin, China, from January to June 2017.

Methodology: TBTB patients, who underwent bronchoscopy during the study period, were included. Their bronchoscopy presentations, histopathology, and induced sputum deoxyribonucleic acid (DNA) and culture results were analysed. Results were expressed as frequency percentage.

Results: There were 110 subjects. Most patients were young females with coughing. The main TBTB subtype was fibrostenotic, while the most common findings were single level lesions and lobar bronchi. The diagnostic rates were 40.43%, 67.86%, and 68.54% for DNA-based analysis of induced sputum, histopathology, and induced sputum culture, respectively. Only in the edematous-hyperemic subtype was the positivity rate of DNA-based analysis of induced sputum higher than in histopathology. In the fibrostenotic subtype, the histopathologic results were superior for diagnosis.

Conclusion: A combination of induced sputum and biopsy using DNA-based methods is a superior and exact method to diagnose TBTB. DNA-based analysis of induced sputum for *Mycobacterium tuberculosis* can be used for preliminary impressions.

Key Words: Tracheo-bronchial tuberculosis, *Mycobacterium tuberculosis*, Pathology, Multiplex polymerase chain reaction, Sputum.

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INTRODUCTION

Tracheo-bronchial tuberculosis (TBTB) is a type of tuberculosis in which *Mycobacterium tuberculosis* (MTB) destroys the mucosa, submucosa, cartilage, smooth muscle, and outer membranes of the trachea and bronchi.¹ Due to its non-specific manifestations, it may be clinically misdiagnosed as asthma, bronchiectasis, or lung cancer. In terms of histopathology, TBTB can be differentiated from sarcoidosis, Wegener's granulomatosis, or other infectious diseases.²

TBTB is estimated to occur in 10-50% of patients with active pulmonary tuberculosis (PTB). Recently, its incidence has been increasing.³ TBTB has many complications, such as tracheo-bronchial stenosis.⁴ Furthermore, its transmissibility is high, underscoring the importance of early diagnosis.

Bronchoscopy is the most useful method in diagnosing TBTB. Presently, bronchoscopic manifestation with histologic features or bacterial findings are considered sufficient to diagnose TBTB. Unfortunately, bronchoscopy is an invasive method that some patients cannot undergo. By contrast, the molecular evaluation of induced sputum (IS) is non-invasive and relatively convenient. However, the molecular testing of IS for TBTB has not been validated, especially in the Chinese population.

In this study, the aim was to compare DNA-based sputum evaluation with histopathology for the diagnosis of TBTB.

METHODOLOGY

This was a study of patients with newly diagnosed TBTB in Tianjin Haihe Hospital, Tianjin, China, from January to June, 2017. The inclusion criteria were administration of TBTB in this period. The

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exclusion criteria were patients who could not undergo bronchoscopies. TBTB was defined as the presence of a visible classic endo-bronchial lesion⁵ (bronchoscopic evidence) as well as meeting one of the following criteria: positive acid-fast stain (AFB) smears from bronchial washing or brushing fluid; pathologic results from the biopsy; positive AFB smear or culture from the sputum; or positive MTB DNA from sputum. TBTB was classified into seven subtypes, based on criteria from Chung *et al.* At the time of diagnosis, medical histories, high-resolution computed tomography (HRCT) scans, and IS examinations were performed. This study was approved by Tianjin Haihe Hospital Institutional Review Board (No. 201814).

IS was performed by a trained nurse. After inhaling a 5% hypertonic saline for 15 min, patients were encouraged to cough in order to induce sputum.⁶ Multiplex polymerase chain reaction (MPCR) was used for some patients with suspected TBTB. MPCR was performed by using specific primers to amplify IS6110 segment and MPB64 gene, which respectively had amplicon sizes of 123 and 240 bp.⁷

Statistical analyses were performed using Microsoft Excel (version 2010) software. Categorical variables were described as frequencies and percentages, while quantitative variables were expressed as the mean \pm standard deviation (SD).

RESULTS

The mean age at diagnosis was 41.17 ± 18.44 years. The subjects consisted of 75 women and 35 men. The main symptom was cough; few patients were asymptomatic.

Table I: Clinical characteristics of 110 patients with TBTB examined by bronchoscopy.

Characteristics	n (%)
Age (years), Mean \pm S.D	41.17 \pm 18.44
Incidence by gender	
Females	75 (68.18)
Males	35 (31.82)
Main symptoms	
Cough	95 (86.36)
Sputum	65 (59.09)
Fever	41 (37.27)
Dyspnea	19 (17.27)
Hemoptysis	5 (4.55)
Typical tuberculosis toxic symptoms	4 (3.64)
Asymptomatic	4 (3.64)
Comorbid disease	21 (19.09)
Symptom duration (Mean \pm S.D, weeks)	8.17 \pm 15.64
Presence of PTB	109 (99.09)
HRCT findings	
Atelectasis	12 (10.91)
No atelectasis	98 (89.09)
Cavitary disease	25 (22.73)
No cavitary disease	85 (77.27)

Only one patient had no evidence of PTB. According to the HRCT scans, 10.91% of patients had atelectasis, while 22.73% had cavities (Table I).

Most patients had lesions in the lobar bronchi, although 15 had lesions in the trachea, 36 in the main bronchi, and 54 in the segmental bronchi. Most patients had single level TBTB.

The positivity rates of IS culture, biopsy, and IS DNA were, 68.54%, 67.86%, and 40.43%, respectively (Table II). Biopsy positivity rates were relatively high in the actively caseating, fibrostenotic, tumorous, ulcerative, and granular subtypes. Sputum DNA positivity rates were relatively high in the tumorous and granular subtypes. Finally, IS culture positivity rates were relatively high in all subtypes except for granular.

DISCUSSION

TBTB is a subtype of pulmonary tuberculosis. It is easily misdiagnosed in the clinic due to its poorly understood pathogenesis and non-specific clinical features. Such diagnostic delays increase the possibility of poor outcomes. Most patients with TBTB develop tracheobronchial stenosis,⁸ so it is vital to diagnose TBTB as early as possible to prevent further deterioration and MTB transmission.

As the present data illustrated, the majority of TBTB patients were young women. This gender imbalance may be due to their unique thinner bronchial structures as well as societal customs. Although the average age at TBTB diagnosis in this study was 41.17 years, most patients are diagnosed between the ages of 20 to 40 years.⁹

The main symptom was cough, followed by expectoration, fever, and dyspnea. Diagnosis was often delayed because of the time taken to differentiate this infection from other respiratory diseases. In this study, symptom duration averaged eight weeks. Nearly 100% of the TBTB patients had active PTB, indicating that active PTB is an important predictor of TBTB occurrence. It remains unclear how to best reduce the onset of TBTB.

In this study, 110 patients underwent HRCT scanning, which detected 12 cases of atelectasis and 25 cases of cavity disease. These findings differed from other reports.¹⁰ A previous study demonstrated that active PTB with cavity lesions was a risk factor for TBTB; cavity lesions are a possible MTB infection pathway. Here, however, cavity disease was not dominant; accordingly, TBTB can occur in the early stages of PTB. Instead, the predominant TBTB subtype in this study was fibrostenotic. Notably, the subtype varies in prior reports, ranging from granular to edematous-hyperemic.⁹⁻¹¹

Bronchoscopy is a useful, albeit invasive, method to visualise lesions as well as to collect histologic and bacteriological evidence. Biopsy *via* bronchoscopy is a main method to diagnose TBTB, and the histopathologic results are considered reliable and highly sensitive. Bronchial biopsies are positive in 30-84% of patients. Even patients with negative sputum smears can be diagnosed with TB.⁹ Altin *et al.* reported that, in a group of 50 TBTB patients, 42 cases (84%) were diagnosed using pathology.¹² In the present study, the positivity rate was 67.86%, higher than the DNA analyses.

A common rapid diagnostic method, used worldwide and recommended by the World Health Organisation, is the microscopic analysis of sputum smears. However, the positivity rate of TBTB from sputum smears is low and variable.

Table II: TB positive rates of different subtypes of TBTB.

Subtype	Cases	Biopsy	Sputum DNA	Sputum Culture
Actively caseating	27	12/17(70.59)	2/7(28.57)	21/25(84)
Fibrostenotic	56	16/21(76.19)	11/25(44)	27/43(62.79)
Oedematous-hyperaemic	11	1/6(16.67)	3/8(37.5)	7/10(70)
Tumorous	8	3/6(50)	2/4(50)	4/6(66.67)
Ulcerative	3	2/2(100)	0/1(0)	1/1(100)
Granular	4	4/4(100)	1/2(50)	0/3(0)
Non-specific bronchitic	1	0/0(0)	0/0(0)	1/1(100)
Overall	110	38/56(67.86)	19/47(40.43)	61/89(68.54)

Its sensitivity is unsatisfactory due to limitations in specimen-acquisition methods; consequently, the false negative rate is artificially high. Improvements in the accuracy of molecular evaluation methods should be prioritised.

IS is becoming more widely used to obtain sputum specimens. It has been reported to improve the diagnosis of PTB in patients, especially in those with problems producing spontaneous sputum.^{13,14} Nonetheless, the research regarding IS in TBTB remains inadequate. In the data, the DNA IS positivity rate was lower than the biopsy positivity rate. This lower rate may have resulted from a low bacillary load. Moreover, not all patients received MPCR assays. Only in the edematous-hyperemic subtype was the IS DNA positivity rate higher than the biopsy's. This result differed from Hou's report,¹⁵ perhaps because the histopathologic features of the edematous-hyperemic subtype are not classical. Notably, the rate of histopathologic positivity is highly operator-dependent. By contrast, IS evaluation using DNA-based methods are easier to collect, and the results do not depend on the operator. In the fibrostenotic subtype, the histopathologic results had a higher positivity rate than the IS DNA. However, when bronchi stenosis is present, sputum specimens are difficult to assess.

This study has several limitations. Firstly, the number of patients was small. Secondly, the diagnostic criteria for TBTB was not particularly strict. Thirdly, infection only had to satisfy clinical diagnoses criteria so that the physicians could refer for these investigations. Hence, many cases might have been missed.

CONCLUSION

Considering all the data, the histopathologic results should be considered sufficient to diagnose TBTB. A combination of IS, biopsy, and bronchoalveolar lavage sample, using DNA-based methods, is the most ideal and accurate approach to diagnose TBTB.

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ETHICAL APPROVAL:

This study was conducted after the approval of Tianjin Haihe Hospital Institutional Review. Board (No. 201814).

PATIENTS' CONSENT:

Since it was designed as a retrospective study, the data were collected from the Hospital archive after approval of the Ethics Committee.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

XL: Concept, design, data analysis, manuscript editing, guarantor.

YX: Conception and design of study, data analysis, manuscript editing and review.

YW: Conception, data acquisition, manuscript preparation and editing.

JW: Design, data acquisition and analysis, manuscript review.

XS: Design, literature search, manuscript editing.

QW: Conception and design, manuscript editing and review, guarantor.

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