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RESEARCH

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GeneXpert analysis of bronchoalveolar lavage (BAL) samples: promising diagnostic modality in patients with smear-negative pulmonary tuberculosis

Mohammed Abdalla Rakha[1,2], Asmaa Ali[3,4,5*], Waleed Hassan[1] and Eman Al-Anbay[1]

Abstract

Purpose: The aim of the study is to assess the diagnostic utility of bronchoalveolar lavage (BAL) samples with GeneXpert test in diagnosis of TB in smear-negative patients.

Method: One-hundred sixty-nine cases were included in this study, as they had a radiological finding suggested to have pulmonary tuberculosis, while the sputum direct smear examination for acid-fast bacilli was negative. All cases prepared to do fiber-optic bronchoscope with bronchoalveolar lavage collection after their consent and post bronchoscope sputum collection for further molecular and microbiological examination.

Results: The combined diagnostic yield of all bronchoscopy procedures was 15.97% (27 cases of 169) and the final diagnosis of TB cases was established in 16 cases of 169 (9.4%) using BAL culture and in 20 cases of 169 (11.83%) by GeneXpert methods. Post bronchoscope direct sputum examination, culture, and GeneXpert evaluation gave diagnostic yield about 1.1%, 5.5%, and 7.1%, respectively. The specificity of GeneXpert examination of BAL and post bronchoscope sputum was 95.42% and 97.38%, respectively.

Conclusion: Early detection of pulmonary tuberculosis using fiber-optic bronchoscope BAL examination and post bronchoscope sputum with GeneXpert method promotes the accurate decision to start antituberculous therapy in patients with smear negative.

Keywords: Smear-negative TB, Bronchoscope, Bronchoalveolar lavage, Post bronchoscope sputum evaluation

Introduction

Tuberculosis as a contagious disease is one of the most common causes of death worldwide [1]; the annual incidence of active pulmonary TB reached to 9.4 million, with mortality incidence about 137 cases per 100,000 populations [2]. TB is a disease of poverty and low-income people; therefore, the highest prevalence countries for TB were located in Asia, mainly in India [3]. Although in developed and high-income countries the health agenda about TB problem is disappeared, the concern is re-emerged in the last few decades because of migration [4, 5]. Kuwait country is located at the northern end of the Arabian Gulf [6]. The ratio of Kuwaitis:non-Kuwaiti's people is 1:2.5, and it is considered as a good example for migration health-associated infectious disease like TB [4, 6–8]. The Kuwait National Tuberculosis Programmed started in 1954, and it worried about screening of TB using tuberculin skin test and radiography; subsequently, the suspected individuals undergo sputum examination using direct smear evaluation and culture to start the specialized antituberculous

*Correspondence: asmaa.ali81@yahoo.com

[3] Department of Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013, China
Full list of author information is available at the end of the article

therapy [4, 6]. However, a considerable proportion of pulmonary TB patients is presented with negative sputum, which ranged from 20 to 60% [9]. The proper diagnosis of TB patients with smear negative is a great challenge toward clinician, to avoid progression to active disease if untreated, or unnecessary empirical antituberculous therapy [10–13]. Currently, several modalities are available to diagnose such cases with different yields and risk association, as sputum induction by hypertonic saline, gastric lavage especially in children, transthoracic needle aspiration, and fiber-optic bronchoscopy (FOB) [11, 14]. The diagnostic utility of FOB and its associated procedures as bronchoalveolar lavage and bronchial brushes is higher in detection of pulmonary infection, and in spite of it is considered as an invasive procedure, it has been proven to be safe with fewer complications in highly skilled hands [14–17]. From that point of view, the primary aim of the work was to evaluate the diagnostic yield of bronchoalveolar lavage culture and GeneXpert, as well as post bronchoscope sputum examination in suspected patients with smear-negative tuberculosis.

Patients and methods

Patients and study type

This is a retrospective cohort study, which was conducted on Pulmonary Rehabilitation Center, Chest Hospital, Kuwait City, and included 169 participants with radiological and clinical features that suggested pulmonary tuberculosis infection irrespective with negative smear for acid fast bacilli (AFB), in the period from January 2019 to January 2020. The data of corresponding cases were extracted from hospital medical records after approval of ethical committee member no. 1394/2020. The study included all cases that were subjected to fiber-optic bronchoscope with collection of bronchoalveolar lavage (BAL) and post bronchoscope sputum sample. The age of participants was above 18 years and below 65 years, and all of them were free from chronic lung disease, advanced lung cancer, or any type of respiratory failure.

Data collection

The following data had been extracted from medical records and included (1) demographic characteristics as age, sex, nationality, smoking habits, and occupation, (2) radiological examination using X-ray, and (3) laboratory investigation including complete cell counts, liver function test, and kidney function.

Bronchoscopy

The bronchoscopic procedure steps were fulfilled as regarding the guideline protocol of Bronchoscopy Unit of Chest Hospital; hence, the cases were assessed clinically and signed informed consent about flexible fiber-optic bronchoscope. The bronchoscope was performed through oral or nasal route in supine position under intravenous conscious sedation. All patients were under continuous monitoring of blood pressure and oxygen saturation. After complete inspection of the bronchial tree, BAL was done with 120 ml of normal saline at the suspected region. The patients had been observed in the recovery room for 2 h after procedure and follow up by chest x-ray PA view before discharge from our bronchoscopy unite. BAL samples and post bronchoscopy sputum samples were sent for AFB smear and culture, gram stain and culture, and GeneXpert PCR for evaluation.

Sputum evaluation

1- *Microscopic examination*

Sputum sample is prepared as regarding the clinical protocol of microscopic examination of mycobacterium tuberculosis; after that, the sample was stained by Ziehl–Neelsen carbolfuchsin (ZN) stain [18].

2- *GeneXpert examination*

The collected samples were subjected to GeneXpert examination as regarding the manufacture guideline protocol of preparation [19].

3- *Sample culture*

The collected samples were subjected to culture on solid media (Lowenstein–Jensen media), which is considered as gold standard test for detection of MTB; the culture time ranged from 6 to 8 weeks [20].

Statistical analysis

Statistical analysis was performed by Minitab 17.1.0.0 for windows (Minitab Inc., 2013, Pennsylvania, USA). The normality of data was examined using Shapiro–Wilk test. Continuous data was presented as mean and standard deviation (SD) while categorical data as number and percentage (%). Sensitivity and specificity were estimated from the equation as follows: "sensitivity = (true positive cases/(true positive cases + false-negative cases) and specificity = (true negative cases/(true negative cases + false-positive cases)." However, positive and negative predictive values depend on the prevalence of the disease in the population of interest and result from equation as follows: "positive predictive value = (true positive cases/(true positive cases + false-positive case) and negative predictive value = (true negative cases/(true negative cases + false-negative cases)." The accuracy of test with binary classification was the result from the

Table 1 General characteristics and clinical presentation of patients

Factors	Total ($n=169$)	
	Mean	SD
Age	43.613	9.03
Sex	N	%
Female	25	14.79
Male	144	85.21
Nationality	N	%
African	7	4.14
Asian	158	93.49
Kuwaiti	4	2.37
Smoking	N	%
Ex-smoker	3	1.78
Nonsmoker	134	79.29
Smoker	32	18.93
Occupation	N	%
Hand working	103	60.95
Office worker	10	5.92
Transportation	56	33.14
Laboratory data	Mean	SD
TLC (10^9/L)	7.36	1.81
HB (g/dL)	14.55	2.32
PLT (10^9/L)	266.92	70.84
Bun (mg/dL)	4.45	1.31
Creatinine (mg/dL)	6.83	1.42
ALT (U/L)	27.48	19.01
AST (U/L)	29.06	20.53
Total bilirubin (mg/dL)	1.36	1.06
Direct bilirubin (mg/dL)	0.23	0.26
X-ray features	N	%
a—Laterality		
Unilateral	120	71.01
Bilateral	49	28.99
b—Lesion site		
RT	74	43.78
LT	46	27.23
Both	49	28.99
c—Zone affection		
Apical zone (yes)	120	71.01
Mid-zone (yes)	15	8.88
Lower zone (yes)	30	17.75
d—Lesion pattern		
Infiltration (yes)	144	85.21
Consolidation (yes)	8	4.73
Cavitation (yes)	1	0.59
Nodules (yes)	4	2.37
Fibrotic scar (yes)	1	0.59
Hilar LN (yes)	2	1.18
Effusion (yes)	6	3.55

Continues data represented as mean and SD and categorical data as number and percentage, *SD* stander deviation, *N* number

grand equation: (true positive + true negative)/(true positive + true negative + false-positive + false negative). All tests were two sided; *P* is considered significant if < 0.05.

Results

General and clinical characteristic of cases

About 169 cases had changes in X-ray as well as symptoms suggested pulmonary tuberculosis; all of them had negative sputum direct microscopic examination for AFB in addition to GeneXpert examination test for sputum. The general and clinical criteria of suspected cases were summarized in Table 1; hence, the mean (SD) age of them was 44 (9) years, with male sex predominance (85.21%) and non-Kuwaiti nationality (97.63%).

Fiber-optic bronchoscope with bronchoalveolar lavage (BAL)

The common gross features abnormality that is detected by bronchoscope was mucosal edema of tracho-bronchial tree (100 cases of 169) and narrowing in some segmental bronchus (40 cases of 169). Post bronchoscopic direct smear examination was positive for AFB in only 2 cases of 169, which has the lower diagnostic yield for TB recognition. However, the GeneXpert test for post bronchoscopic sputum and BAL sample was positive in 12 and 20 cases of 169 respectively, which enhance the diagnostic yield up 7.1% and 11.83, respectively. Moreover, the rate of pulmonary TB in patients with negative sputum increased up to 5.33% and 9.47% respectively after culture of post bronchoscopic sputum and BAL sample. The overall diagnostic utility of all fiber-optic bronchoscope technique in identifying TB infection increased to 15.97% (Table 2).

The specificity and accuracy of all diagnostic tests after fiber-optic bronchoscope were more than 90% (Table 3).

Table 2 Diagnostic yield of fiber-optic bronchoscopic technique

Factors	Total ($n=169$)	
Technique	*N*	%
A—BAL		
BAL culture	16	9.47
BAL *GeneXpert*	20	11.83
B—Post bronchoscope		
Post bronchoscope direct smear	2	1.18
Post bronchoscope culture	9	5.33
Post bronchoscope *GeneXpert*	12	7.1
C—Combined technique	27	15.97

BAL bronchoalveolar lavage, *N* number

Table 3 Utility of GeneXpert and post bronchoscope sputum examination

Test	Sensitivity	Specificity	PPV	NPV	ACC
BAL *GeneXpert*	81.25	95.42	65	97.98	94.08
Post bronchoscope direct smear	12.5	100	100	91.61	91.71
Post bronchoscope culture	43.75	98.69	77.77	94.37	93.49
Post bronchoscope GeneXpert	50	97.38	66.66	94.9	92.89

BAL bronchoalveolar lavage, *N* number, *PPV* positive predictive value, *NPV* negative predictive value, *ACC* accuracy

Discussion

The current study included 169 cases with radiological evidence suggested pulmonary TB infection; however, three-sputum sample direct smear and GeneXpert were negative. All of them subjected to fiber-optic bronchoscope with BAL for further microbiological and molecular evaluation for TB. Bronchoscopic examination showed 100 (59.1%) cases with mucosal edema and 40 (23.6%) cases with segmental narrowing that came in consistence with previous study [21], as 42% of evaluated cases showed normal bronchoscopic inspection, while 32% of cases had unhealthy mucosa. On the other hand, Kumar et al. [11] found that edema with or without narrowing of a bronchial segment is present in 10 (7.5%) patients while mucus pooling, endobronchial nodules, and hyperemia in 6, 5, and 4 patients, respectively. The discrepancy of our results and aforementioned Kumar et al. study may be due to the criteria of cases selection, as all cases had changes in chest X-ray, which subsequently denotes corresponding changes in gross picture of fiber-optic bronchoscopy.

The present work reported 15.97% diagnostic yield of all FOB-associated technique for confirming TB diagnosis in patients with smear negative, which is considered as one of a lower diagnostic yield studies; however, the diagnostic yield of bronchoscopic procedures has ranged from 9 to 75% [11, 17, 22]. Chan et al. [23] study reported 14% diagnostic yield of FOB, as well as similar results reported by Russel et al. [24] and Khoo et al. [25], 12% and 9% respectively, while Palenque et al. [26] results showed a slightly higher yield; 34% of his cases were diagnosed as TB by FOB. On the other side, the studies with higher diagnostic yield were originated from India [17, 27–29], where the TB was endemic. The diagnostic yield was more than 45% of all examined cases.

The current data highlights the diagnostic utility of BAL examination by GeneXpert test; hence, beside the rapid interpretation of Xpert molecular test, its sensitivity and specificity were higher too, 81.25% and 95.42%, respectively. In study conducted by Walters et al. [30], to evaluate the potential role of GeneXpert on BAL samples, it include 40 cases, and the results revealed that BAL Xpert was positive in 7 (17.5%) cases with sensitivity of about 78%, which was close similar to our results. Moreover, Gowda et al. [31] examined the diagnostic utility of GeneXpert on BAL of patients with negative smear for TB and enrolled 60 patients. TB was confirmed by culture in 16 (26.7%) cases, with sensitivity and specificity of Xpert test reached to 81% and 73%, respectively in culture-confirmed cases. In the same line, Kilaru et al. [32] study the diagnostic role of GeneXpert in 56-suspected pulmonary TB cases with BAL samples; 17.8% of cases confirmed to have PTB based on culture. The sensitivity of Xpert test was closer to our results (90%), while the specificity was low (52.2%). This may be due to study design difference, as he considered all cases with positive Xpert or direct smear test of BAL sample plus the radiological changes as a possible TB case and subsequently sent for culture, in contrast with our study, since all BAL samples were subjected to TB culture evaluation from the start.

The strength of the present work rested on using acceptable sample size (169) that subjected to the same technique without selection baize, in addition to use GeneXpert test, which provide rapid results for decision-making for consequent treatment application.

In spite of different diagnostic facilities that were provided in our center, the study showed some limitations: the first of them was the study type; retrospective cohort, as, the research team data had been limited to which was accessible in record. The second limitation was neglecting the potential role of BAL collecting quality as well as the level of experience of bronchoscopist, which affected the results of all bacteriological tests.

Conclusion

GeneXpert test in bronchoalveolar lavage sample as well as on post bronchoscope sputum provided rapid results especially in cases with negative smear for TB, which could be enhanced by the significant decision for antituberculous treatment initiation.

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Authors' contributions
All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by AA. The first draft of the manuscript was written by MAR and AA, and all authors commented on previous versions of the manuscript. The authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
The study was approved by National Research Ethics Committee of Pulmonary Rehabilitation Center, Chest Hospital, Kuwait City, with reference number 1394/2020.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Pulmonary Rehabilitation Center, Chest Hospital, Kuwait City, Kuwait. [2]Department of Chest Disease, Al-Azhar University, Cairo, Egypt. [3]Department of Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013, China. [4]Department of Pulmonary Medicine, Abbassia Chest Hospital, MOH, Cairo, Egypt. [5]Department of Respiratory Allergy, Al-Rashed Allergy Centre, MOH, Kuwait City, Kuwait.

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