The Professional Medical Journal
www.theprofesional.com

DOI: 10.29309/TPMJ/2020.27.3.3279

ORIGINAL PROF-0-3279

DIAGNOSIS OF TUBERCULOSIS PATIENTS BY TRANSBRONCHIAL BIOPSY AND BRONCHOALVEOLAR LAVAGE IN SPUTUM SMEAR NEGATIVE PULMONARY TUBERCULOSIS.

Mahwish Niaz[1], Mumtaz Ahmad[2], Kanwal Fatima[3], Faiza Kazi[4], Hassan Salim[5], Irum Sohail[6]

1. MBBS, FCPS (Histopathology)
Assistant Professor
Foundation University Medical College, Islamabad.
2. MBBS, MCPS, FCPS
Professor Pathology
Foundation University Medical College, Islamabad.
3. MBBS, MCPS, MRCP, FCPS
Assistant Professor Pulmonology
Fauji Foundation Hospital, Rawalpindi.
4. MBBS, MCPS, FCPS
Professor Pathology
Foundation University Medical College, Islamabad.
5. MBBS, FCPS
Registrar Medicine
Fauji Foundation Hospital, Rawalpindi.
6. MBBS, FCPS
Assistant Professor Pathology
Foundation University Medical College, Islamabad.

Correspondence Address:
Dr. Mahwish Niaz
Senior lecturer Pathology
Foundation University Medical College, Islamabad.
mahwishhassan83@gmail.com

Article received on:
12/02/2019
Accepted for publication:
15/08/2019

ABSTRACT… Objectives: To calculate and compare the diagnostic accuracy of transbronchial biopsy and bronchoalveolar lavage smear in sputum negative patients. **Study Design:** Cross-sectional validation study. **Setting:** Department of histopathology, Foundation University Medical College, Islamabad and Department of Pulmonology and Microbiology, Fauji Foundation Hospital, Rawalpindi. **Period:** From May 2016 to May 2017. **Materials & Methods:** It comprised 96 patients who underwent bronchoscopy. Transbronchial biopsy, bronchoalveolar lavage smear preparation and bronchoalveolar lavage culture was performed on specimens of all patients. **Results:** Out of 96 patients 22 (22.91%) patients were actually having tuberculosis whereas 74 (77%) had only clinical and radiological suspicion of tuberculosis. The mean age of patients was 43 years with a standard deviation of ±19.1 .The age range was 12-80 years. The sensitivity, specificity, positive predictive value, negative predictive value and true positives of transbronchial biopsy were 68.1%, 77%, 46.8%, 89% and15.62% while the values for bronchoalveolar lavage were 50%, 97.29%, 84.6%, 86.7% and 11.45% respectively. Thus, the diagnostic accuracy calculated for transbronchial biopsy and bronchoalveolar lavage was 75% and 13.54% respectively. **Conclusions:** Bronchoscopy should be done in all sputum negative tuberculosis patients having strong clinical and radiological suspicion to obtain transbronchial biopsy and bronchoalveolar lavage for timely diagnosis, treatment and prevention of disease transmission as well as to avoid empirical treatment and its side effects in patients having no tuberculosis. The diagnostic accuracy of transbronchial biopsy is almost 5 times more compared to bronchoalveolar lavage smear.

Key words: Bronchoalveolar Lavage, Sensitivity, Specificity, Transbronchial Biopsy.

Article Citation: Niaz M, Ahmad M, Fatima K, Kazi F, Salim H, Sohail I. Diagnosis of tuberculosis patients by transbronchial biopsy and bronchoalveolar lavage in sputum smear negative pulmonary tuberculosis. Professional Med J 2020; 27(3):499-505. **DOI:** 10.29309/TPMJ/2020.27.3.3279

INTRODUCTION

Tuberculosis (TB) was declared a global health emergency by World Health Organization (WHO). According to WHO Global TB Report 2015, there were an estimated 10.4 million incident cases of TB globally.[1] Pakistan ranked 5th among the the list of 22 high TB burden countries.[2] It occupies 4th position in mortality caused by tuberculosis with a death rate of 34 per 100,000 population[3], incidence rate of 181 cases / 100,000 and a prevalence of 398 cases /100,000 population.[4] Approximately 620, 000 people in Pakistan have tuberculosis, 410, 000 are newly infected cases and 59 000 die of it yearly.

The causative organism of TB is Mycobacterium tuberculosis. In usual routine the diagnosis of tuberculosis is established by obtaining sputum sample from suspected patients.[5] A sputum smear is then prepared and stained for acid fast bacilli which stain the Mycobacterium. If the stain is positive then patient is considered to be sputum positive. However 40-60 % of patients with TB would not show a positive stain with AFB and will be labeled as sputum negative patients.[6] According to WHO guidelines, sputum negative pulmonary tuberculosis is defined on the basis of at least three negative smears for AFB but with radiographic abnormalities consistent with active pulmonary tuberculosis and lack of clinical response to at least two weeks of broad spectrum antibiotics.

Sputum negative patients are a persistent source of infection for the community.10-20% of disease transmission at population level is attributed to smear negative pulmonary tuberculosis cases, so they must be diagnosed on urgent basis to reduce transmission to healthy individuals as well as to decrease morbidity and mortality in suffering individuals.[7]

The clinician will suspect tuberculosis when patient has two or more of the symptoms like cough for more than 3 weeks, fever, weight loss, heavy night sweats, tiredness and loss of appetite.[8]

The radiological predictors of tuberculosis can be anyone or all of the following: cavitations, consolidation, pleural effusion, atelectasis and mediastinal lymphadenopathy.[9] When these clinical and radiological findings are present but sputum for AFB is negative, fiberoptic bronchoscopy, is a diagnostic modality, indicated for rapid diagnosis in suspected TB patients.

Transbronchial biopsy (TBB) and bronchoalveolar lavage (BAL) is obtained after bronchoscopy. Microscopy of bronchoalveolar lavage smear is done for detection of AFB and histopathology of transbronchial biopsy is done to detect caseating granuloma. BAL Culture is a gold standard test for the diagnosis of tuberculosis with a sensitivity of 92.7%[10], specificity of 100%, positive predictive value (PPV) of 100% and negative predictive value (NPV) of 63%[11], but it takes about 6-8 weeks to give a positive result.

In this study, we diagnose sputum smear negative patients by transbronchial biopsy and bronchoalveolar lavage and also compared the diagnostic accuracy of these two modalities. BAL smear and TBB have variable sensitivities and specificities in different previous studies. The sensitivities of BAL smear are 19%[11], 34%[12], 38%[13] and 88%.[12] The specificities of BAL smear are 79%[12], 96%[11], and 100%[13], similarly the sensitivities of TBB are 42%[11] and 89%[14] and specificities are 92%[11] and 100%.[13] Although bronchoscopy done to obtain BAL smear and TBB, is an invasive procedure but it gives quick results compared with culture which takes about 6-8 week to give a positive result, thus will help in early initiation of treatment, reduction in transmission, morbidity and mortality of patients with a negative sputum for AFB.

MATERIALS AND METHODS

This cross-sectional validation study was conducted at the department of histopathology, Foundation University Medical College, Islamabad and department of pulmonology and microbiology, Fauji Foundation hospital, Rawalpindi for a duration of one year from May 2016 to May 2017. Non-probability, consecutive sampling technique was applied.

The number of patients underwent bronchoscopy were 96.The inclusion criteria were all patients irrespective of gender and age, strong clinical suspicion of tuberculosis, chest X-ray suspicious of tuberculosis and 3 consecutive negative sputum smear for AFB.

The exclusion criteria were HIV positive cases, patients with history of bleeding diathesis, myocardial infarction or arrhythmias, asthma, suspected or known pregnancy, signs of cardiac and respiratory failure and any malignancy, patients with history of antituberculous treatment for more than one month, any contraindication for transbronchial biopsy and un co-operative patients.

Fiberoptic Bronchoscopy was done to obtain transbronchial biopsy and bronchoalveolar lavage fluid. After informed consent patients underwent bronchoscopy and were nil by mouth for4 to 6 hours prior to the procedure. 30-45 minutes prior to bronchoscopy patients were premedicated with 0.6mg atropine and nebulization with 2% xylocaine was done. Bronchoscopy was carried out under local anesthesia and transbronchial biopsy was performed under fluoroscope by General Electronics Brivo OEC 850.

The biopsy was put in formalin and was received by the department of Histopathology. BAL was obtained and was send to Microbiology

department for smear preparation and for culture on Lowenstein Jensen media. At the end of procedure, patients were observed for the development of any complications like pneumothorax, hemorrhage or cardiac arrhythmias for 24-48 hours.

Statistical Analysis

All the data was analyzed using the software SPSS version 17. Descriptive statistics were used to describe the data for quantitative and qualitative variables. For age, mean and standard deviation was calculated. For qualitative variables i.e. sex, true positives and true negatives, frequency and percentages were calculated .2x2 tables were constructed to find out the validity of transbronchial biopsy and BAL smear for AFB which is given below with the calculation formulas.

Transbronchial Biopsy	BAL Culture	
	Culture Positive	Culture Negative
Granuloma positive	True positive / a	False positive / b
Granuloma negative	False negative / c	True negative / d

BAL Smear for AFB	BAL Culture	
	Culture Positive	Culture Negative
AFB positive	True positive / a	False positive / b
AFB negative	False negative / c	True negatives / d

Sensitivity = a/a+c x100
Specificity =d/d+b x 100
Positive predictive value= a/a+b x100
Negative predictive value=d/c+d x100
Diagnostic accuracy= a+d/a+b+c+d x100

RESULTS

The female to male ratio was 3:1 with 72 female patients and 24 male patients. In 22 out of 96 patients, which were actually having tuberculosis proved by culture, 18 patients were females and 4 were males.74 (77%) of 96 cases, had clinical and radiological suspicion of tuberculosis and only 22 (22.91%) patients actually had tuberculosis. Mean age was 43 years with standard deviation of ± 19.18. The age range was 12-80 years.

32 patients exhibited granulomatous inflammation (caseating as well as non-caseating) on transbronchial biopsy. Out of 32 individuals, 15 cases were actually having tuberculosis as proven by a subsequent positive BAL culture.17 of 32 patients had granulomatous inflammation on transbronchial biopsy but don't have tuberculosis which was declared after a negative BAL culture.

There were 7 patients who had a positive BAL culture but no granuloma was found on transbronchial biopsy. 57 patients had no granuloma on biopsy and their BAL culture was also negative (Table-I). From these values we calculated that transbronchial biopsy had a sensitivity of 68.1%, specificity of 77%, positive predictive value of 46.8% and negative predictive value of 89% (Table-II).

BAL smear of only 13 patients revealed Mycobacterium tuberculosis with Ziehl-Neelsen stain.11 of 13 cases were actually having tuberculosis which was later on proved by culture positivity in these patients. 2 of 13 cases don't have tuberculosis because the culture results were negative.

11 patients were culture positive but BAL smear negative and 72 patients were BAL smear as well as culture negative (Table-III). BAL smear had a sensitivity of 50%, specificity of 97.29%, positive predictive value of 84.6%, and negative predictive value of 86.7% as (Table-II).

Transbronchial Biopsy	BAL Culture	
	Culture Positive	Culture Negative
Granuloma positive	15 (a)	17(b)
Granuloma negative	7(c)	57(d)

Table-I. 2x2 table showing tranbronchial biopsy and culture results.

Sensitivity = (a/a+c) x 100 = {15/(15+7)} x 100 = 68.1%
Specificity = (d/b+d) x 100 = {57/(17+57)} x 100 = 77%
Positive Predictive Value = (a/a+b) x 100 = {15/(15+17)} x 100 = 46.8%
Negative Predictive Value = (d/c+d) x 100 = {57/(7+57)} x 100 = 89%
Diagnostic Accuracy = (a+d)/(a+b+c+d) x 100 = 75%

Parameter	Transbronchial Biopsy	BAL Smear
True Positives	15	11
False Positives	17	2
True Negatives	57	72
False Negatives	7	11
Sensitivity	68.1%	50%
Specificity	77%	97.29%
Positive Predictive Value	46.8%	84.6%
Negative Predictive Value	89%	86.7%
Diagnostic Accuracy	75%	13.54%

Table-II. Table showing comparison of diagnostic accuracy of TBB and BAL smear for AFB in sputum smear negative pulmonary tuberculosis patients (n=96).

BAL smear for AFB	BAL Culture	
	Culture Positive	Culture Negative
AFB positive	11(a)	2(b)
AFB negative	11(c)	72(d)

Table-III. 2x2 table showing bronchoalveolar lavage and culture results.

Sensitivity = (a/a+c) x 100 = {11/(11+11)} x 100 = 50%
Specificity = (d/b+d) x 100 = {72/(2+72)} x 100 = 97.29%
Positive Predictive Value = (a/a+b) x 100 = {11/(11+2)} x 100 = 84.6%
Negative Predictive Value = (d/c+d) x 100 = {72/(11+72)} x 100 = 86.7%
Diagnostic Accuracy = (a+d)/(a+b+c+d) x 100 = 13.54%

DISCUSSION

Sputum negative patients are unaware about having tuberculosis and therefore their accurate diagnosis and treatment is delayed. This leads to increase morbidity and mortality among these patients. Furthermore they continue to pose a threat of transmitting the disease to other people. Therefore accurately diagnosing these patients is very important for the timely management and prevention of disease spread.

While comparing mean age and age range with our study we found that a study was conducted by CaymmiAL[14] which showed a mean age value of 39 years. Likewise, a study carried out in china, provided a mean age value of 47.2 years[15] So, age range of patients of our study, is almost comparable with values given by international studies.

The most frequent symptoms with which the patient presented in this series, were fever and cough which constituted 37.1% while a study done by Waheed K et al in 2011 gave a value of 22.5%. Ansari J et al also showed cough and fever as the most frequent presenting complaint in his study.[16] Other clinical features included weight loss, dyspnea, hemoptysis, weakness, lethargy and pleural chest pain in ours series as well as in various other studies.

Cavitations and consolidation were the most frequent radiological observation in our study. Wekesa C et al also give consolidation as the most frequent finding but provided a value of 88.2% which is quite high compared to our study and cavitations as the second most frequent chest X-ray pattern with a value of 11.8%.[17] Another study done by Eini P gives a percentage of 30.4 for consolidation and 20.9[18] forcavitary lesions.8.33% patients, in our study presented with hilar and mediastinal lymphadenopathy while in Wekesa C et al studies 3.9% cases showed hilar lymphadenopathy on chest X-ray.[17]

Comparison of transbronchial biopsy with other studies shows variable results. A study done by Jacomelli et al in 2012 done on bronchoscopically obtained specimens in sputum negative tuberculous patients found 42% sensitivity and 53% negative predictive value of transbronchial biopsy.[11] These values were lower as compared to our cases and a specificity of 92% and positive predictive value of 88%which were higher than our studies.[11] Similarly Figueiredo et al did a research project in 2005 which also showed lower values of sensitivity of 31.8% and a negative predictive value of 38.7% and higher values of 100% specificity and positive predictive value for transbronchial biopsy.[19] Another study showed 89% sensitivity of transbronchial biopsy in HIV negative patient and 67% sensitivity for HIV positive patients.[14]

Gupta N et al in his research work gave diagnostic yield of transbronchial biopsy of 66.6% which is very close to the value obtained in our study.[20] Evidence from another study done in 2010 by Altaf B A et al came with a diagnostic accuracy of

61.6% which is a little lower than our series but is comparable.[21]

The Iran journal of pathol published an article of Nikbakhsh N et al which provided a value of sensitivity of 60% for BAL smear slightly higher than our study, the specificity of 91%, positive predictive value of 89% which was comparable to our study whereas a lower negative predictive value of 64% contrasted to the current study.[22]

Conde MB et al concluded that BAL smear had a sensitivity of 38.1 %, specificity and positive predictive value of 100% and a negative predictive value of 54.9%.[13] This study revealed a lower sensitivity and negative predictive value, matching specificity and a higher positive predictive value compared to our study.

A study conducted in University of Sao Paulo showed a sensitivity of 19%, specificity of 96%, positive predictive value of 84% and negative predictive value of 54% for BAL smear. The sensitivity and negative predictive values of this study were lower than current study but specificity and positive predictive value were comparable.[11]

In Brazil, Figueiredo et al carried out a study on 34 patients and revealed 13.6% sensitivity, 100% specificity and positive predictive value and 38.7% negative predictive value for BAL smear. This study showed a lower sensitivity and negative predictive value, almost equal specificity and a higher positive predictive value compared to our study.[19]

We also observed that, a single test alone, that is direct examination of BAL smear for AFB and histopatholgical examination of TBB has low diagnostic sensitivity of 50% and 68.1% respectively. So, while doing bronchoscopy both these specimens should be collected in order to diagnose maximum number of sputum smear negative pulmonary tuberculosis patients.

In the end of our study we come to the conclusions that bronchoscopy must be done in the sputum negative tuberculous suspects and TBB has a higher diagnostic accuracy compared to BAL smear. Whenever bronchoscopy is done, transbronchial biopsy specimen should be collected because it provides a greater quantity of material to the histopathologist for analysis and expedite the identification of tuberculosis granuloma. These granulomas in tissue along with clinical and radiological findings allow treatment initiation before culture results are available.

CONCLUSIONS

Bronchoscopy should be done in all sputum negative tuberculosis patients, having strong clinical and radiological suspicion, to obtain transbronchial biopsy and bronchoalveolar lavage for timely diagnosis, treatment and prevention of disease transmission as well as to avoid empirical treatment and its side effects in patients having no tuberculosis.

The diagnostic accuracy of transbronchial biopsy is almost 5 times more compared to bronchoalveolar lavage smear. These modalities alone have low diagnostic sensitivities but it can be increased when both tests are performed together. Therefore, both tests should be done in all patients who undergo bronchoscopy to diagnose maximum number of sputum negative tuberculous patients.

ACKNOWLEDGEMENT

I would like to pay my gratitude to all my colleagues especially Dr. Mahwish Javed, Dr. Asma and all laboratory attendants especially Mr. Mushtaq Hussain who have been very helpful in the completion of my research work.

Limitations and conflict of interest

There are no limitation and conflict of interest in our study

REFERENCES

1. Kyu HH, Maddison ER, Henry NJ, Mumford JE, Barber R, Shields C et al. **The global burden of tuberculosis: Results from the global burden of disease study 2015.** Lancet Infect Dis. 2018; 18(3):261-284.

2. Ahmad T Haroon, Khan M, Khan MM, Ejeta E, Karami M, Ohia C. **Treatment outcome of tuberculosis patients under directly observed treatment short course and its determinants in Shangla, Khyber-Pakhtunkhwa, Pakistan:** A retrospective study. Int J Mycobacteriol. 2017; 6(4):360-364.

3. Khan AH. Tuberculosis control in Sindh, **Pakistan: Critical analysis of its implementation.** J Infect Public Health. 2017; 10(1):1-7.

4. Tahseen S, Shahnawaz H, Riaz U, Khanzada FM, Hussain A, Aslam W et al. **Systematic case finding for tuberculosis in HIV-infected people who inject drugs: Experience from Pakistan.** Int J Tuberc Lung Dis. 2018; 22(2):187-193

5. Rasool G, Riaz M, Mahmood Z, Mohy-Ud-Din R, Akhtar J, Javed I. **Effects of household bleach on sputum smear microscopy to concentrate acid fast bacilli for the diagnosis of pulmonary tuberculosis.** J Biol Regul Homeost Agents. 2018; 32(3):607-611.

6. Keeratichananont W, Nilmoje T, Keeratichananont S, Rittatorn J. **Diagnostic yield and safety of sputum induction with nebulized racemic salbutamol versus hypertonic saline in smear-negative pulmonary tuberculosis.** The R Adv Respir Dis. 2015; 9(5):217-23.

7. Campos LC, Rocha MV, Willers DM, Silva DR. **Characteristics of patients with smear-negative pulmonary tuberculosis (TB) in a region with high TB and HIV prevalence.** PLoS One. 2016; 11(1):e0147933.

8. Badane AA, Dedefo MG, Genamo ES, Bekele NA. **Knowledge and healthcare seeking behavior of tuberculosis patients attending Gimbi General Hospital, West Ethiopia.** Ethiop J Health Sci. 2018; 28(5):529-538.

9. Mathur M, Badhan RK, Kumari S, Kaur N, Gupta S. **Radiological manifestations of pulmonary tuberculosis - A comparative study between immuno compromised and immuno competent patients.** J Clin Diagn Res. 2017 Sep; 11(9):TC06-TC09.

10. Ceyhan I, Simşek H, Tarhan G. **[Comparison and evaluation of Lowenstein-Jensen medium and 2% Ogawa medium for the diagnosis of tuberculosis].** Mikrobiyol Bul. 2012; 46(1):33-8

11. Jacomelli M,Silva PR,Rodrigues AJ,Demarzo SE,Seicento M,Figueiredo VR. **Bronchoscopy for the diagnosis of Pulmonary tuberculosis in patients with negative sputum smear microscopy results.** J Bras Pneumol 2012; 38:167-73.

12. Hung HC, Chan CH, Tsao SM, Chen SC, Wu TC, Lu MC et al. **Effectiveness of the BD Probe Tec ET system for detection of Mycobacterium tuberculosis complex in sputum and bronchoalveolar lavage specimens.** Braz J Infect Dis 2012; 16: 242-9.

13. Conde MB, Soares SL, Mello FC, Rezende VM, Almeida LL, Reingold AL et al. **Comparison of sputum induction with fiber optic bronchoscopy in the diagnosis of tuberculosis: Experience at an acquired immune deficiency syndrome reference center in Rio deJaneiro, Brazil.** Am J Respir Crit Care Med 2000; 162:2238-40.

14. Caymmi AL, Silveira MAS, Montal G, Lemos ACM. **Role of the fiber optic bronchoscopy in the diagnosis of patients with suspected pulmonary.** J Bras P neumol 2004; 30:39-45.

15. Lu Y, Zhu Y, Shen N, Tian L, Sun Z. **Evaluating the diagnostic accuracy of the Xpert MTB/RIF assay on bronchoalveolar lavage fluid: A retrospective study.** Int J InfectDis. 2018; 71:14-19.

16. Ansari J, Anwar N, Abbas SW. **Diagnostic yield of fiber optic bronchoscopy in smear negative new cases of pulmonary tuberculosis.** Pak J Chest Med 2005; 11:27-38.

17. Wekesa C, Kirenga BJ, Joloba ML, Bwanga F, Katamba A, Kamya MR. **Chest X-ray vs. Xpert ® MTB/RIF assay for the diagnosis of sputum smear-negative tuberculosis in Uganda.** Int J Tuberc Lung Dis. 2014; 18:216-9.

18. Eini P, Osquee HO, Nasab MS, Khiyabani FN, Rahighi AH. **Chest radiological features among patients with smear positive pulmonary tuberculosis.** Caspian J Intern Med. 2013; 4:777–80.

19. Figueiredo VR, Vargas FS, Seiscento M, Kawakama J, Acencio M M, Moreira CL et al. **Diagnosis of pulmonary tuberculosis in patients with clinical or radiological evidence of disease and negative sputum.** Chest 2005; 128:400S.

20. Gupta N, Singh GC, Rana MK. **Histopathological yield in different types of bronchoscopic biopsies in proven cases of pulmonary tuberculosis.** Indian J Pathol Microbiol.2015; 58(4):439-42

21. Altaf BA, Gupta R, Haq I, Varudkar HG. **Diagnosing sputum/smear- negative pulmonary tuberculosis: Does fiberoptic bronchoscopy play a significant role?** Lung India 2010; 27:58-62.

22. Nikbakhsh N, Bayani M, Siadati S. **The value of bronchoalveolar lavage in the diagnosis of sputum smear-negative pulmonary tuberculosis.** Iran J Pathol. 2015; 10(1):35-40.

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Mahwish Niaz	Conception and design of work.	
2	Mumtaz Ahmad	Conception and design of work.	
3	Kanwal Fatima	Work design and revision of concept.	
4	Faiza Kazi	Final approval of version.	
5	Hassan Salim	Drafting of work.	
6	Irum Sohail	Drafting of work.	