Open Access

Original Article

Assessing Endobronchial Washing Mycobacterium Tuberculosis Gene Xpert's Role in Diagnosing Pulmonary Tuberculosis in Sputum Smear Negative Patients

Muhammad Faizan, Muhammad Amir, Muhammad Iqbal, Ehsan Elahi, Muhammad Faraz, Owais Amjad Janjua

Department of Medicine, Pak Emirates Military Hospital, Rawalpindi/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To see diagnostic accuracy of GeneXpert MTB in diagnosing pulmonary tuberculosis in sputum smear negative patients.

Study Design: Cross-sectional Study.

Place and Duration of Study: Department of Pulmonology, Pakistan Emirates Military Hospital Rawalpindi, Pakistan from May to Oct 2024.

Methodology: Patients having at least any of two clinical criteria and at least one radiological finding suspected of TB and were included. The clinical criteria comprised of intermittent fever or persistent cough for greater than 3 weeks, drenching night sweats for more than 2 weeks, HIV positive patients, weight loss greater 5% of body weight, and haemoptysis. The radiological criteria included cavitatory lesions, consolidation, pleural effusion or hilar adenopathy on chest radiographs. After initial history and investigations, all patients went through fiber optic bronchoscopy. The Lowenstein Jensen (LJ) medium was used for sample inoculation and incubated for 8 weeks. The GeneXpert MTB samples were processed as per manufacturer's specifications.

Results: In this study, one hundred and twenty-two (n=122) patients median age of 46.00(28.75-59.25) years were included. The cough 95(77.87%), fever 79(64.75%) and weight loss 74(60.66%) were common symptoms respectively. The upper lung zone 84(68.85%) and middle lung zone 12(9.84%) were commonly involved. The GeneXpert MTB was reported high positive in 41(33.61%) patients. The GeneXpert MTB was found to be 92.64% sensitive, 85.71% specific and 90.98% accurate. The positive predictive and negative predictive values were 91.30% and 90.56% respectively.

Conclusion: GeneXpert MTB is a reliable tool to test smear-negative patients suspected of tuberculosis.

Keywords: Diagnostic tests, GeneXpert MTB, Microscopy, Microbiology, Pulmonary tuberculosis.

How to Cite This Article: *Faizan M, Amir M, Iqbal M, Elahi E, Faraz M, Janjua OA. Assessing Endobronchial Washing Mycobacterium Tuberculosis Gene Xpert's Role in Diagnosing Pulmonary Tuberculosis in Sputum Smear Negative Patients. Pak Armed Forces Med J 2026; 76(Suppl-1): S112-S115. DOI: https://doi.org/10.51253/pafmj.v76iSUPPL-1.12959*

INTRODUCTION

Pulmonary Tuberculosis (TB) is communicable and contagious disease caused by a fastidious and difficult to culture acid fast bacillus known as Mycobacterium Tuberculosis (MTB) also know a Koch's Bacillus.[1] TB is known for being the leading cause of death from single agent and also comes in top 10 disease causing morbidity and mortality.[2,3] Pakistan is fifth largest host of the TB and comes under the Eastern Mediterranean Region (EMRO). The government of Pakistan offers nationwide free diagnostics and treatment for TB through 1500 healthcare facilities and by similar number of laboratories as party of TB control and prevention initiative.[4] The timely diagnosis not only decreases morbidity and mortality but also lessens the financial burden on health-care delivery system.

Correspondence: Dr Muhammad Faizan, Department of Medicine, Pak Emirates Military Hospital, Rawalpindi Pakistan

Received: 12 Apr 2024; revision received: 23 Feb 2025; accepted: 26 Feb 2025

The sputum smear is the most common and widely used method for diagnosis but with very variable sensitivity.[5] The MTB culture is gold standard but it very time taking. It takes from 21 days in liquid medium to 56 days in standard culture mediums.[6] Recently, a new TB diagnostic test, GeneXpert MTB has been introduced that is also recommended by World Health Organization for TB diagnostic purposes and same has been recommended by International Standard for TB care (ISTB) for smear negative cases.[7,8]

The GeneXpert MTB involves nucleic acid amplification technique (NAAT) / polymerase chain reaction (PCR) for detecting MTB deoxyribonucleic acid (DNA). It is rapid (48 hours) and safe (bactericidal medium) compared to smear and culture.[8,9] Difficulty usually arises when there is scarce or no sputum production or compromised cough reflex in a patient with high suspicion of pulmonary TB in cases when smear is negative for MTB or culture is negative and pulmonary TB is still suspected. To cater this

bronchoalveolar washings (EBW) with or without bronchial biopsy are taken via bronchoscopy.

Keeping above in view and high burden of Pulmonary TB in our community, this study was planned to see the sensitivity and specificity of GeneXpert MTB in bronchoalveolar lavage/ washings in smear negative patients suspected with TB. This study will not only help clinicians make better management decisions in suspected cases, but it will also help in making early diagnosis.

METHODOLOGY

This cross-sectional study was conducted at Department of Pulmonology and Bronchoscopy, Pak Emirates Military Hospital (PEMH) Rawalpindi after ethical approval form Institutional Ethical Committee (vide certificate number # A/28/ERC/85/24) This study spanned over 6 months from May 2024 to October 2024. The sample size was calculated using online WHO Sample Size calculator and sensitivity calculator with confidence interval of 95%, margin of error 5%, and GeneXpert MTB sensitivity of 81.6% and culture sensitivity of 86% as used by Khalil KF *et al*., the sample size came out to be 93.[10] The informed consent was taken from all suspected TB smear negative patients for screening and inclusion into the study while written consent was taken as per departmental guidelines for bronchoscopy. The convenient sampling technique was used for patient enrollment.

Inclusion Criteria: Patients greater than 18 years of age from both genders having at least any of two clinical criteria and at least one radiological finding suspected of TB were included. The clinical criteria comprised of intermittent fever or persistent cough for greater than 3 weeks, drenching night sweats for more than 2 weeks, HIV positive patients, weight loss greater than 5% of body weight, and haemoptysis. The radiological criteria included cavitatory lesions, consolidation, pleural effusion or hilar adenopathy on chest radiographs.

Exclusion Criteria: Individuals on TB treatment, coagulopathy, respiratory failure, lung cancer, hemodynamically unstable patients and those not consenting for bronchoscopy were excluded from the study. Moreover, pregnant females and those unfit for bronchoscopy were also excluded.

The demographic details, clinical history and relevant examinations of all patients were done. The chest radiograph, coagulation profile, anti-HbsAg, and anti-HCV antibodies of all patients were done. Patients were prepared as per departmental guidelines for bronchoscopy. The flexible fiber optic bronchoscopy was done under conscious sedation. After careful examination of bronchial tree, 0.9% normal solution was instilled at or around lesion site and minimum of three bronchoalveolar lavage samples were collected. The patients' vitals were continuously monitored during the procedure and in recovery room after the procedure for 02 hours. The samples were processed as per the hospital's laboratory standard operating procedures. The Lowenstein Jensen (LJ) medium was used for sample inoculation and incubated for 8 weeks. According to manufacturer instructions and specifications, the GeneXpert MTB samples were processed and interpreted as very low positive, low positive, medium and high positive.

The data was analyzed using Statistical Package for Social Sciences (SPSS) Version 25:0. The Shapiro-Wilk test was used to check normality of quantitative variables. The normally distributed quantitative variables were represented using Mean±SD while for skewed quantitative data, median and interquartile range was used. The categorical data was represented using frequency and percentages. The sensitivity, specificity was calculated using standard formulas for GeneXpert MTB using culture as gold standard.

RESULTS

In this study, one hundred and twenty-two (n=122) patients suspected of pulmonary TB were included. The median age was 46.00 (28.75-59.25) years. The sample population was male predominant 66(54.10%) and 56(45.90%) were females. Among the sampled, 22(18.03%) were smokers. The cough 95(77.87%) was the most common symptom followed by fever 79(64.75%), weight loss 74(60.66%) and night sweats 68(55.74%) respectively. On chest radiographs, upper lung zone 84(68.85%) and middle lung zone 12(9.84%) were commonly involved. The GeneXpert MTB was reported high positive in 41(33.61%), medium positive 17(13.93%) and low positive in 8(6.56%). Rest of the details are given in Table -I.

Detailed analysis showed, there were 63(51.64%) true positive (TP) cases while 48(39.34%) were true negative (TN) cases. The sensitivity of GeneXpert MTB came out to be 92.64% while specificity was 85.71% with MTB culture on LJ Medium as a gold standard. Details are given in Table-II.

Table-I: Characteristics of Sample Population (n=122)

Characteristics	Values
Median Age (years)	46.00 (28.75-59.25)
Gender, n (%)	
Male	66(54.10%)
Female	56(45.90%)
Smoking History, n (%)	
Yes	22(18.03%)
No	100(81.97%)
Clinical Symptoms, n (%)	
Cough	95(77.87%)
Fever	79(64.57%)
Weight Loss	74(60.66%)
Night Sweats	68(55.74%)
Dyspnoea	47(38.52%)
Anorexia	20(16.39%)
Haemoptysis	8(6.56%)
Chest Radiograph Findings, n (%)	
Upper Zone Infiltrates	84(68.85%)
Middle Zone Infiltrates	12(9.84%)
Lower Zone Infiltrates	6(4.92%)
Pleural Effusion	8(6.56%)
Cavitatory Lesion	7(5.74%)
Consolidation	5(4.10%)
GeneXpert MTB Assay	
High Positive	41(33.61%)
Medium Positive	17(13.93%)
Low Positive	8(6.56%)
Very Low Positive	5(4.10%)
Negative	51(41.80%)

Table-II: Sensitivity, Specificity, PPV, NPV and Accuracy of GeneXpert MTB (n=122)

	Culture Positive	Culture Negative
GeneXpert MTB Positive	63 (51.64%)	6 (4.92%)
GeneXpert MTB Negative	5 (4.10%)	48 (39.34%)

*Sensitivity= TP/(TP+FN)= 63/(63+5)*100=92.64%*
*Specificity= TN/(TN+FP)= 48/(48+6)*100=85.71%*
*Positive Predictive Value= TP/(TP+FP)*100= 63/(63+6)*100= 91.30%*
*Negative Predictive Value= TN/(TN+FN)*100= 48/(48+5)*100= 90.56%*
*Diagnostic Accuracy=(TP+TN)/All patients*100 = (63+48)/122*100=90.98%*

DISCUSSION

This study had a cohort of 122 patients, 66 (54.10%) were males. The cough 95(77.87%) was most common pulmonary manifestation of the disease followed by fever 79(64.57%) and weight loss 74(60.66%). The anorexia 20(16.39%) and haemoptysis 8(6.56%) were the least common reported symptoms. The GeneXpert MTB was negative in 51(41.80%) patients. The GeneXpert MTB was reported high positive in 41(33.61%), medium positive in 17(13.93%), low positive in 8(6.56%), and very low positive in 5(4.10%). The GeneXpert MTB was found 92.64% sensitive, 85.71% specific and 90.98% accurate. The positive predictive and negative predictive values were 91.30% and 90.56% respectively. These results show the GeneXpert MTB to be reasonably sensitive, specific and accurate. The findings were comparable to the observations reported by Omar A, *et a*l.[11] However the specificity in our study was less compared to the one in their study (94.4%).

A study conducted in Nepal also looked for diagnostic accuracy of GeneXpert MTB in smear negative patients, the researchers found very promising specificity, PPV and NPV while sensitivity (74.3%) was low.[12] Our study showed comparable results in case of NPV, PPV while it came the opposite in case of sensitivity and specificity. This change in values might be due procedural differences as in our study we used BAL samples while they used carefully collected standard sputum samples. In another research, the GeneXpert MTB/ RIF was found highly sensitive and specific for detecting MTB, the findings are coherent with our findings.[13]

In a study by Rakha et al done in China also tested the GeneXpert MTB role in detecting MTB in smear negative TB suspected cases.[14] They had GeneXpert MTB sensitivity (81.25%), specificity (95.42%) and accuracy (94.08%) respectively. Similarly, research done by Malik *et al*., showed a sensitivity (86.36%) and specificity (84.1%) for GeneXpert MTB in smear negative patients.[15] These findings are close to the observations of our study. Thus, potentiating the role of GeneXpert MTB in diagnosis of such cases. The lower specificity in this study is due to comparison with gold standard i.e. culture on LJ Media. Similar differences with variable increment or decrement in specificity has been reported by other researchers as well.[16-18] Moreover, these near cut differences in end results can be plausibly explained by different techniques of sampling, sample handling and culture media and populations demographics.

This study had comparatively low specificity due to increased number of false positive results. Gowda *et al*., conducted a study which showed false positive results were more commonly seen in patients previously treated TB treated patients. The reason for false positive results can also be explained by use of prophylactic antibiotics which might also affect the MTB.[19] Moreover in another study it was postulated that the patients who were previously treated in a 5 years time yield false positive results as a dead MTB can be present in bronchial tree.[17] Zhang *et al.,* in meta-analysis reported the lower specificity of GeneXpert MTB might be due to higher sensitivity.[20] This comes

at a cost as these false positive results like 4.92% in this study can lead to unnecessary long and cumbersome treatment of anti-tuberculous therapy.[21] Despite all these limitations of GeneXpert MTB, it remains as one of the most suitable option as it is safe, quick and has promising diagnostic accuracy.

LIMITATIONS OF STUDY

This study has certain limitations. In this study, we took sputum smear negative patients and did not do any smear studies on BAL or washings. We suggest a large, multicentre clinical trial should be done to study patients which a suspected of having TB as in our population injudicious use of antibiotics, steroids and incomplete antituberculosis therapy remains a concerning issue.

CONCLUSION

The higher number of pulmonary TB positive patients (55.73%) on bronchoalveolar lavage/ washings in smear negative patients shows the importance of GeneXpert MTB. To conclude, the GeneXpert MTB can be used as a diagnostic modality in smear negative patients who are highly suspected of pulmonary TB.

Conflict of Interest: None.

Funding Source: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

MF & MA: Data acquisition, critical review, approval of the final version to be published.

MI & EE: Conception, study design, drafting the manuscript, approval of the final version to be published.

MF & OAJ: Data analysis, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. TeBeek LA, van der Werf MJ, Richter C, Borgdorff MW. Extrapulmonary tuberculosis by nationality, The Netherlands, 1993-2001. Emerg Infect Dis 2006; 12(9): 1375-1382. https://doi.org/10.3201/eid1209.050553
2. WHO. The top 10 causes of death [Internet]. World Health Organization: WHO. 2020. Available from: https://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death
3. Mello FCQ, Silva DR, Dalcolmo MP. Tuberculosis: where are we? J Bras Pneumol 2018; 44(2): 82. https://doi.org/10.1590/s1806-37562017000000450
4. DGHS. TB Control Program. DGHS Gop 2014. Available from: https://dghs.punjab.gov.pk/tb_control Das PK, Ganguly SB, Mandal B. Sputum smear microscopy in tuberculosis. Biomed Biotechnol Res J 2019; 3: 77-79.
5. Moore DF, Guzman JA, Mikhail LT. Reduction in turnaround time for laboratory diagnosis of pulmonary tuberculosis by routine use of a nucleic acid amplification test. Diagn Microbiol Infect Dis 2005; 52(3): 247-254. https://doi.org/10.1016/j.diagmicrobio.2005.02.014
6. World Health Organization. International Standard for Tuberculosis Care. 3rd ed. Geneva: World Health organization; 2014 www.who.int/TB/publications/standards-TB-care-2014
7. Mustafa H, Shah NN, Shahnawaz M, Yousuf M. Role of Gene Xpert in smear negative pulmonary tuberculosis. Indian J Tuberc 2022; 69(4): 552-557. https://doi.org/10.1016/j.ijtb.2021.08.035
8. World Health Organization. Xpert MTB/RIF Assay for Diagnosis of Pulmonary and Extrapulmonary TB in Children and Adults: Policy Update. Geneva: World Health Organization; 2013.
9. Khalil KF, Butt T. Diagnostic yield of Bronchoalveolar Lavage gene Xpert in smear-negative and sputum-scarce pulmonary tuberculosis. J Coll Physicians Surg Pak 2015; 25(2): 115-118.
10. Omar A, Elfadl AEA, Ahmed Y, Hosny M. Valuing the use of GeneXpert test as an unconventional approach to diagnose pulmonary tuberculosis. Egypt J Bronchol 2019; 13(3): 403–407. https://doi.org/10.4103/ejb.ejb_88_18
11. Rimal R, Shrestha D, Pyakurel S, Poudel R, Shrestha P, Rai KR, et al. Diagnostic performance of GeneXpert MTB/RIF in detecting MTB in smear-negative presumptive TB patients. BMC Infect Dis 2022; 22(1): 321. https://doi.org/10.1186/s12879-022-07287-5
12. Barnard DA, Irusen EM, Bruwer JW, Plekker D, Whitelaw AC, Deetlefs JD, et al. The utility of Xpert MTB/RIF performed on bronchial washings obtained in patients with suspected pulmonary tuberculosis in a high prevalence setting. BMC Pulm Med 2015; 15: 103. https://doi.org/10.1186/s12890-015-0086-z
13. Rakha MA, Ali A, Hassan W, Al-Anbay E. GeneXpert analysis of bronchoalveolar lavage (BAL) samples: promising diagnostic modality in patients with smear-negative pulmonary tuberculosis. Egypt J Bronchol 2022; 16(1): 68. https://doi.org/10.1186/s43168-022-00171-5
14. Malik MI, Ejaz T, Ahmed J, Arshad K, Jamal Y, Zohfreen Z et al. Diagnostic accuracy of GeneXpert assay and comparison with smear AFB on bronchial washings in sputum negative suspected pulmonary tuberculosis. Pak Armed Forces Med J 2019; 69(4): 857-862.
15. Ullah I, Javaid A, Masud H, Ali M, Basit A, Ahmad W, et al. Rapid detection of Mycobacterium tuberculosis and rifampicin resistance in extrapulmonary tuberculosis and sputum smear-negative pulmonary suspects using Xpert MTB/RIF. J Med Microbiol 2017; 66(4): 412-418. https://doi.org/10.1099/jmm.0.000449
16. Pan X, Yang S, Deighton MA, Qu Y, Hong L, Su F et al. A Comprehensive Evaluation of Xpert MTB/RIF Assay With Bronchoalveolar Lavage Fluid as a Single Test or Combined With Conventional Assays for Diagnosis of Pulmonary Tuberculosis in China: A Two-Center Prospective Study. Front Microbiol 2018; 9: 444. https://doi.org/10.3389/fmicb.2018.00444
17. Theron G, Peter J, Meldau R, Khalfey H, Gina P, Matinyena B, et al. Accuracy and impact of Xpert MTB/RIF for the diagnosis of smear-negative or sputum-scarce tuberculosis using bronchoalveolar lavage fluid. Thorax 2013; 68(11): 1043-1051. https://doi.org/10.1136/thoraxjnl-2013-203485
18. Gowda NC, Ray A, Soneja M, Khanna A, Sinha S. Evaluation of Xpert®Mycobacterium tuberculosis/rifampin in sputum-smear negative and sputum-scarce patients with pulmonary tuberculosis using bronchoalveolar lavage fluid. Lung India 2018; 35(4): 295-300. https://doi.org/10.4103/lungindia.lungindia_412_17
19. Zhang M, Xue M, He JQ. Diagnostic accuracy of the new Xpert MTB/RIF Ultra for tuberculosis disease: A preliminary systematic review and meta-analysis. Int J Infect Dis 2020; 90: 35-45. https://doi.org/10.1016/j.ijid.2019.09.016
20. Osei Sekyere J, Maphalala N, Malinga LA, Mbelle NM, Maningi NE. A Comparative Evaluation of the New Genexpert MTB/RIF Ultra and other Rapid Diagnostic Assays for Detecting Tuberculosis in Pulmonary and Extra Pulmonary Specimens. Sci Rep 2019; 9(1): 16587. https://doi.org/10.1038/s41598-019-53086-5