Print ISSN: 2321-6379
Online ISSN: 2321-595X
DOI: 10.17354/ijss/2017/464

Original Article

Role of Cartridge-based Nucleic Acid Amplification Test in Diagnosis of Tuberculous Pleural Effusion Compared to Tuberculous Empyema in HIV-seronegative Patients

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Abstract

Introduction: The tuberculous pleural effusion is the second most common form of extrapulmonary tuberculosis (TB) in high TB burden country such as India. Since the endorsement of cartridge-based nucleic acid amplification test (CBNAAT) in the diagnosis of extrapulmonary TB by the World Health Organization, there are several publications assessing the diagnostic accuracy of CBNAAT in pleural effusion. However, there is very little data on its role in tuberculous empyema which is a very common clinical presentation in this part of the world. The objective of the present study is to reevaluate the role of CBNAAT in the diagnosis of tuberculous pleural effusion compared to tuberculous empyema.

Materials and Methods: This was a prospective observational study where patients with a clinical and radiological diagnosis of pleural effusion attending the outpatient department and emergency of a tertiary care hospital were enrolled in the study after obtaining consent and satisfying the set inclusion and exclusion criteria over a period of 1 year. All the pleural fluid samples were sent for CBNAAT for *Mycobacterium tuberculosis* and BACTEC culture along with other routine tests. Statistical analysis was done using SPSS version 20.0 (SPSS Inc., Chicago, IL) software for MS-Windows.

Results: Among a total of 105 patients of tuberculous pleural effusion (male 68 and female 37) with the mean age 36.23 ± 13.45 years, 10 (male 5, female 5) had tuberculous empyema. Pleural fluid acid-fast bacilli smear, mycobacterial culture, and CBNAAT were positive in 8.57%, 20%, and 15.23%, respectively. The sensitivity and specificity of CBNAAT, considering mycobacterial culture positivity as standard reference, were 4.76% (95% confidence interval [CI] 0.99-13.29) and 87.5% (95% CI 71.01-96.49), respectively, for tuberculous pleural effusion, while they are 100% (95% CI 66.37-100) and 100% (95% CI 2.5-100) in tuberculous empyema, respectively. A single case of rifampicin resistance was detected among tuberculous effusion without empyema which was later confirmed by solid and liquid culture.

Conclusion: CBNAAT is a useful rapid diagnostic tool for suspected tuberculous pleural effusion/empyema considering the advantage of rapid test result and information about drug resistance pattern, especially in high burden country such as India.

Key words: Cartridge-based nucleic acid amplification test, Mycobacterial culture, Tuberculous empyema, Tuberculous pleural effusion

Access this article online

www.ijss-sn.com

Month of Submission : 07-2017
Month of Peer Review : 08-2017
Month of Acceptance : 09-2017
Month of Publishing : 09-2017

INTRODUCTION

The tuberculous involvement of pleura[1] The diagnosis of tuberculous pleural effusion with confidence is still a challenge before physicians even more than 100 years after the discovery of TB bacilli. The demonstration of *Mycobacterium tuberculosis* (MTB) in pleural fluid or

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demonstration of granuloma in biopsy specimen is considered as a gold standard; however, the sensitivity and specificity of such investigations are widely varied ranging from >10% in pleural fluid smear,[2] 15-35% for pleural fluid culture,[3,4] and ranging from 60% to 100% in biopsy specimen depending on the procedure adapted.[2,5-7] The World Health Organization (WHO) has endorsed cartridge-based nucleic acid amplification test (CBNAAT) as a rapid diagnostic test for the detection of MTB and rifampicin resistance on December 2010. Later, the WHO published a policy update on 2013 emphasizing the role of Xpert MTB/RIF in early diagnosis of extrapulmonary TB.[8] Since then, several studies and meta-analysis reports have been published assessing sensitivity and specificity of CBNAAT in pleural effusion, ranging from 15-55% and 98-100%, respectively.[5,9-11] This wide variation is partly due to the use of different reference standards adapted for the analysis. Recently, the index-TB guidelines on extrapulmonary TB, published in 2016, suggest strong recommendation against routine use of Xpert MTB/RIF in the diagnosis of pleural TB.[1] Although there are several publications on CBNAAT in pleural effusion, there is a paucity of data among tuberculous empyema.

The purpose of the present study is to reevaluate the role of CBNAAT in the diagnosis of tubercular pleural effusion with special reference to tuberculous empyema.

MATERIALS AND METHODS

The present study was a prospective observational and analytical study conducted in a tertiary care hospital in eastern India over a span of 1 year extending from January 2016 to December 2016. The study population was recruited from those attending the outpatient department (OPD) of the Department of Pulmonary Medicine or Emergency Department of the hospital.

The diagnosis of tuberculous pleural effusion was established by a composite reference standard (CRS) which was defined as any one of the following in the present study: (1) Demonstration of tubercle bacilli in the pleural fluid or any specimen collected from other body site except sputum, by Ziehl-Neelsen stain or fluorescent microscopy or CBNAAT or line probe assay or culture (solid or liquid media) or demonstration of caseating granuloma in biopsy specimen and (2) exudative lymphocytic pleural effusion (according to Light's criteria) with adenosine deaminase assay (ADA) >40 IU/L with lymphocyte-to-neutrophil ratio of more than 0.75 and other probable causes of pleural effusion excluded with reasonable certainty with complete radiological and clinical resolution of symptoms at the end of full course of antitubercular drugs (ATDs). The demonstration of either of acid-fast bacilli (AFB) in culture or smear was considered as a gold standard in the diagnosis of tubercular etiology.

The diagnostic criteria for tuberculous empyema are the presence of frank pus with or without demonstration of MTB in smear or culture and/or MTB detected in CBNAAT and/or radiological lesions consistent with diagnosis of active pulmonary TB on chest X-ray or computed tomography (CT) of the thorax (nodular consolidation with or without cavity more in apex, tree in bud appearance) and other obvious causes of empyema excluded.

The inclusion criteria for the study was the age above 12 years, with a clinical and radiological diagnosis of exudative pleural effusion with or without evidence of tuberculous involvement of pulmonary or other extrapulmonary site in the form of lymphadenopathy, and cold abscess.

However, those with HIV seropositive, exudative pleural effusion due to parapneumonic effusion, confirmed malignancy, and transudative effusion were excluded from the study. The patients who did not consent for the full workup or remained undiagnosed at the end of the complete workup were also excluded from the study.

Study Protocol

The patients attending OPD or emergency with clinical and radiological evidence of pleural effusion were recruited for initial screening. After ruling out, cardiac, hepatic, or renal etiology for the effusion a diagnostic thoracentesis was performed under ultrasonography guidance after obtaining consent for the procedure. A minimum of 20-25 ml of pleural fluid sample was collected and sent for physical analysis, chemical tests such as protein, sugar, and ADA, and microbiological tests such as Gram-stain, Ziehl-Neelsen stain, and aerobic and mycobacterial culture, in tube without anticoagulant (red-topped). The pleural fluid cytology and differentials were sent in EDTA-treated vial (purple-topped), and another 5 ml for CBNAAT for MTB in falcon tube and CBNAAT was tested by Cepheid, GX-IV Processing Unit*:* 11.00" weeks × 12.00" h × 11.70" days, GXIV-4-D. The samples were transferred to respective laboratories at the earliest. The samples collected from other sites were transferred to the nearest laboratory as per the Revised National Tuberculosis Control Policy (RNTCP) guideline as relevant to the presenting symptoms. The investigations such as blood glucose, urea, creatinine, baseline liver function test, and complete hemogram were obtained in all. The contrast-enhanced CT thorax, ultrasonography of the abdomen, closed pleural biopsy

with Abram's needle, and other site-specific advanced investigations were done as indicated in selected patients. Blood was also sent for HIV screening at the integrated counseling and testing center of our hospital.

Those who were diagnosed with tuberculous pleural effusion were started on ATDs according to the RNTCP guideline. Those who were diagnosed to have tuberculous empyema were treated with water-seal intercostal tube drainage along with ATDs. All the patients were followed up on a regular basis till the completion of ATD regimen.

The study was conducted after obtaining permission from the Ethics Committee of the Institute and informed consent from the patients.

Statistical Analysis

The statistical analysis was done using SPSS version 20.0 (SPSS inc., Chicago, IL) software for MS-Windows. The descriptive statistical analyses were done to summarize the age-sex distribution of the study population and presence of comorbid conditions. The diagnostic accuracy of CBNAAT as a diagnostic test for tubercular pleural infection was expressed as sensitivity, specificity, positive predictive value and negative predictive value, and likelihood ratios with special emphasis on tuberculous empyema.

RESULTS

A total of 200 patients were admitted with the diagnosis of pleural effusion (male 115 and female 85) in this 1 year period. Six were excluded for HIV seropositivity, 42 as parapneumonic effusion, 33 with malignant pleural effusion, 10 patients refused to consent for advanced invasive diagnostic tests, and in 4 cases, confirmed diagnosis could not be reached at the end of workup and was lost to follow-up. Among 105 patients with diagnosis of tuberculous etiology, 10 patients were diagnosed as tuberculous empyema. The demographic details of the patients with tuberculous effusion are given in Table 1. The pleural fluid AFB smear was positive in 9 out of 105 (8.57%), with only 2 out of 95 (2.1%) in patients with tuberculous pleural effusion subgroup, and mycobacterial culture was positive in 21/105 (20%) cases, of which nine was in empyema subgroup. The pleural biopsy was performed in 35 patients, and the diagnosis was confirmed in 18 (51%). Four out of 10 (40%) patients with empyema had sputum smear positive for AFB. 21 out of 105 (20.9%) had confirmed the diagnosis of TB in other organs like lymph node TB and caries spine by histology and, whereas CBNAAT confirmed diagnosis in 16 out of 105 (15.23%) cases of pleural effusion. The ADA level was more than 70 IU/L in 45 out of 95 (47.4%) cases of pleural effusion with lymphocyte neutrophil ratio more than 0.75 and rest had ADA level between 40 and 70 IU/L. The diagnosis of tuberculous pleural effusion was confirmed by culture and/or HPE of pleural biopsy in 27 out of 105 (25.7%). The efficacy of CBNAAT in detecting MTB as compared to pleural fluid smear staining for AFB is shown in Table 2. The details of CBNAAT result along with status of rifampicin resistance are shown in Table 3. The resistance pattern was later confirmed by liquid and solid culture sensitivity test in the intermediate reference laboratory. In one patient with empyema, both CBNAAT and culture were negative; however, diagnosis of tubercular etiology was confirmed by demonstration of AFB in cold abscess aspirate. The diagnostic accuracy of CBNAAT in comparison with mycobacterial culture positivity as a standard reference for tuberculous empyema as compared to tuberculous effusion is shown in Table 4.

Table 1: Demographic profile of the patients

Demographic profile	TB pleural effusion (*N*=95)	TB empyema (*N*=10)	Significance
Male	63, 66.3%	5, 50%	*P*=0.3064
Female	32, 33.6%	5, 50%	
Age, mean±SD	37.1±13.50726	27.4±10.17841	*P*=0.0291
Proportion of comorbid conditions			
DM	13	1	*P*=0.7506
HTN	9	0	*P*=0.3103

TB: Tuberculosis, SD: Standard deviation, DM: Diabetes mellitus, HTN: Hypertension

Table 2: comparison of AFB smear and CBNAAT examination of tubercular pleural fluid

CBNAAT result	Pleural fluid AFB smear		Grand total
	AFB smear+	AFB smear−	
MTB detected	7	9	16
MTB not detected	2	87	89
Total	9	96	105

AFB: Acid-fast bacilli, CBNAAT: Cartridge-based nucleic acid amplification test, MTB: *Mycobacterium tuberculosis*

Table 3: CBNAAT result of pleural effusion and empyema

CBNAAT	Tubercular	
	Empyema	Pleural effusion
MTB detected		
Sensitive	9	6
Resistant	0	1
MTB not detected	1	88
Total	10	95

CBNAAT: Cartridge-based nucleic acid amplification test, MTB: *Mycobacterium tuberculosis*

DISCUSSION

The pathogenesis of tuberculous pleural effusion was once thought to be due to delayed hypersensitivity reaction. However, it is now believed to be due to direct invasion of pleura space by TB bacilli that initiate a cascade of protracted lymphocyte driven immunological reactions which leads to accumulation of pleural fluid.[2] On the other hand, tuberculous empyema is an active infection of pleural space caused by either rupture of subpleural focus into pleural space or direct extension from adjacent lymph node or subdiaphragmatic foci or hematogenous spread and thus have high bacillary load.[2] The paucibacillary nature of pleural fluid and partly due to presence of certain inhibitors in pleural fluid may be the reason for low sensitivity of CBNAAT in tuberculous pleural effusion without empyema.[12]

The comparison of the sensitivity and specificity of CBNAAT in the diagnosis of tubercular pleural effusion in several previous studies is shown in Table 5. To the best of the author's knowledge, there is no previous report published on CBNAAT as a diagnostic test in tuberculous empyema even after extensive Medline and Pubmed search. The results vary widely depending on the reference standard adapted for analysis. The pooled sensitivity of CBNAAT in pleural fluid was 46.4% (95% confidence interval [CI] 26.3-67.8) against culture as the reference standard and 21.4% (95% CI 8.8-33.9) when analyzed against CRS as shown in a meta-analysis report.[16] Sehgal *et al.* published a meta-analysis report compiling the list of sensitivity and specificity of CBNAAT of pleural fluid considering culture and CRS as reference standard from different studies since year 2010.[5] The pooled sensitivity was found to be more when culture positivity was used as benchmark compared to CRS (51.4% in culture sub group and 22.7% in CRS subgroup), whereas specificity was almost same (98.6% in culture group and 99.8% in CRS subgroup).[5] The definition of CRS was very different in different studies.[9,10,13,17]

Pravin *et al.* performed Gene-Xpert assay on 164 pleural fluids and found a positive result in 15 out of 164 samples (10%) including four with rifampicin resistance.[18] On considering CRS, the sensitivity of pleural fluid smear in the present study was 9/105 (8.6%) overall and 7/10 (70%) in empyema subgroup; CBNAAT was overall 16/105 (15.23%) and 9/10 (90%) in empyema cases. Although the sensitivity of pleural fluid smear (2%) and CBNAAT (7.3%) was low in pleural effusion considering CRS as reference standard, it could detect one case with rifampicin resistance. The sensitivity and specificity of CBNAAT were 77.78% (95% CI 39.99-97.19) and 90.62% (95% CI 82.95-95.62), respectively, with pleural fluid smear positivity was considered as reference standard.

Although the overall sensitivity is much low in the present study as compared to several published studies and meta-analysis reports, the sensitivity and specificity are 100% in empyema cases.

However, the major limitation of the present study was that the study did not consider pleural biopsy and culture of the

Table 4: Diagnostic accuracy of CBNAAT in overall tubercular effusion and tubercular empyema in comparison with culture positive

Statistic	Tubercular pleural effusion (overall)		Tubercular empyema		Tubercular effusion without empyema	
	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity (%)	36.36	20.4-54.88	100	66.37-100	12.5	2.66-32.36
Specificity (%)	94.4	86.38-98.47	100	2.5-100	94.37	86.2-98.4
Positive likelihood ratio	1.05	0.40-2.79			0.38	0.09-1.6
Negative likelihood	0.99	0.83-1.18			1.09	0.94-1.25
Positive predictive value (%)	75	51.12-89.59	100		25.26	16.91-35.22
Negative predictive value (%)	76.4	71.32-80.83	100		42.86	15.3-75.69

CBNAAT: Cartridge-based nucleic acid amplification test, CI: Confidence interval

Table 5: Comparison with the previous studies

Study	Place	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Rosso *et al*. 2011[9]	Brazil	42.8	94.2	93.3	48.5
Porcel *et al*. 2013[10]	Spain	15	100		
Shital *et al*. 2014[13]	India	92.86	33.33	35.13	92.3
Du *et al*. 2015[14]	China	43.6	98.6		
Rufai *et al*. 2015[11]	India	54.8	100		
Saeed *et al*. 2017[15]	Pakistan	84.3	100		

PPV: Positive predictive value, NPV: Negative predictive value

biopsy material in every patient which might have improved diagnostic yield. Moreover, the sample size of tuberculous empyema was too small. The result needs be validated in a larger patient population. The efficacy of CBNAAT as a diagnostic tool in suspected tuberculous pleural effusion/empyema cases in HIV-seronegative patients compared to HIV-seropositive patients may be considered in the future.

CONCLUSION

CBNAAT is a useful rapid diagnostic tool for suspected TB. The authors would like to conclude that despite low sensitivity and specificity of CBNAAT in suspected tuberculous pleural effusion, the test should be considered in the routine diagnostic workup considering the advantage of early detection of drug resistance pattern, especially in a high burden country such as India. For suspected tuberculous empyema, CBNAAT provides a rapid confirmed diagnosis within 2 h including drug susceptibility status when compared with conventional culture and drug sensitivity tests.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Sonali Das, District Tuberculosis Officer, North 24 Parganas, West Bengal, India, Chandan Mondal, Senior Treatment and Laboratory Supervisor, RNTCP, College of Medicine and Sagar Dutta Hospital, Kolkata, West Bengal, India, and Rajib Chatterjee, Laboratory technician, RNTCP, B. N. Bose Hospital, Barrackpore, West Bengal, India.

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How to cite this article: Biswas D, Mukherjee S, Begum S, Paul A, Ghosh P, Sarkar S. Role of Cartridge Based Nucleic Acid Amplification Test in Diagnosis of Tuberculous Pleural Effusion Compared to Tuberculous Empyema in HIV Seronegative Patients. Int J Sci Stud 2017;5(6):125-129.

Source of Support: Nil, **Conflict of Interest:** None declared.