ORIGINAL ARTICLE

To Study Correlation between Results of GeneXpert and AFB Culture in Bronchial Aspirate Samples in the Diagnosis of Pulmonary Tuberculosis

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ABSTRACT

Aims and Objectives: The aim of the study was to compare the Nucleic Acid Amplification Assay (GeneXpert) with acid fast bacilli (AFB) in patients with suspected pulmonary tuberculosis (TB) and assess the sensitivity, specificity, positive predictive value, and negative predictive value. **Materials and Methods:** Inclusion criteria: Patients with clinical suspicion of pulmonary TB, including symptoms of weight loss, weariness, hemoptysis, and loss of appetite, as well as cough with or without expectoration for more than 2 weeks, were included in the study. Exclusion criteria: The following criteria were excluded from the study: (1) Samples obtained with no clinical background, (2) samples for all three tests were received without a request, and (3) patient with a history of fungus infections or lung cancer. Pulmonary samples of 150 patients with suspected pulmonary TB were reviewed between January 2021 and November 2021 after being received retrospectively for liquid AFB culture and GeneXpert requests from various wards, icus, and centers to the Microbiology laboratory and DMC Laboratory of Smt Kashibai Navale Medical College and General Hospital, Pune. One hundred and fifty BAL samples were included in the pulmonary specimens. Information pertaining to patients was gathered from the patients attendants. **Results:** One hundred and fifty samples in all were examined; of these, 22 showed both culture and gene expert positivity, eight showed just gene expert positivity, and five showed only culture positivity. Test requisition forms were delivered with the sample culture. **Conclusion:** Gene expert and AFB culture have approximately identical specificities, although gene expert has a substantially higher sensitivity in bronchial aspirates than AFB culture.

Key words: GeneXpert, AFB culture, Pulmonary tuberculosis

INTRODUCTION

The World Health Organization's 2014 global tuberculosis (TB) report states that *Mycobacterium tuberculosis* (MTB), which causes TB, continues to be one of the deadliest infectious illnesses in the world. Pulmonary TB is a disease that often affects the lungs and is transferred through the air by those who already have it. In 2013, there were an estimated 9 million new cases of TB worldwide. For effective patient outcomes and early patient management, an early diagnosis is essential. In underdeveloped countries, false negative results and misinterpretation of TB suspects are frequent since the majority of TB control programs utilize the insensitive ZN smear microscopy, which necessitates numerous visits and raises the default rate.

Although mycobacterial culture is the gold standard, it is slow – it typically takes 2–6 weeks to get a final result — and it requires specialized equipment and infrastructure.

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Numerous nucleic acid amplification techniques have been created for the quick identification of MTB in clinical samples from instances of extrapulmonary and pulmonary TB. These methods not only offer the benefit of speedy identification but also identify even minute genomic copies of MTB in a variety of specimens.

Aim

The aim of the study was to compare with acid fast bacilli (AFB) culture and assess the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Nucleic Acid Amplification Assay (GeneXpert) utilizing bronchial aspirate samples from patients with suspected pulmonary TB.

MATERIALS AND METHODS

Inclusion Criteria

Patients with clinical suspicion of pulmonary TB, including symptoms of weight loss, weariness, hemoptysis, and loss of appetite, as well as cough with or without expectoration for more than 2 weeks, were included in the study.

Exclusion Criteria

The following criteria were excluded from the study:
1. Testing of samples received without a clinical history
2. Samples of all three tests received without request
3. Patient with a history of fungal infections or lung malignancies.

Pulmonary samples of 150 patients with suspected pulmonary TB were reviewed between January 2021 and November 2021 after being received retrospectively for liquid AFB culture and GeneXpert requests from various wards, icus, and centers to the Microbiology laboratory and DMC Laboratory of Smt Kashibai Navale Medical College and General Hospital, Pune. 150 BAL samples were included in the pulmonary specimens. The test requisition forms that were received with the sample were used to gather patient-related data.

Laboratory Methods

According to the laboratories collection and transportation policy, each BAL sample is received from wards and the ICU was split into two pieces. On the same day, a portion was tested for gene expertise and a subsequent portion for AFB culture. The manufacturer's recommendations were followed when conducting the gene expert testing. 2 mL of the inactivated material was transferred to the test cartridge, then put into the test platform, after the sample reagent had

been manually agitated for 10 min at room temperature. Only digital outcomes were utilized for comparison.

According to the manufacturer's instructions, the second half was processed using the N acetyl -l cysteine – sodium hydroxide procedure, grown on MGIT media, and incubated in MGIT BACTEC 320 liquid culture system. Sodium hydroxide is an emulsifier and a decontaminating agent, and NALC functions as a mucolytic agent and lowers the concentration of Naoh needed. As instructed by the manufacturer, AFB culture on 5% sheep blood agar was carried out directly from the tubes when the system identified them as positive to check for contamination.

Till 42 days, every tube was examined for positive. According to the manufacturer's instructions, fast immunochromatography test kit employing MPT 64 antigen was used to screen for MTB from positive culture tubes.

Analysis

The data were collated in a master chart using a Microsoft Excel spreadsheet, and correlation was looked for. With the help of the SPSS17.0 statistical software for the social science system, a stochastic analysis of the data was carried out. Calculations were made for sensitivity, specificity, PPV, and NPV. The AFB culture and gene expert calculated the sensitivity, specificity, PPV, and NPV for the diagnosis of pulmonary TB.

RESULTS

One hundred and fifty bronchial aspirate specimens were analyzed, and 22 samples tested positive for both genes and cultures, eight samples tested positive for genes only, and five samples tested positive for cultures only. For continuous variables, data for categorical variables are displayed as *n* (%) or median (interquartile range). To compare the AUC of gene Xpert and culture to find MTB in bronchial aspirate samples, receiver operating characteristic curves are plotted [Figure 1]. The ideal cutoff point for calculating sensitivities and specificities was found using Youden's index.

$P < 0.05$ is considered statistically significant. Analysis is done using R version 4.2.2.

DISCUSSION

In this retrospective investigation, we compared the diagnosis yield of AFB culture, which was used as the gold standard, to

Test	Sensitivity (95% CI)	Specificity (95% CI)	Negative predictive value (95% CI)	Positive predictive value (95% CI)	AUC (95% CI)
Gene Xpert	0.99 (0.98–1.00)	0.98 (0.96–1.00)	0.99 (0.97–1.00)	0.93 (0.85–1.00)	0.99 (0.98–1.00)
Culture	0.79 (0.64–0.93)	0.96 (0.93–0.99)	0.95 (0.92–0.98)	0.82 (0.68–0.95)	0.82 (0.68–0.95)
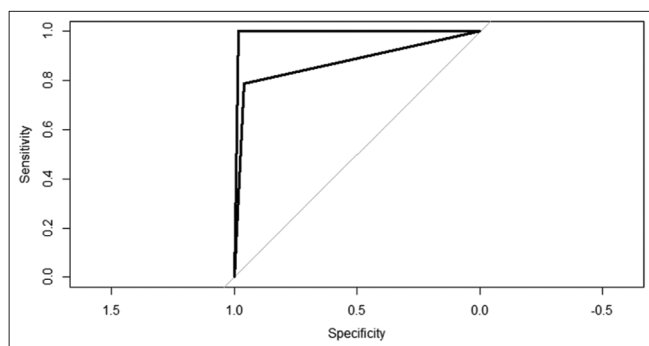
Figure 1: Receiver operating characteristic curve for gene Xpert (upper) and culture (lower)

the diagnosis yield of gene expert to detect MTB in bronchial aspirate samples. Mycobacterial cultures can be made using a solid broth system (LJ Media) or a liquid broth method (MGIT 320) to identify MTB. Results from the MGIT liquid culture medium are available earlier than those from the LJ Medium. MGIT 320 culture was used in our investigation. Gene Xpert is a straightforward bench top point of care diagnostic test that requires little training to use. Unlike cultures, which often take days to produce good results, the results are ready in 2 h.

In our investigation, the gene Xpert had overall sensitivity, specificity, PPV, and NPV that were, respectively, 99%, 98%, 99%, and 93%. These results showed that the overall sensitivity and specificity of gene expert are more than that of AFB culture in bronchial aspirate samples, with values of 79%, 96%, 95%, and 82% when compared to the overall sensitivity, specificity, PPV, and NPV of AFB culture.

Standard microscopy, culture, and anti-tubercular drug sensitivities are still required to monitor the efficacy of treatment and spot drug resistance to drugs other than rifampicin.

Limitations
1. Because the investigation was conducted retrospectively, radiographic data and histopathological reports could not be correlated with the findings
2. Because we did not receive a request for rifampicin sensitivity by the phenotypic technique in all the positive samples, the sensitivity and specificity of the MTB/RIF assay to identify rifampicin resistance in our study were not examined and not included in our aim
3. Since there were fewer bronchial aspirate samples in this study, future research with more samples is required.

CONCLUSION

Although Gene Xpert and AFB culture essentially have the same specificity, Gene expert has substantially higher sensitivity in bronchial aspirate samples than AFB culture. Even though culture is regarded as the gold standard method, it cannot simultaneously identify rifampicin resistance because it takes days to become positive. On the other hand, because to its speed and simultaneous identification of rifampicin resistance, gene Xpert can be a helpful diagnostic tool in patients with suspected pulmonary TB, whether their AFB smear results are positive or negative. This is especially true for patients with MDR and HIV related TB. In low-income nations like India with a high prevalence of TB, gene expert research must be cost-effective. Results from a positive gene expert but a negative culture must be carefully interpreted and should be well connected with the patient's clinical and treatment history.

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