Original Article

The Yield of Thoracoscopic Biopsy Truenat in the Diagnosis of Tuberculous Pleural Effusion

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Abstract

Background and Objective: Extrapulmonary tuberculosis (EPTB) affects about 25% of patients presenting with Tuberculosis (TB). Tuberculous pleural effusion is the second-most common type of EPTB, after lymph node TB. Although the molecular TB diagnostics have lower turnaround time compared to traditional testing methods, the sensitivity in microscopy negative specimens are low. Higher cost and infrastructure requirements are other disadvantages. Truenat, developed by Mobilio diagnostics and validated by ICMR, is a rapid, polymerase chain reaction (PCR)-based diagnostic test to detect Mycobacterium Tuberculosis (MTB) and also rifampicin resistance. According to the WHO, the accuracy of Truenat is comparable to Xpert PCR. We report our experience in using Truenat for EPTB, specifically, pleural TB. **Methods:** We retrospectively analyzed data from thoracoscopy over the past 2 years. All data pertaining to the use of Truenat for TB in pleural fluid and thoracoscopic biopsy specimens, histopathology, and mycobacterial cultures were analyzed. **Results:** We had a total of 114 patients with undiagnosed pleural effusion who underwent thoracoscopy during the study. Forty-five patients (39%) had a diagnosis of TB, among the total 114 patients. The sensitivity of tissue Truenat was 51.11 (95% confidence interval [CI]: 35.77–66.30), tissue culture 37.50% (95% CI: 22.73–54.20), pleural fluid Truenat 20% (95% CI: 8.44–36.94), and fluid culture 14.29% (95% CI: 5.43–28.54). The specificities of all the confirmatory tests were 100% when compared to a reference standard which was taken as a combination of histology and culture as the reference standard with or without acid-fast bacilli in the histology samples. Tissue Truenat was significantly more sensitive than fluid Truenat ($P < 0.05$). Likewise, tissue culture was more sensitive than fluid culture ($P < 0.05$). Among all microbiology confirmatory tests performed, Truenat of pleural tissue had the highest yield (51.11). **Conclusion:** Thoracoscopic pleural biopsy Truenat results in improved sensitivity in cases of EPTB.

Keywords: *Pleural effusion, pleural tuberculosis, thoracoscopy, Truenat, tuberculosis*

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Introduction

Globally, an estimated 10.0 million people fell ill with TB in 2018, a number that has been relatively stable in recent years. The burden of tuberculosis (TB) varies enormously among countries, from fewer than five to more than 500 new cases per 100,000 population per year, with the global average being around 130. There were an estimated 1.2 million (range, 1.1–1.3 million) TB deaths among HIV-negative people in 2018 (a 27% reduction from 1.7 million in 2000), and an additional 251,000 deaths among HIV-positive people (a 60% reduction from 620 000 in 2000).[1] Extrapulmonary

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TB (EPTB) affects about 25% of patients presenting with TB. Most of these are lymph node and pleural TB.[2] Tuberculous pleural effusion is the second-most common type of EPTB, after lymph node TB. The bacillary burden in the pleura is low, and pleural effusion is thought to occur due to hypersensitivity to tubercular proteins.

The gold standard for diagnosis of pleural TB is the detection of *Mycobacterium* TB in pleural fluid, biopsy specimens, or the histological finding of caseating granulomas in the pleura. In high burden settings, however, the diagnosis is solely done on the basis of a lymphocytic predominant exudate and a high adenosine deaminase (ADA) level.[3] The disadvantage of biomarkers such as ADA or interferon-gamma is that it is not specific and therefore is bound to have false positive as well as false-negative values.

Although the molecular TB diagnostics have lower turnaround time compared to traditional testing methods, the sensitivity in microscopy negative specimens is low.[4] Higher cost and infrastructure requirements are other disadvantages. Truenat, developed by Mobilio diagnostics and validated by ICMR is a rapid, polymerase chain reaction (PCR)-based diagnostic test to detect MTB and also rifampicin resistance. According to the WHO, the accuracy of Truenat is comparable to Xpert PCR. It is a portable machine which does not require stringent laboratory conditions and can be carried for field surveillance for rapid detection of MTB and rifampicin resistance.

We undertook this study to calculate the yield of thoracoscopic biopsy Truenat in diagnosing tuberculous effusion keeping histology as the gold standard.

Methods

All patients who underwent medical thoracoscopy during the period January 1, 2018–May 31, 2020 were included in the study. We analyzed the data retrospectively for this duration from our database at the Department of Pulmonology, Lisie Hospital, Kochi, a tertiary care hospital in India. This study was approved by the institutional ethics committee. As this was a retrospective study, the need for an informed consent was waived by the institutional ethics committee.

Medical thoracoscopy was performed using the Evis Exera Pleuravideoscope LTF-160 manufactured by Olympus (Tokyo, Japan). The patients were assessed with a chest X-ray and an ultrasound of the chest to check for the presence of loculations and the approximate amount of pleural fluid. The site of entry was marked on the chest wall on the day prior to the procedure.

Pleural fluid was collected for cell counts, biochemistry, cytology, gram stain and culture, AFB stain, Truenat for TB, and culture for TB. All cultures were performed in the Department of Microbiology, Lisie Hospital using commercially prepared Lowenstein Jensen media SL001 from Himedia. QC document is obtained from the manufacturer for each lot and the same is maintained in the laboratory. Positive control *Mycobacterium* TB ATCC 25177 and negative control *Escherichia coli* 25,922 are set up for each lot. The Truenat test was done with the Truelab DUO machine with Instrument ID no: TLDU0436 and is calibrated with standard calibration chip once every 6 months and more frequently in case any maintenance work is performed. Biopsies were taken from the abnormal areas visualized on medical thoracoscopy. When present, the loculations were broken with the biopsy forceps and the pleural cavity was irrigated with saline. If there were no pleural abnormalities, random areas were sampled and sent for histopathology in formalin and for the Truenat test. Around five pieces were collected for histopathological examination and three were collected for Truenat testing. The Truenat testing was done on the pleural tissue without any further processing.

We used a combination of histology and culture as the reference standard with or without acid-fast bacilli in the histology samples.

Results

We had a total of 114 patients with undiagnosed pleural effusion who underwent thoracoscopy during the study. The mean age of all the patients was 46.8.

Forty five patients (39%) had a diagnosis of TB, among the total of 114 patients. Ninety one percent of these were histology proven. Moreover, the rest were culture proven [Table 1].

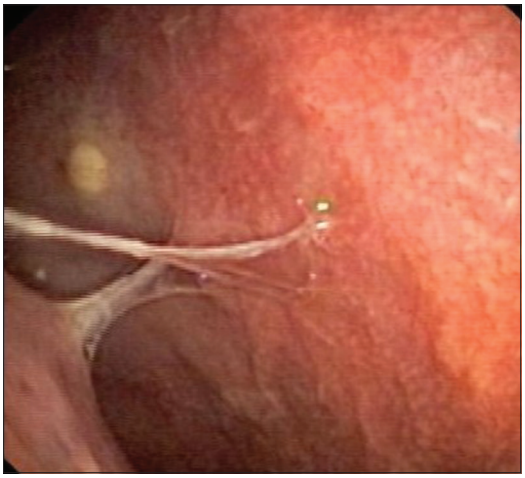

Figure 1: Tubercular nodule - caseous

Of these patients of extra-pulmonary TB, 28 cases (62%) were of right-sided pleural effusion, and 17 were left-sided effusion (38%). Ultra sonological evidence of septations was seen in 33 patients (73%). All patients were HIV negative.

The diagnostic yields of the microbiological tests are shown in Tables 2 and 3. The sensitivity of tissue Truenat was 51.11 (95% confidence interval [CI]: 35.77–66.30), tissue culture 37.50% (95% CI: 22.73–54.20), pleural fluid Truenat 20% (95% CI: 8.44–36.94), and fluid culture 14.29% (95% CI: 5.43–28.54). The specificities of all the confirmatory tests were 100% when compared to a reference standard which was taken as a combination of histology and culture as the reference standard with or without acid-fast bacilli in the histology samples. Tissue Truenat was significantly more sensitive than fluid Truenat ($P < 0.05$). Likewise, tissue culture was more sensitive than fluid culture ($P < 0.05$). Among all microbiology confirmatory tests performed, Truenat of pleural tissue had the highest yield (46.3%). Truenat confirmed the diagnosis in 4 patients on the basis of tissue Truenat positivity and 5 patients on the basis of tissue and/or fluid Truenat positivity, when mycobacterial cultures from both fluid and tissue samples were negative [Table 4].

Discussion

To the best of our knowledge, this is the first study to report the yield of Truenat from pleural biopsy samples. Christopher *et al*., published a study regarding the yield of Xpert MTB/RIF on thoracoscopic pleural biopsy.[5] The study showed a sensitivity of tissue Xpert of 45% and of pleural fluid to be 14%. The specificities of all the confirmatory tests were 100%. Tissue Xpert was found to be more sensitive than fluid Xpert, and tissue culture was more sensitive than fluid culture. The low sensitivities of the Xpert technique might be due to the presence of inhibitors in the pleural fluid or because of intracellular sequestration of the mycobacteria.[2]

We also noticed a low yield from mycobacterial cultures of pleural tissue and pleural fluid which could be due to the low bacterial load in EPTB specimen. Such paucibacillary samples may not yield high culture positivity as compared to pulmonary TB samples. Another reason might also have been a clinician bias toward sending the larger and more adequate specimens for Truenat. The classical thoracoscopic findings described for tuberculosis are sago grain appearance, and caseous nodules [Figure 1 and 2].

Xpert developed by Cepheid has been considered to be one of the best molecular methods for the diagnosis of TB. It is a cartridge-based method, and has the advantage of ease of sample handling, but is limited by the higher cost of the instrument and the cost per test. Truenat PCR is also a cartridge-based test for the diagnosis of TB along with the detection of rifampicin resistance,

Table 1: Final diagnosis in patients who underwent thoracoscopy (*n*=114)

	Number of patients, *n* (%)
Tuberculosis	45 (39.4)
Others*	17 (14.9)
Malignancy	52 (45.6)

Table 2: Microbiological diagnostic yield from pleural tissue and pleural fluid

Tuberculosis	Number of patients, *n* (%)
PT Truenat positive (*n*=45)	23 (46)
PT MC positive (*n*=40)	15 (37)
PF Truenat positive (*n*=35)	7 (20)
PF MC positive (*n*=42)	6 (14)

MC: Mycobacterial culture, PF: Pleural fluid, PT: Pleural tissue

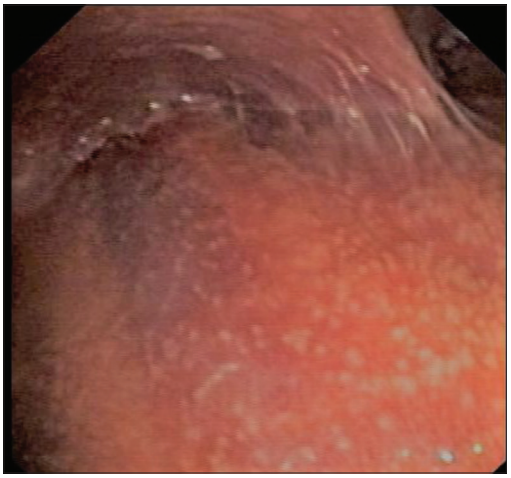

Figure 2: Sago grain appearance on thoracoscopy

Table 3: Sensitivity, specificity, positive predictive value, and negative predictive value of microbiological tests

	Sensitivity	Sensitivity (95% CI)	Specificity	Specificity (95% CI)	PPV	NPV
PF Truenat	20	8.44-36.94	100	95.44-100	100	73.83
PF MC	14.29	5.43-28.54	100	96.64-100	100	66.67
PT Truenat	51.11	35.77-66.30	100	95.44-100	100	78.22
PT MC	37.50	22.73-54.20	100	95.75-100	100	87.27

MC: Mycobacterial culture, NPV: Negative predictive value, PF: Pleural fluid, PPV: Positive predictive value, PT: Pleural tissue, PT MC: Pleural tissue mycobacterial culture, CI: Confidence interval

Table 4: Truenat contribution in culture-negative patients

	Number of patients, *n* (%)
PF Truenat alone positive	2 (5.7)
PT Truenat alone positive	4 (9.7)
PF and PT Truenat positive	1 (2.8)

PF: Pleural fluid; PT: Pleural tissue

which is developed in India.[6] The DNA extraction by Truenat takes about 25 min and it takes another 35 min to diagnose TB. Rifampicin resistance testing takes an additional 1 h. Truenat MTB test is extremely useful in resource-limited health-care settings and helps get results quickly with a significantly reduced cost.[6] Truenat is battery operated and portable, unlike Xpert, which needs reliable electricity supply and air-conditioning.

Various studies have looked at a composite reference score and pleural fluid culture as the reference standards. We used a combination of histology and culture as the reference standard with or without acid-fast bacilli in the histology samples. The histology diagnosis of TB is not always very easy to make, especially when the sampled areas look normal. Granulomas may be seen in sarcoidosis, tularemia, and rheumatoid pleuritis.[2] The Truenat test in these cases, with an appropriate clinical background helps the clinician arrive at the final diagnosis.

The other advantage of Truenat test is the quick arrival at a diagnosis of MTB, with very high specificity so that the anti TB treatment is initiated without delay. The results are available by the time the patient reaches their rooms from the thoracoscopy suite, which also helps in allaying the anxiety of the patient and relatives regarding the diagnosis.

One of the important strengths of the Truenat assay is its ability to detect the presence of resistance to rifampicin rapidly.[7] Rifampicin resistance by culture methods take weeks for the final report, and this method circumvents the issue. In cases where the pleural effusion increases during treatment, it is clinically difficult to differentiate it from immune reconstitution inflammatory syndrome and drug-resistant TB. The Truenat at these times would help the clinician, as the rifampicin resistance report is also available early, much before the culture sensitivity. Conventional DST results take at least 2 months from the time when the culture is inoculated.

To the best of our knowledge, there have been only two articles that have studied the yield of molecular techniques in the diagnosis of pleural EPTB by pleural biopsy, by Du *et al*. in Chinese patients and by Christopher *et al*. in Indian patients. The Chinese study had taken the reference standard as pleural biopsy MTB culture. In their study, the overall sensitivity, specificity, positive predictive value, and negative predictive value of Xpert assay using pleural fluid specimens for pleural TB diagnosis were 43.6%, 98.6%, 96.0%, and 69.3%, respectively. The sensitivity of Xpert assay on pleural fluid specimens was lower to that obtained with pleural biopsy specimens ($P < 0.01$) as in our study. In addition, a comparison of Xpert assay with smear-microscopy revealed that the sensitivity of Xpert was significantly higher than that of the smear-microscopy on pleural biopsy specimens (85.5% vs. 27.3%; $P < 0.01$), and on pleural fluid specimens (43.6% vs. 7.3%; $P < 0.01$).[7]

They concluded that accurate quantification of the MTB load in patient samples may allow for the evaluation of the patient's infectiousness, the evaluation of the disease severity, and the monitoring of treatment. One limitation of this technique is that, in detecting MTB DNA, they cannot distinguish between viable and nonviable microorganisms. However, higher Xpert assay loads were associated with decreased MGIT culture time to positivity, consistent with previous data indicating that the Xpert assay's semi-quantitative results could be used to estimate the MTB load.[7]

A recent meta-analysis, concluded that using pleural fluid culture and composite reference standard, which were poor reference standards for diagnosing tubercular pleural effusion were used in most studies and suggested that histology of the tissue be kept as the reference standard.[8] Histology was kept as the reference standard in the study conducted by Christopher *et al*. Our study also used histology as the reference standard.

Conclusion

Truenat is a very specific test which can be advocated in patients with suspected tubercular pleural effusion given its high specificity although it is found lacking in sensitivity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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