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The incremental value of bronchoalveolar lavage for the diagnosis of pulmonary tuberculosis in a high-burden urban setting

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SUMMARY

Objectives: We prospectively evaluated the use of bronchoalveolar lavage fluid (BALF) specimens to assess their added incremental value to pulmonary tuberculosis (TB) diagnostic strategies used currently in a high-burden urban setting in China.

Methods: A prospective study was conducted of patients with presumptive pulmonary TB registered at the Fifth Hospital of Suzhou between March 2018 and July 2018. BALF samples from patients with initial Xpert-negative sputum results were tested to diagnose TB.

Results: Of 440 participants, 316 (71.8%) were initially diagnosed with TB from sputum, including 245 (55.7%) definitive TB cases based on a positive culture and/or Xpert result(s) and 71 (16.1%) positive cases based on clinical diagnosis. Of 153 patients with initial positive cultures, a significantly higher proportion were confirmed as TB-positive using Xpert (94.1%) versus smear microscopy (45.8%, $P < 0.01$). Xpert testing of BALF from 182 Xpert-negative cases exhibited greater detection sensitivity (97.4%) than did smear microscopy (23.4%, $P < 0.01$). Meanwhile, 74.1% of TB patients initially diagnosed as TB-negative via smear microscopy were identified using Xpert testing of BALF at reduced diagnostic cost/patient (from USD 266.9 to 171.5).

Conclusions: BALF samples added incremental value to pulmonary TB diagnostic strategies for patients with Xpert-negative sputum. Xpert outperformed smear microscopy for tubercle bacilli detection in both sputum and BALF.

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB) complex, remains one of the most common and deadliest diseases worldwide, with an estimated global mortality rate of 1.6 million deaths in 2017.[1,2] Efforts to reduce mortality should focus on early diagnosis and immediate initiation of effective treatment in TB patients.[2] Despite recent advances, pulmonary TB is primarily diagnosed via identification of MTB using sputum smear microscopy. However, this simple method lacks both sensitivity and specificity that are urgently needed in high-incidence settings.[3,4] Mycobacterial culture, the gold standard diagnostic technique, yields high sensitivity for detecting MTB from clinical specimens.[3] Unfortunately, the lengthy procedure, due to the slow growth rate of MTB, cannot meet clinical needs for point-of-care diagnostics[5] and requires an extensive laboratory infrastructure that is lacking in resource-limited settings.[3]

Recently, the Xpert MTB/RIF (Xpert) assay (Cepheid, Sunnyvale, CA) has been developed as an integrated system incorporating a disposable cartridge that performs both specimen processing and molecular detection.[6,7] By targeting the *rpoB* gene sequence of MTB, Xpert detects both the presence of MTB and its susceptibility

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to rifampin within 2 h.[6] Several multicenter studies have demonstrated that this assay offers a level of detection sensitivity approaching that of the culture-based gold standard method.[6,8] As a consequence, Xpert is endorsed by the World Health Organization (WHO) for use in TB diagnosis, particularly for HIV-infected individuals and suspected MDR-TB cases.[9] Although the application of molecular diagnostics greatly accelerates the timely acquisition of results needed for patient management decision-making, a large number of pulmonary TB patients remain undiagnosed due to a lack of microbiological evidence.[1] According to a recent global TB epidemic annual report, nearly half of reported cases were diagnosed based only on patient symptoms and chest X-ray findings.[1] The situation is even worse in China, where laboratory results can confirm MTB organisms in only 30% of suspected TB cases, leaving no room for doubt that the current diagnostic algorithm based on non-specific clinical manifestations results in either over- or under-diagnosis of pulmonary TB.[10] Therefore, there is an urgent need to formulate a new diagnostic algorithm that adds incremental value to currently recommended TB diagnostic strategies.

Bronchoalveolar lavage fluid (BALF) is a promising alternative to the commonly used sputum specimen for detecting MTB in individuals with suspected TB, especially for patients who fail to provide sputum specimens of adequate volume or quality.[11] Indeed, there is strong evidence that BALF is superior to sputum for use in bacteriological confirmation of pulmonary TB, especially for patients with smear-negative TB results.[11] Thus, it would be meaningful to investigate whether the use of BALF samples collected from patients without bacteriological evidence of TB would be beneficial for clinical TB diagnostic decision-making. Indeed, little has been reported regarding this issue to date, especially with regard to strategies incorporating both Xpert and BALF. Therefore, in this study we prospectively evaluated the use of BALF specimens to assess their added incremental value to pulmonary TB diagnostic strategies used currently in a high-burden urban setting in China.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of the Fifth Hospital of Suzhou. All patients enrolled in this study provided written informed consent.

Patients

A prospective study was conducted of patients with presumptive pulmonary TB who were registered in the Fifth Hospital of Suzhou between March 2018 and July 2018. All patients with symptoms suggestive of pulmonary tuberculosis were consecutively enrolled in this study. Eligible patients were at least 18 years of age and exhibited clinical symptoms suggestive of pulmonary TB.[12] Each patient provided three sputum samples (spot, night and morning sputum) at the initial visit. For BALF sample collection, bronchoscopy was conducted by placement of the bronchoscope into an airway of an affected lung segment. Next, 60 mL of sterile saline (0.9%) was instilled into the lung segment and the returned aspirate was collected into a sterile 50-mL tube for further laboratory examination. Detailed demographic and clinical characteristics of patients were obtained from electronic medical records.

TB case definitions

A person with presumptive TB was defined as someone presenting to the clinic with clinical TB symptoms and a clinical-radiologic picture suggestive of TB. Each person with presumptive TB was classified into one of three diagnostic categories: (i) definitive TB patients: clinical TB symptoms with positive isolation and identification of MTB and/or the presence of MTB detected by Xpert; (ii) clinically diagnosed TB patients: with clinical TB symptoms, pulmonary infiltrates visible on chest X-ray or chest computed tomography (CT) scans and a positive response to anti-TB treatment; (iii) non-TB patients: with no evidence of TB on the basis of clinical examination (Table 1). Clinical TB symptoms included a cough of 2 weeks or longer duration, hemoptysis, fever, chest pain, dyspnea, weight loss and/or night sweats.[13]

Laboratory methods

Direct smears from each sputum and BALF specimen were examined using Auramine O staining for acid fast bacilli (AFB) according to the National Guidelines for TB Laboratories endorsed by the Chinese Center for Disease Control and Prevention.[14]

Specimens were digested with N-acetyl-L-cysteine and sodium hydroxide (NALC–NaOH) for 15 min then decontaminated specimens were neutralized by the addition of PBS buffer. After centrifugation for 15 min at $3000 \times g$ the supernatant was discarded and the pellet was suspended in 1.5 mL of PBS buffer. Next, 0.2 mL of the suspension was inoculated onto the surface of Löwenstein–Jensen (L–J) medium. Each culture was read weekly until positive growth was observed or scored as negative if no growth was observed by 8 weeks. Growth of bacterial colonies was recorded each week to assess the presence of live tubercle bacilli.[15] Fresh colonies were collected for species identification using the MPT64 antigen kit (Genesis, Hangzhou, China) as previously reported.[5]

For the Xpert MTB/RIF assay, 1.0 mL of specimen was mixed with 2.0 mL of Xpert sample reagent followed by incubation for 15 min at room temperature. Next, 2.0 mL of digested specimen was added to an Xpert MTB/RIF cartridge for analysis (Cepheid, Sunnyvale, CA, USA).[16] Final results were automatically reported by the Xpert system within 2 h.

Data analysis

The costs of conventional and newer diagnostic algorithms for identification of definitive pulmonary TB were compared using costs paid for different clinical and laboratory techniques through medical insurance rebate payments for these procedures in China. To identify the most cost-effective algorithm, we calculated the cost per case diagnosis using each algorithm. For evaluation of diagnostic accuracy of Xpert and smear microscopy, mycobacterial culture was considered the gold standard for results comparisons. Diagnostic test results, including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), were also compared to the mycobacterial culture gold standard. The PPV for a given diagnostic tool was calculated as: true positive (positive culture result) divided by the number of patients with a positive test result obtained using the given diagnostic tool. The NPV for a given diagnostic tool was calculated as: true negative (negative culture result) divided by the number of patients with a negative test result for the given diagnostic tool. The chi-squared test was used to compare performances among the various laboratory methods evaluated in this work. All statistical analysis was conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Differences were declared significant for P values less than 0.05.

Results

Participants

Of 440 individuals enrolled in this study, 311 (70.7%) patients were male. The median age was 48 years (range 18–89) and 19

Table 1
Diagnostic classification of active tuberculosis patients.

Characteristic	Definitive TB case	Clinically diagnosed TB case
I. Clinical symptoms[a] II. Chest radiological features[b] III. Smear positive or culture positive IV. Presence of MTB detected by Xpert V. Response to anti-TB treatment	In addition to Characteristic I and II, the cases with suspected TB had either Characteristic III or VI.	In addition to Characteristics I and II, the cases with suspected TB had Characteristic V.

[a] Clinical symptoms include a cough of 2 weeks or more, hemoptysis, fever, chest pain, dyspnea, weight loss or night sweats.
[b] Chest radiological features include mediastinal and/or hilar lymphadenopathy with pulmonary parenchymal infiltration, bronchial obstruction, cavity or miliary lesions and calcification.

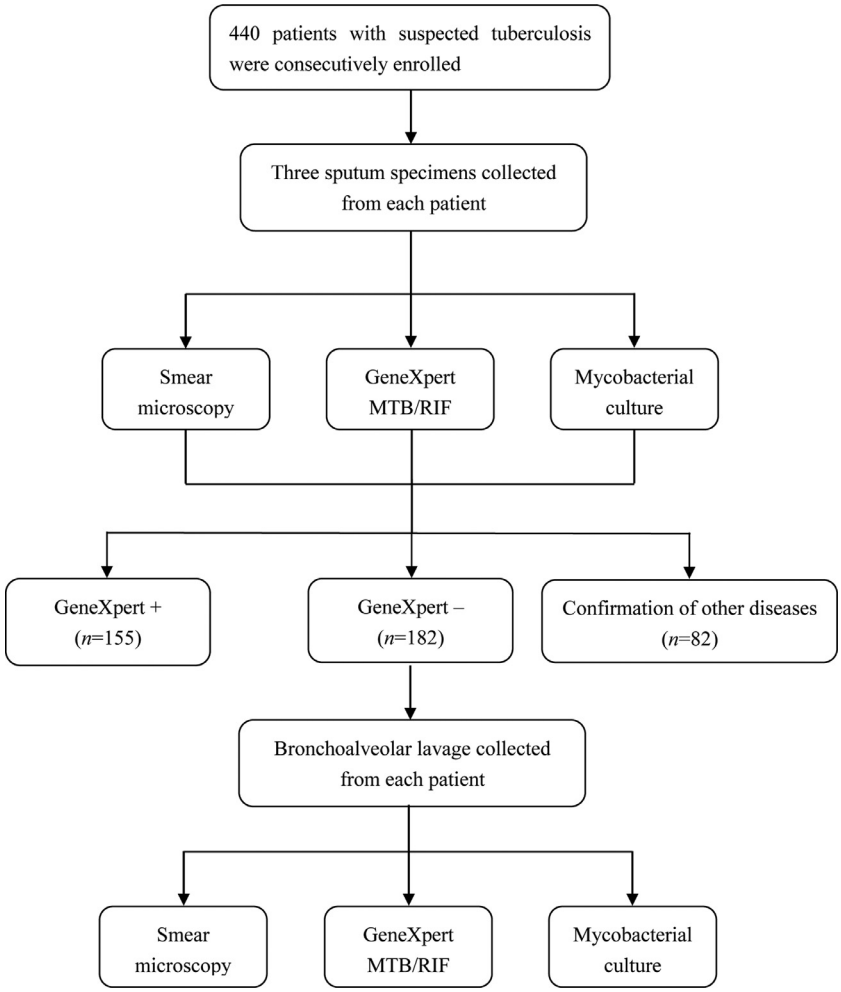

Fig. 1. Enrolment of the study participants.

(4.3%) cases were excluded from final analysis, including 7 due to contaminated cultures, 2 with invalid Xpert results and 10 with nontuberculous mycobacteria (NTM) infections. Of the remaining 421 cases, initial testing supported diagnoses of 245 (55.7%) as definitive TB cases due to a positive culture and/or Xpert result and 71 (16.1%) clinically diagnosed TB cases (Fig. 1).

Diagnostic accuracy of Xpert and smear microscopy performed on sputum specimens

Of 153 culture-positive patient sputum samples, 144 yielded positive Xpert results, for a test sensitivity of 94.1% (144/153; 95% CI: 90.4–97.8%) that was significantly higher than that of smear microscopy (45.8%, 70/153; 95% CI: 37.9–53.6%) ($P < 0.01$). Sputum samples of eleven of 268 culture-negative cases were positive by Xpert, yielding a specificity of 95.9% (257/268; 95% CI: 93.5–98.3%) that was significantly lower than the specificity of smear microscopy, which was 99.3% (266/268; 95% CI: 98.2–100.0%; $P = 0.02$). For sputum samples, the positive predictive value (PPV) and negative predictive value (NPV) were 92.9% (144/155; 95% CI: 88.9–96.9%) and 96.6% (257/266; 95% CI: 94.4–98.8%), respectively (Table 2).

Diagnostic accuracy of Xpert and smear microscopy performed on BALF specimens

Of the 266 patients with negative Xpert results, 82 were confirmed to have other diseases and did not receive bronchoscopy. Only results for BALF samples collected from the other 182 patients were subjected to statistical analysis to compare the diag-

Table 2
Accuracy of Xpert for the detection of culture-positive TB in various clinical specimens.

Specimen[a]	Method	Sensitivity[b] (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)
Sputum[b] (*n* = 421)	**Smear microscopy**	45.8(37.9–53.6)70/153	99.3(98.2–100.0)266/268	97.2(93.4–100.0)70/72	76.2(71.8–80.7)266/349
	Xpert	94.1 (90.4–97.8)144/153	95.9 (93.5–98.3)257/268	92.9(88.9–96.9)144/155	96.6(94.4–98.8)257/266
BALF[c] (*n* = 182)	**Smear microscopy**	23.4(13.9–32.8)18/77	100.0(100.0–100.0)105/105	100.0(100.0–100.0)18/18	50.2(43.5–57.0)105/209
	Xpert	97.4(93.8–100.0)75/77	97.1(94.0–100.0)102/105	96.2(90.1–99.8)75/78	98.1(95.4–100.0)102/104
Total (*n* = 421)	**Smear microscopy**	38.3(32.0–44.5)88/230	98.9(97.4–100.0)179/181	97.8(94.7–100.0)88/90	55.8(50.3–61.2)179/321
	Xpert	95.2(92.5–98.0)219/230	92.7(89.0–96.4)177/191	94.1(90.8–97.5)177/188	93.6(90.5–96.7)219/234

[a] BALF, bronchoalveolar lavage fluid; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[b] $\chi^2 = 85.11$, $P < 0.01$ (Smear sensitivity vs. Xpert sensitivity for sputum); $\chi^2 = 6.39$, $P = 0.02$ (Smear specificity vs. Xpert specificity for sputum).
[c] $\chi^2 = 88.20$, $P < 0.01$ (Smear sensitivity vs. Xpert sensitivity for BALF); $\chi^2 = 3.04$, $P = 0.25$ (Smear specificity vs. Xpert specificity for BALF).

Table 3
Cost of diagnostic procedures.

Diagnostic procedure	Cost per person ($US)
Bronchoscopy	66.1
Smear microscopy for sputum[a]	8.8
Smear microscopy for BALF	13.2
GeneXpert MTB/RIF	73.5

[a] According to the National Tuberculosis Programme in China, three separate smears are completed for each patient with presumptive pulmonary tuberculosis.

nostic accuracy of Xpert to that of smear microscopy. As shown in Table 2, 18 culture-positive cases were identified as positive using smear microscopy, resulting in a sensitivity of 23.4% (18/77; 95% CI: 13.9–32.8%), which was significantly lower than the sensitivity of 97.4% obtained using Xpert (75/77; 97.4%; 95% CI: 93.8–100.0%; $P < 0.01$). The estimated specificities of Xpert and smear microscopy methods, useful for achieving accurate TB diagnostic results from BALF samples, were 96.2% (102/106; 95% CI: 92.6–99.9%) and 100.0% (105/105; 95% CI: 100.0–100.0%), respectively. Statistical analysis revealed no significant difference in specificity between the two methods ($P > 0.05$).

We also compared accuracies between Xpert and smear microscopy for identification of MTB using the diagnostic algorithm that included testing of BALF specimens. Overall, a total of 230 BALF patient specimens tested culture-positive via the mycobacterial culture method. Of these culture-positive BALF samples, Xpert and smear microscopy could identify 219 and 88 culture-positive TB patients, for overall sensitivities of 95.2% (95% CI: 92.5–98.0%) and 38.3% (95% CI: 32.0–44.5%), respectively. In addition, all positive cases reported by smear microscopy were also positive by Xpert testing. In addition, the overall specificity values of Xpert and smear microscopy for identification of MTB were 92.7% (95% CI: 89.0–96.4%) and 98.9% (95% CI: 97.4–100.0%), respectively.

Cost analysis

The cost of each diagnostic procedure is listed in Table 3, where calculations of different diagnostic strategies are compared based on various combinations of diagnostic test with detected specimen type. As shown in Table 4, comparisons among eight different diagnostic strategies demonstrated that the sole use of smear microscopy to test sputum was the most cost-effective strategy, with an average cost per case of USD 52.8. However, approximately 80% of TB cases were missed using this strategy. Meanwhile, strategies based on BALF specimen collection combined with Xpert could detect the highest number of TB patients, with 74.1% (234/316) of TB patients accurately identified as MTB-positive, but with high cost a concern. Notably, to reduce costs associated with Xpert cartridges, Xpert testing performed only after obtaining negative smear microscopy results could significantly decrease the cost of MTB detection per patient. As compared with the highest cost of strategy 7 (USD 266.9), the cost per case for strategy 8 was much lower (USD 175.1) and similar to that of strategy 4 (USD 172.7) which included Xpert testing limited to sputum specimens that had initially tested negative for MTB via smear microcopy.

To assess how product price influenced diagnostic cost, we analyzed the effect of adjusting the price of Xpert per case on overall costs when Xpert was added to strategies with highest TB detection rates. As summarized in Table 5, the unit price of Xpert had a great effect on the cost of TB diagnosis for strategies 6 and 7. For example, when the price of Xpert was hypothetically reduced from USD 70 to USD 10, the costs of diagnosing TB decreased by 66.7% for strategy 6 and by 60.7% for strategy 7, while for strategy 8 the cost only decreased from USD 171.1 to USD 102.7.

Discussion

In China the diagnosis of tuberculosis mainly relies on the clinical symptoms and chest radiology.[17] The fact that approximately 70% of TB cases without microbiological evidence highlights the urgent need for formulation of a new diagnostic algorithm to add incremental value to current recommended strategies for TB diagnosis.[1] In this study, our results demonstrated that for testing of BALF samples, Xpert significantly outperformed smear microscopy and detected TB in 97.4% of smear-negative TB cases. The sensitivity observed in the present study of 92−93% for Xpert testing of BALF samples is on par with sensitivities obtained in recent studies.[11,18] The relatively lower sensitivity reported by Lee et al. (~82%)[19] may reflect statistically weaker results obtained in that study due to small sample size.

In addition, when BALF specimens were collected for follow-up testing of cases with initial Xpert-negative sputum test results, the detection rate significantly increased from 49.1% to 74.1%. Our findings may thus provide important insights to improve tuberculosis control and prevention in China. On the one hand, use of BALF with Xpert as a strategy revealed that Xpert can play an essential role in improving timely detection of TB cases. However, this advantage is offset by the relatively high cost of Xpert testing, which elevated diagnostic costs per pulmonary TB case by three-fold. Therefore, greater efforts should be expended to develop more cost-effective nucleic acid amplification-based assays. On the other hand, in 2016 the Chinese government issued the five-year National Tuberculosis Control Plan (2016–2020), which aims to increase the proportion of cases accompanied by microbiological confirmation to at least 50% by 2020. Based on our observations, even if Xpert, the most sensitive test, is integrated into the diagnostic algorithm, only 49% of cases would be detected, leaving a 1% gap to fill in order to meet the 50% required threshold outlined in 2020 national planning goals. However, this gap could be closed if BALF specimens were used for initial MTB detection to improve early TB diagnosis accuracy.

Although a comparative study of BALF versus induced sputum revealed that induced sputum was more sensitive than BALF for

Table 4
Costs of diagnostic algorithms for detection of TB from patients with presumptive pulmonary tuberculosis.

Diagnostic algorithm	Cases detected (%)	Cost of investigations (USD)	Cost per case diagnosis (USD)	Cases missed
1. Smear microscopy on sputum	70(22.2)	3704.8	52.9	246(77.8)
2. Xpert on sputum	155(49.1)	30943.5	199.6	161(50.9)
3. Smear microscopy plus Xpert on sputum	155(49.1)	34648.3	223.5	161(50.9)
4. Smear microscopy plus subsequent Xpert on sputum[a]	155(49.1)	26773.7	172.7	161(50.9)
5. Smear microscopy on sputum and BALF	88(27.8)	18137.4	206.1	228(72.2)
6. Xpert on sputum and BALF	234(74.1)	56350.7	240.8	82(25.9)
7. Smear microscopy plus Xpert on sputum and BALF	234(74.1)	62457.9	266.9	82(25.9)
8. Smear microscopy plus subsequent Xpert on sputum and BALF	234(74.1)	40978.0	175.1	82(25.9)

[a] The subsequent Xpert represents the usage of Xpert as a subsequent method for detecting MTB in specimens identified as smear-negative.

Table 5
Effect of the unit price of Xpert MTB/RIF on the cost of diagnosis.

Diagnostic algorithm	Cost per case diagnosis at various unit prices of Xpert MTB/RIF (USD)						
	10	20	30	40	50	60	70
6. Xpert on sputum and BALF	77.2	102.9	128.7	154.5	180.3	206.0	231.8
7. Smear microscopy plus Xpert on sputum and BALF	99.9	125.6	151.4	177.2	202.9	228.7	254.5
8. Smear microscopy plus subsequent Xpert on sputum and BALF	102.7	114.1	125.5	136.9	148.3	159.7	171.1

detecting active pulmonary TB,[20] these results differ from those of Anderson and Schoch whereby bronchoscopy provided better diagnostic yield than did sputum induction.[21,22] In order to produce comparable performance to that obtained using BALF, collection of two induced sputum specimens from smear-negative cases is recommended to maximize the likelihood of obtaining positive results.[22] Unfortunately, the additional detection sensitivity obtained using Xpert testing of BALF specimens cannot meet cost-effectiveness considerations in resource-limited endemic TB settings. Meanwhile, several previous reports have found that the presence of lignocaine in BALF samples has a negative impact on mycobacterial growth, thereby resulting in false-negative culture results.[21,23] Nevertheless, the results of Xpert for follow-up testing using BALF samples would be unaffected by the concentration of lignocaine and would improve the accuracy of pulmonary TB diagnosis.

We acknowledge that bronchoscopy is a cough-inducing procedure that produces infectious droplet nuclei, thus increasing health care worker (HCW) exposures to MTB.[24,25] Therefore, appropriate infection control precautions are necessary to reduce MTB exposure of patients and HCWs during and after BALF collection, such as through the use of suitable exhaust ventilation and respiratory protection.[24,26] Additionally, although the use of bronchoscopy is increasing, bronchoscopy is not universally available in resource-limited clinical settings.[20]

This study has several obvious limitations. First, it was only conducted in one tertiary TB-specific hospital, possibly weakening the validity of the study conclusions. Therefore, further evaluation is required to clarify the incremental value of BALF for pulmonary TB diagnosis in other settings. Second, although mycobacterial culture remains the gold standard for establishing the presence or absence of MTB in clinical specimens, we did not incorporate it into our diagnostic algorithm design in view of its long turn-around time. Third, cost analysis in the present study was crude and relied only on medical insurance rebates for procedures, without considering potential long-term cost-saving benefits associated with early TB diagnosis. Thus, the cost analysis model used in this study may exaggerate the cost of Xpert-based diagnostic strategies. Nevertheless, this study provides an alternative for improving bacteriological confirmation of pulmonary TB in a TB-epidemic setting.

In conclusion, our data demonstrate that implementation of BALF specimen collection adds incremental value to strategies currently used to diagnose suspected pulmonary TB patients with initial Xpert-negative sputum results. The Xpert assay outperforms conventional smear microscopy for detection MTB in both sputum and BALF specimens and thus plays an essential role in improving timely TB case detection, although its high cost is a barrier to its widespread implementation. Further evaluation is required to clarify the incremental value of BALF for pulmonary TB diagnosis in other clinical settings.

Conflict of Interest

None.

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