

Bronchial brushing Xpert improves the diagnostic efficiency of sputum Xpert in patients with pulmonary tuberculosis

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Abstract

Background: The role of bronchial brushing Xpert MTB/RIF (hereafter referred to as Xpert) in patients with pulmonary tuberculosis (PTB) remains unclear. Therefore, a retrospective study was conducted aiming to evaluate the diagnostic efficiency of bronchial brushing Xpert in patients with PTB.

Methods: Between July 2018 and August 2019, suspected PTB patients who were admitted to our hospital and had bronchial brushing and matched sputum collection for acid-fast bacilli (AFB) smear, mycobacterial culture, and Xpert were included for further analysis. Subsequently, PTB was defined based on mycobacterial culture, and if an alternative diagnosis was established, 'non-tuberculosis (TB)' was considered. Comparison of bronchial brushing and matched sputum examination was performed between groups. Then, the differences in the sensitivities between bronchial brushing and sputum Xpert were examined using the chi-square test.

Results: A total of 111 patients were included and divided into TB and non-TB groups (52 versus 59 patients). The sensitivities of Xpert against culture were calculated as follows: sputum, 44.2% (23/52); bronchial brushing, 59.6% (31/52); sputum and bronchial brushing, 69.2% (36/52). The specificities of all Xpert assays were the same (100.0%, 59/59). A significant difference was found in the comparison of the sensitivities of Xpert using sputum, bronchial brushing and both, and the sensitivity of Xpert on both sputum and bronchial brushing was higher than that on sputum alone ($p < 0.05$). Moreover, it appears that bronchial brushing Xpert was more sensitive than sputum Xpert in the detection of PTB.

Conclusion: Bronchial brushing Xpert improves the diagnostic efficiency of sputum Xpert in the detection of PTB.

Keywords: bronchial brushing, diagnosis, pulmonary tuberculosis, sensitivity, Xpert

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Introduction

Tuberculosis (TB) remains one of the most prevalent infectious diseases worldwide, which causes high morbidity and mortality. According to the Global Tuberculosis Report 2020, an estimated 10 million people fell ill with TB, and TB-related deaths were reported at 1.2 million in 2019.¹ Studies have shown that a successful TB control requires effective anti-TB treatment as well as proper and effective diagnosis.²

However, conventional microbiological methods, such as acid-fast bacilli (AFB) staining and culture, have their own limitations.

To identify TB, smear examination for AFB has a low sensitivity.³ Mycobacterial culture is considered as a gold standard method for TB detection. However, this assay is not recommended in resource limited countries because of a relatively high bio-safety requirements and unavailable

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resources (e.g. equipment).⁴ In addition, the culture usually requires several weeks, which is time-consuming and labor-intensive, resulting in a significant delayed diagnosis and therapy. The nucleic acid amplification test (NAAT) is considered more sensitive than routine microbiological tests and has a good specificity. Moreover, NAAT has a short turnaround time, mostly a few hours.

Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA, USA) is a World Health Organization (WHO)-recommended, rapid and automated NAAT that is used widely for simultaneous detection of *Mycobacterium tuberculosis* and rifampicin resistance.⁵ In a meta-analysis, the pooled sensitivities were calculated as follows: 98% for smear-positive, culture-positive TB and 68% for smear-negative, culture-positive TB.⁶ In addition, TB patients benefit from early detection and initiation of treatment, because Xpert usually takes 2 h and has a short turnaround time.⁷

Bronchial brushing is an increasingly used tool for the diagnosis of neoplastic and non-neoplastic diseases.⁸ It is a common practice and is often obtained during fiberoptic bronchoscopy. Bronchial brush is relatively safe and well tolerated in humans.⁹ To date, few studies have investigated the diagnostic efficiency of bronchial brushing Xpert in patients with pulmonary tuberculosis (PTB). Therefore, this retrospective study was conducted aiming to evaluate the diagnostic accuracy of Xpert using bronchial brushing and compare it with Xpert using sputum in PTB patients.

Materials and methods

This retrospective study was conducted at Shandong Provincial Chest Hospital (SPCH). SPCH is a tertiary referral TB hospital, with approximately 1000 beds. Half of the patients admitted to the center are there due to TB. The hospital is located at the eastern district of China, with a TB prevalence of 66 (52–84) per 100,000 population.¹⁰ This study was approved by the Ethics Committees of SPCH (no. 2020XKYYEC-29). Written informed consent was waived by the Ethics Committees of SPCH because of the de-identified data and retrospective nature of the study. The investigations were carried out in accordance with the Declaration of Helsinki.

Between July 2018 and August 2019, suspected PTB patients who were admitted to our hospital and had bronchial brushing and matched sputum collection for AFB smear, mycobacterial culture, and Xpert were included for further analysis. PTB was defined as microbiologically confirmed TB on the basis of positive cultures for *M. tuberculosis* (sputum or bronchial brushing). Patients were considered ‘non-TB’ if an alternative diagnosis (without TB) was established. Patient characteristics such as demographics and underlying diseases were obtained from electronic medical records.

Bronchial brushing *via* fiberoptic bronchoscopy (Olympus, Tokyo, Japan) and matched sputum were all examined by AFB smear (Auramine O stain), mycobacterial culture (Lowenstein–Jensen method) and Xpert. Xpert assay was performed following the manufacturer’s instructions. Briefly, the sample was mixed with the reagent and then incubated for about 10 min at room temperature. After that, the mixture was lodged into the cartridge, and the tests were performed automatically.

Patient characteristics were summarized using means and standard deviations. The differences in the sensitivities between bronchial brushing and sputum Xpert were examined using the chi-square test. Cohen’s kappa analysis between bronchial brushing and sputum Xpert was determined using the chi-square test. In addition, the positive predictive value (PPV) and negative predictive value (NPV) were also estimated. A *p* value < 0.05 was considered significant. Data analysis was carried out using SPSS 16.0 (IBM Corp., Armonk, United States).

Results

A total of 111 patients were enrolled in the study and subsequently divided into TB and non-TB groups (52 *versus* 59 patients). Men comprised 50.5% of the patients, and the mean age was 44.3 ± 18.0 years old (range 11–79 years old). Six (11.5%) were retreatment cases. The mean period between the initiation of active TB requiring retreatment and previous treatment completion was 1.9 years (range from 1 month to 7 years). One hundred and six patients were tested for HIV antibodies, and all of them were HIV negative. The baseline characteristics are shown in Table 1. Of those with non-TB diseases, 32 patients have

Table 1. The baseline characteristics of patients and the diagnostic accuracy of AFB smear and Xpert in PTB.

		TB group (n)	Non-TB group (n)
Number		52	59
Sex, male		22 (42.3%)	34 (57.6%)
Age, years		39.3 ± 17.0	48.8 ± 17.7
HIV status		0 (0%, 0/51)	0 (0%, 0/55)
Previous treatment	Initial treatment	46 (88.5%)	
	Retreatment	6 (11.5%)	
AFB	Sputum (+)	14 (26.9%)	0 (0%)
	Bronchial brushings (+)	15 (28.8%)	
Xpert	Sputum (+, %)	23 (44.2%)	0 (0%)
	Bronchial brushings (+)	31 (59.6%)	
	Sputum or bronchial brushings (+)	36 (69.2%)	

AFB, acid-fast smear; PTB, pulmonary tuberculosis.

community acquired pneumonia, 16 lung cancer, four lung fungal infection, two sarcoidosis, and four other diseases.

Fourteen patients (26.9%) were AFB sputum smear positive, and 15 (28.8%) were AFB brushing smear positive. The PTB patients were all culture positive. Then, the sensitivities of Xpert against culture were calculated as follows: (a) sputum, 44.2% (23/52); (b) bronchial brushing, 59.6% (31/52); (c) sputum and bronchial brushing, 69.2% (36/52). The specificities of all Xpert assays were the same (100.0%, 59/59). In addition, the sensitivities and specificities of Xpert against AFB smear were also calculated as follows: (a) sputum, 71.4% (10/14) and 86.6% (84/97); (b) bronchial brushing, 93.3% (14/15) and 82.3% (79/96). There was no discordant result in the rifampicin resistance between sputum and bronchial brushing Xpert, and 18 patients had concordant Xpert rifampicin resistance results (two showing rifampicin resistance and the other with susceptibility to this drug). Among the 52 PTB patients, 13 (25.0%) of them were sputum Xpert negative and bronchial brushing positive, five (9.6%) of them were sputum Xpert positive and bronchial brushing negative. Therefore, an incremental diagnostic value of bronchial brushing Xpert was found in 13 (25.0%) of 52 patients. PPV and NPV were also estimated for Xpert as follows: 100% (23/23) and 67.0% (59/88) for

bronchial brushing Xpert, and 100% (31/31) and 73.8% (59/80) for sputum Xpert.

Statistical analysis showed that: (a) the Xpert was superior to AFB smear in the detection of PTB in both of sputum and bronchial brushing (all $p < 0.05$); (b) a significant difference was found in the sensitivity of Xpert using sputum, bronchial brushing and both, and the sensitivity of Xpert on both sputum and bronchial brushing was higher than that on sputum alone ($p < 0.05$). Moreover, further analysis was performed and it was discovered that there was no significant difference in the sensitivity between sputum and bronchial brushing ($p > 0.05$). However, bronchial brushing Xpert has a high sensitivity of 59.6%, while sputum Xpert has a sensitivity of 44.2%. Cohen's kappa analysis was used to evaluate the agreement between bronchial brushing and sputum Xpert, and Cohen's kappa index was calculated as 0.563 ($p < 0.001$).

Discussion

Many studies have discovered that Xpert assay is a promising method for the diagnosis of PTB and extrapulmonary TB using several specimens, such as sputum, body fluids, tissues, and others.¹¹⁻¹⁴ However, very limited data exist on the Xpert assay using bronchial brushing for the diagnosis of PTB. Our findings show that the Xpert

assay has a high diagnostic yield in the combination of sputum and bronchial brushing, and 69.2% of TB cases were identified by Xpert using both sputum and bronchial brushing samples.

In the study, 26.9% of PTB patients were AFB sputum smear positive, and 28.8% were AFB brushing smear positive. Further analysis showed that the Xpert outperformed AFB smear and established the diagnosis in a significant proportion of patients who were smear negative. As we know, the earlier diagnosis was associated with improved TB treatment initiation among smear-negative presumptive TB patients. Therefore, Xpert could significantly improve patient-centered outcomes.¹⁵

In a recent meta-analysis, the diagnostic accuracy of Xpert was evaluated against culture, and it was reported that Xpert has a pooled sensitivity of 98% in smear-positive and 67% in smear-negative, culture-positive participants.¹⁶ The sensitivity of sputum Xpert identified in this study was lower than that reported in the meta-analysis. Besides the bacterial burden and the quality of samples, another possible explanation was that the positivity of Xpert was associated with the local level of TB burden.¹⁴ In addition, antibiotic abuse in China is also an important factor in decreasing bacterial burden in the corresponding specimens.¹⁷ In the study, most of the patients before diagnosis of TB had been given antibiotics.

Overall, the sensitivity of Xpert on sputum or bronchial brushing alone seemed to be lower than that of Xpert on both, and the sensitivity in brushing samples seemed to be higher than that in sputum samples for Xpert or AFB smear. Furthermore, Xpert using sputum plus bronchial brushing can improve the diagnostic sensitivity for *M. tuberculosis* detection in PTB. A similar result was reported by Zhang *et al.*,¹⁸ and it discovered that: (a) regarding the performance of smear, the brushing (32.8%) was superior to sputum (13.1%) for the detection of TB; (b) brushing Xpert had a sensitivity of 57.4% in detecting TB. Although this previous study evaluated the role of brushing Xpert in the detection of TB;¹⁸ however, comparison of brushing or bronchial tissues and sputum Xpert in the detection of TB has not been performed. Remarkably, other bronchoscopic samples, such as bronchoalveolar lavage fluid (BALF), have also been evaluated by

Xpert,^{19–21} and this special sample was recommended in these studies. In our study, BALFs were collected from very few patients, and a very low proportion of them have been examined by Xpert. Therefore, the comparison of brushing and BALF Xpert was not performed in the study.

There were no discordant results in the rifampicin resistance between sputum and bronchial brushing Xpert. However, caution should be exercised because of false-positive resistance detection and mixed infection. For example, Williamson *et al.*²² found that the Xpert test incorrectly detected rifampicin resistance in 31% of the evaluated cases; two isolates with different resistance profiles may exist in brushing and sputum, respectively.²³

This study has some limitations. First, the study was designed aiming to evaluate the diagnostic accuracy of the Xpert assay using sputum or bronchial brushing, not to detect drug resistance. Therefore, further studies are required to be conducted. Second, our study was a single-center study. However, we think the results may generalize to other districts with similar TB burden. Third, bronchial brushing should be performed based on the abnormal appearance on bronchoscopic observation or on radiological assessment, this may limit the use of this approach in some cases. Fourth, the patients investigated in this study were not grouped according to disease severity, which may influence the performance of the Xpert assay. Fifth, although bronchial brushing Xpert is useful for diagnosing PTB, the cost-effectiveness remains unclear. The strategy of the utility of bronchial brushing Xpert should be developed to ensure an adequate use. Finally, we suggest that future studies should focus on sample collection and processing methods to improve the efficiency of Xpert.

Compared with smear microscopy, Xpert offers better sensitivity for the diagnosis of PTB in both sputum and bronchial brushing. When bronchial brushing Xpert is applied, it improves the diagnostic efficiency of sputum Xpert in the detection of PTB. This would speed up the diagnosis of PTB, and a significant impact may be seen in patients' outcomes.

Author contribution statement

WMS and GHZ conceived the study. HY and WMS collected the data. HC performed the data

analysis. WYH contributed the revision. WMS wrote the manuscript and all authors have approved the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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