

## Original Research Article

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## Diagnostic Role of Polymerase Chain Reaction and Inadequacy of Mycobacterium Growth Indicator Tube culture in Tuberculous Pleuritis

Gandhi Kandhakumari<sup>1\*</sup> and Selvaraj Stephen<sup>2</sup><sup>1</sup>Kanti Devi Medical College and Research Centre, Mathura, Uttar Pradesh-281406, India<sup>2</sup>Department of Microbiology, Mahatma Gandhi Medical College & Research Institute, SBV University, Pillaiyarkuppam, Pondicherry - 607403, India

\*Corresponding author

### ABSTRACT

Diagnosis and management of patients with tuberculous pleuritis needs a rapid and sensitive diagnostic tool. Our aim was to diagnose cases of tuberculous pleuritis from patients employing ZN smear, culture (LJ & MGIT) and to compare with PCR. Pleural fluid collected from 36 patients with clinical and/or radiological suspicion of TB pleuritis was included in this study. Pleural fluid collected from nine non-tuberculous pleuritis cases was included as negative controls. Pleural fluid samples were analyzed for ZN smear, LJ and automated MGIT culture and results compared with MTB PCR targeting IS6110 region. Positivity for smear, LJ, MGIT and PCR individually were 5.6%, 2.8%, 16.7% and 25% respectively. Combination of all above techniques together increased the percentage positivity to 30.6%. Negative controls were negative by all procedures. In the context of paucibacillary specimens like pleural fluid, smear microscopy and LJ are less sensitive. MGIT has moderate sensitivity and rapidity compared to LJ. PCR is rapid and highly sensitive, but needs interpretation of PCR reports with “gold standard” culture and clinical history to arrive at an early and accurate diagnosis.

#### Keywords

PCR,  
*Mycobacterium tuberculosis*,  
Pleural fluid, LJ,  
IS6110

#### Article Info

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### Introduction

India, a developing country with high tuberculosis endemicity accounts for highest incident cases of 27% among the total cases reported worldwide (WHO, 2018). Pleural tuberculosis ranks second next to tuberculous lymphadenitis and India accounts for 30-80% of cases. Though pleural biopsy is reported as the optimum specimen for definitive

diagnosis of tuberculous pleural effusion, pleural fluid is commonly collected for diagnostic purpose (SK Sharma *et al.*, 2004; P James *et al.*, 2010). Because pleural fluid tends to be paucibacillary it turns out to be negative in 80 to 90% cases making conventional microbiological techniques like smear and culture least sensitive. But molecular techniques which detect and amplify specific regions of *M. tuberculosis*

genome are considered more sensitive when compared to conventional diagnostic modalities (Sharma *et al.*, 2004; Makesh Kumar *et al.*, 2014; Siddiqui *et al.*, 2013; Shital *et al.*, 2014; Kandhakumari *et al.*, 2015). Hence in this study Ziehl-Neelsen smear (ZN) microscopy, culture on Lowenstein-Jensen media (LJ) and Mycobacterium Growth Indicator Tube 960 (MGIT 960) were carried out and compared with that of Polymerase Chain Reaction (PCR) targeting IS6110 region for diagnosis of tuberculous pleuritis.

### **Materials and Methods**

This work was approved by Institutional Human Ethical Committee and the work was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from patients before collection of clinical samples. Patients with clinical/radiological suspicion of TB pleuritis were included. Patients suspected for tuberculous pleuritis with pulmonary tuberculosis, sputum positive cases and those treated with ATT (Anti-tuberculous treatment) drugs were excluded. With sterile precautions, pleural fluid from 36 patients attending our hospital with clinical and/or radiological suspicion of TB pleuritis between June 2011 and October, 2014 was included in this study. Pleural fluid from nine non-TB patients (five cases of pneumonia and four cases of Chronic Obstructive Pulmonary Disease) were included as negative controls. The specimens were centrifuged, and deposits stained by ZN. Inoculation of the deposit was made on LJ and MGIT 960 tubes and incubated up to eight and six weeks respectively. Identification of *M. tuberculosis* complex (MTBC) recovered in LJ and MGIT was confirmed by standard biochemical tests and a rapid immunochromatography based SD Bioline MPT64Ag detection kit (G Kandhakumari *et al.*, 2015).

### **Nested Polymerase chain reaction (nPCR):**

DNA from pleural fluid was extracted as per the guidelines of the manufacturer (Amplification Reagent Set for Mycobacterium tuberculosis, Bangalore Genei, Bangalore, India) for nPCR. This is single-tube nested PCR with two – step sequential assay. In the first step, 220 bp Insertion sequence (IS6110) was amplified and in second step nested primers were added to the amplicon to further amplify the 123 bp region. Gel electrophoresis was carried out and amplified products were observed using gel documentation system (BIO-RAD, Hercules, CA, USA). Positive and negative controls were included and monitored to avoid false positivity and carry over contamination.

### **Statistical analysis**

Sensitivity, specificity, positive predictive value and negative predictive value of different diagnostic methods were determined and compared using Chi-square test with or without Yate's correction. P values < 0.05 were considered statistically significant.

### **Results and Discussion**

Among 36 pleural fluids processed, 5.6% was positive by smear, 2.8% by growth on LJ and 16.7% by growth in MGIT (Table. 1). All six isolates were *M. tuberculosis* and none belonged to non tuberculous Mycobacteria (NTM) group. Two pleural fluid samples which were positive by smear did not grow *M.tuberculosis* on LJ, but were isolated in MGIT 960 system. Among 34 smear negative pleuritis patients, four isolates of *M.tuberculosis* (11.08%) were recovered from MGIT, and one from LJ (2.8%). PCR alone yielded 25% positivity. All techniques together increased the positivity to 30.6%. Among the 34 smear negative samples, only 2.9% were positive by LJ, but 11.08% by

MGIT and 23.5% by PCR (Table-1). All negative control samples were negative by all techniques. Keeping culture as the gold standard, the sensitivity specificity, PPV &

NPV for ZN smear was 60%, 100%, 100% and 86% whereas for PCR it was 66%, 83%, 44% and 92% respectively.

**Table.1** Comparison of PCR results with conventional techniques

S	Smear	LJ	MGIT	PCR	No. of cases	Percentage positivity
1	+	-	+	+	1	2.8
2	+	-	+	-	1	2.8
3	-	+	+	+	1	2.8
4	-	-	+	+	2	5.6
5	-	-	+	-	1	2.8
6	-	-	-	+	5	13.9
7	-	-	-	-	25	69.4
<b>Total positive</b>	2	1	6	9	36	100

Diagnosis of TB pleuritis needs high index of clinical suspicion and appropriate specimen for effective diagnosis. TB pleuritis mainly occurs as allergic reaction to tuberculo-protein and hence the bacilli load is very low in pleural fluid. Though pleural biopsy is the preferred specimen, pleural fluid is still routinely submitted for diagnosis. In India, smear microscopy is commonly used for screening purposes. It is cheap and rapid, but less sensitive due to the paucibacillary nature of pleural fluid and cannot differentiate *M.tuberculosis* from other non tuberculous mycobacterium species. The sensitivity of microscopy for pleural fluid and pleural biopsy were very less around < 10% and < 50% respectively (L Li *et al.*, 2019). According to S Mirza *et al.*, 2003; smear microscopy of pleural fluid requires  $10^4$  bacilli/ml and cultures needs 10-100 viable bacilli/ml. Several researchers reported smear positivity ranging from 0% to 31% (Makeshkumar *et al.*, 2014; Siddiqui *et al.*, 2013; Shital *et al.*, 2014; Maurya *et al.*, 2012; Kumar *et al.*, 2010; Khosravi *et al.*, 2017; Lee *et al.*, 2015; Maurya *et al.*, 2011). Hence for timely diagnosis, smear microscopy of pleural

fluid alone is not dependable and there is a need for better diagnostic techniques like culture and PCR.

Though LJ culture is less sensitive for pleural fluid, researchers have reported positivity ranging from 0%-70% (Sharma *et al.*, 2004; Makeshkumar *et al.*, 2014; Siddiqui *et al.*, 2013; Shital *et al.*, 2014). In the present study, we could report only 2.8% positivity on LJ. Among the 36 pleural fluids processed, two smear positive samples have not grown on LJ. This may be due to the non-uniform distribution of bacilli in the inoculum and could have been erroneously inferred as due to dead bacilli, if liquid culture system were not employed. This underlines the need for inclusion of liquid culture. Gopi *et al.*, suggested bed side inoculation and incorporation of liquid culture system for pleural fluid samples (Gopi *et al.*, 2007). In our study MGIT recorded 16.7% positivity. Table-1 compares PCR results with smear and culture. Around 25% culture positivity from pleural fluid was reported in MGIT system (Siddiqui *et al.*, 2013; Goyal *et al.*, 2015). Among the 34 smear negative samples

processed in the present study 11.8% isolates have grown in MGIT. Bunger *et al.* reported MGIT as a better system for detecting MTB in smear negative samples (R Bunger *et al.*, 2013).

As reviewed by Gill *et al.*, PCR sensitivity targeting IS6110 region was near 100% and specificity ranged from 60%-90% (Gill *et al.*, 2013). A sensitivity of 66% and specificity of 83% was observed in our study. As a standalone technique, PCR was positive in 25% of our cases, but when combined with other conventional techniques it was 30.6%. In this study, two culture positive samples were negative by PCR and hence the sensitivity and specificity for PCR was 66% and 83% respectively. This decreased sensitivity may be due to undetectable amount of DNA and/or presence of less copies of target IS6110 region. PCR alone was positive in 16.7% of the 30 smear and culture negative specimens. Since the EPTB specimens are paucibacillary, PCR helps in the detection of smear and culture negative specimens. False positivity is very common in PCR due to carry over contamination, dead bacilli etc. and since nested PCR is carried out in the present study the chances of false positivity is comparatively less. Khosravi *et al.*, reported that the detection of several target genes will increase sensitivity and eliminate false positive and false negative results (AD Khosravi *et al.*, 2017). PCR can be used as an adjunct test for diagnosis of TB pleuritis along with other parameters and correlate with clinical conditions.

Today molecular techniques like Xpert MTB/RIF is used for rapid identification of MTB and rifampicin resistance with conditional recommendations for EPTB specimens. However, WHO and CDC recommend Xpert MTB/RIF positive cases to be confirmed by culture and drug susceptibility testing.

To conclude it may be said that smear microscopy and LJ are less sensitive tools for diagnosis of TB pleuritis. MGIT automated system helps in the early isolation, but less sensitive for pleural fluid compared to PCR. Till now TB PCR is not approved by FDA. TB PCR plays a role where conventional techniques remain negative. Hence PCR can be used as an adjunct test along with smear and culture reports and should be correlated with clinical history, radiological evidence, etc. so as to initiate prompt treatment.

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