Diagnostic Performance of GenoType® MTBDRplus Line Probe Assay in Bronchoalveolar Lavage for Pulmonary Tuberculosis Diagnosis in Sputum Scarce and Smear-negative Patients

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Abstract

Background: MTBDRplus line probe assay (LiPA) has been endorsed by the World Health Organization for pulmonary tuberculosis (TB) diagnosis. However, its value for Mycobacterium tuberculosis (MTB) detection in bronchoalveolar lavage (BAL) needs exploration. This study determined the diagnostic performance of MTBDRplus in BAL for MTB complex detection and isoniazid/rifampicin resistance in smear-negative and sputum scarce patients. Materials and Methods: Retrospective evaluation of data (January–December 2013) from patients who underwent bronchoscopy was done. Of these, patients with high TB suspicion with available data on acid-fast bacilli (AFB) smear/culture and MTBDRplus were selected. Results of MTBDRplus were compared with AFB smear/culture and drug susceptibility. Sensitivity and specificity of MTBDRplus was determined using TB culture as gold standard. Results: Data on 383 patients who underwent bronchoscopy were collected. Of these, 154 previously untreated TB suspect patients that were either smear negative on sputum microscopy or sputum scarce were selected. Out of 154 patients, 11 were smear positive and 34 patients were AFB culture positive. MTBDRplus detected MTB in 23/34 cases, the sensitivity and specificity being 67.6% and 85% (P < 0.001) versus 32.4% and 100% (P < 0.001) compared to smear microscopy. All smear-positive cases (n = 11) were detected by MTBDRplus. There were no discrepancies between phenotypic drug susceptibility testing and LiPA for isoniazid and rifampicin resistance in patients. Two cases of multidrug-resistant TB were detected. Conclusion: MTBDRplus detected TB more rapidly and accurately than smear microscopy with significant accuracy for isoniazid and rifampicin resistance. Its use in clinical practice would lead to rapid detection and effective management.

Keywords: Bronchoalveolar lavage, MTBDRplus line probe assay, pulmonary tuberculosis

INTRODUCTION

Tuberculosis (TB) results in high morbidity and mortality worldwide with ~10.4 million new TB cases and 1.4 million deaths as reported in global TB report 2016.[1] Among these deaths, ~190000 could be attributed to multidrug-resistant (MDR) TB. Pakistan has high TB and MDR-TB burden with ~323,856 cases reported in 2015. The rate of MDR-TB among untreated patients is estimated to be 4.2%.[1]

With increasing TB drug resistance, effective management relies heavily on accurate and early diagnosis.[3] The situation becomes even more challenging when a patient with a high TB suspicion is either smear negative or sputum scarce.[3,4] In such patients, specimen collection using bronchoscopy has been recommended. Diagnostic yield of TB culture in bronchoalveolar lavage (BAL) of smear-negative cases has been reported from 15% to 24%.[5,6]

A number of rapid nucleic acid amplification-based TB diagnostic methods have been developed and validated.[7-10] The World Health Organization (WHO) has recommended Xpert MTB/RIF assay for pulmonary TB diagnosis and rifampicin resistance detection in both smear negative and positive patients.[11] Studies evaluating Xpert MTB/RIF assay performance for BAL report sensitivity of 80%–93% in...
culture-positive patients and recommended this assay for TB diagnosis in BAL.[12–15]

Use of MTBDRplus line probe assay (LiPA) was recommended in 2008 by the WHO for pulmonary TB diagnosis. However, it was not recommended in smear-negative cases due to low sensitivity of earlier version of this assay in this patient population. A later study comparing the performance of MTBDRplus LiPA version 2 with Xpert MTB/RIF assay has shown equivalent performance in both smear-negative and smear-positive pulmonary TB patients.[16] MTBDRplus has an advantage over Xpert MTB/RIF as it can detect both isoniazid and rifampicin resistance.[10] So far, the performance of MTBDRplus has been validated mainly on sputum samples, and its role in diagnosing TB in sputum scarce patients requiring BAL has not been assessed.[17–19]

This study was performed to evaluate the diagnostic performance of MTBDRplus in BAL using TB culture as a reference standard.

Materials and Methods

Study design
The study was conducted at Aga Khan University (AKU) Hospital, Karachi, Pakistan. Records were evaluated for all bronchoscopies performed in the hospital from January 2013 to December 2013 after taking approval from the Ethical Review Committee of AKU.

Identification of patients with suspected TB was done through file review. Only those cases were selected who were sputum smear negative (sputum microscopy negative twice) or sputum scarce and on whom BAL samples had been sent with a request for acid-fast bacilli (AFB) smear, culture, and MTBDRplus on the same sample. Patients who had received previous TB treatment or were on current treatment for 2 weeks or more were excluded from the study.

Bronchoscopy procedure
All bronchoscopy procedures were performed by faculty and staff in the pulmonology section of hospital. A flexible bronchoscope with a 5.9 mm diameter (Olympus Optical Co., Tokyo, Japan) was used. After inspecting bronchial trees, samples were collected from the segment or subsegment that showed abnormal lesions on chest X-ray or computed tomography scan. Bronchial washing fluid was obtained by instillation of 20 ml of normal saline, and for BAL, 50–100 ml of normal saline was instilled and aspirated from the lung segments involved. BAL was collected and sent to AKU laboratory in a sterile container. A number of patients also underwent bronchial biopsy along with BAL, and data on histopathology for those specimens were also collected.

Microbiological investigations
AKU laboratory is a Supranational Reference Laboratory for TB diagnosis in the region. All three tests, microscopy, culture, and MTBDRplus were performed at this laboratory.

Specimen processing

Acid-fast bacilli smear and isolation of Mycobacterium tuberculosis
BAL fluid samples submitted for AFB smear and culture underwent digestion and decontamination using 5% NaOH-NALC solution and centrifuged. The pellet was resuspended in phosphate buffer saline to inoculate culture media and smear preparation. Auramine-based fluorescent stain was used to report AFB smear results as recommended by Global Laboratory Initiative in Mycobacteriology Laboratory Manual (http://www.stoptb.org/wg/gli/assets/documents/gli_mycobacteriology_lab_manual_web.pdf). Last accessed July 9, 2016. Last updated April 2014). During the study period, Mycobacterium tuberculosis (MTB) culture was performed using the standard methods. Pulmonary specimens were inoculated on Lowenstein–Jensen and MGIT (Becton Dickinson). MTB identification was confirmed using the absence or presence of growth on Middlebrook 7H10 Agar containing 0.5 mg/ml L-nitrophenyl butyrate (Sigma-Aldrich, St. Louis, USA) prepared in-house.[20]

Antimicrobial susceptibility test
Antituberculous drug susceptibility testing (DST) was performed according to CLSI M24-A2 guidelines[21] by agar proportion technique on enriched Middlebrook 7H10 medium (BBL). The concentration that was used was rifampicin, 1 µg/ml; isoniazid, 0.2 and 1 µg/ml; ethambutol, 5 and 10 µg/ml; and streptomycin, 2 and 10 µg/ml. Pyrazinamide resistance was detected at 100 µg/ml using the BD BACTEC™ MGIT 960 PZA test Medium and Kit (Becton Dickinson, USA). For quality control of susceptibility testing, MTB H37Rv was tested.

Line probe assay
LiPA was performed using GenoType® MTBDRplus version 2.0 (Hain Lifescience, Germany). About 0.5 ml of decontaminated specimen was centrifuged, heat inactivated, and DNA released by enzymatic lysis. Multiplex polymerase chain reaction was run on 5 µl of the supernatant (the extracted DNA) according to the instructions provided by manufacturers. Amplicons were hybridized to the DNA probe-labeled strip with conjugate; amplification and gene locus controls for MTB complex, rpoB, inhA, and katG; and wild type and mutation probes for inhA promoter, katG, and rpoB genes. An isolate was categorized as sensitive to both drugs if all wild-type probes tested positive and absence of hybridization with mutation detection probes. If at least one wild-type probe is absent or a mutation probe is present, resistance of the strain to the respective antibiotic was considered.

Statistical analysis
The data were obtained from the computerized records and file review. Descriptive analysis was done for demographic and clinical features. Data were entered and analyzed by Statistical Package for the Social Sciences (SPSS) software version 19 IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp. Results were expressed as number (percentage) as all variables were categorical.
Measures of diagnostic accuracy (sensitivity, specificity, positive predictive value [PPV], and negative predictive value) were calculated with 95% confidence interval taking culture as a gold standard. For further analysis, a composite standard was developed combining culture positivity, histology consistent with TB, and response to TB treatment.

**Results**

**Characteristics of patients**

Of the 383 patients who underwent bronchoscopy between January 1, 2013, and December 31, 2013, 160 TB suspects were selected in whom BAL was sent for all three tests: AFB smear, TB culture, and MTBDRplus. Six patients who had been on antitubercular therapy (ATT) were excluded from the study. Out of 154 study patients, 67.53% were male and 32.47% were female. About 96 (62.33%) were unable to produce sputum and 58 (37.66%) had previously been smear negative on two occasions. In addition, bronchial biopsy or cytology of bronchial brushings was performed on 34 patients [Figure 1]. The clinical details of 154 study patients are shown in Table 1.

On clinical and radiological feature, 72 patients were diagnosed to have pulmonary TB and were started on ATT. Eight two patients were excluded as they have alternate diagnosis and not treated as TB [Table 1]. Cultures of BAL from 34 patients yielded growth of MTB, whereas BAL from 38 patients remained negative on culture. All 72 patients were started on anti-TB therapy. Of these, 64 successfully completed their treatment. However, eight patients were lost to follow-up between 2 and 4 months of therapy, despite having shown signs of improvement during the initial 2 months of treatment.

**Diagnos tic accuracy of acid-fast bacilli smear, tuberculosis culture, and MTBDRplus**

MTB was detected with MTBDRplus in 23 (67.6%) of the 34 culture-positive patient samples. There were three cases of culture-proven MDR-TB, in which MTB was not detected by MTBDRplus. TB smear was positive in 11 of the 34 (32.4%) culture-positive patients. All smear-positive cases (11/11; 100%) were detected by MTBDRplus. Sensitivity, specificity, PPV, and PPV of TB smear and MTBDRplus in comparison to culture are shown in Tables 2 and 3.

The study included six MTBDRplus-positive and culture-negative cases. Two of these patients were expired during the 1st week of hospital stay with a final diagnosis of squamous cell carcinoma of lung in first patient and antineutrophil cytoplasmic antibodies positive granulomatosis with polyangiitis in second patient. Four patients were started on ATT. Of the 4 who were started on ATT, two patients showed chronic granulomatous inflammation on endobronchial biopsy and two were started on clinical grounds. Three patients had successfully completed ATT and were labeled cured and one was lost to follow-up. When these four cases were included, sensitivity and specificity of MTBDRplus increased to 79.41% and 94.74%, respectively.

The drug susceptibility pattern of 34 culture-positive samples is shown in Table 4. Two cases of MDR-TB were

**Table 1: Demographic and clinical characteristics of 154 patients with suspected pulmonary tuberculosis**

| Characteristic | n (%) |
|----------------|-------|
| Age in years, median (range) | 48 (19-83) |
| Male | 104 (67.53) |
| Presenting complaints | |
| Cough | 122 (79.2) |
| Fever | 98 (63.6) |
| Weight loss | 63 (40.9) |
| Abnormal chest X-ray | 148 (96.1) |
| Bilateral infiltrates | 38 (24.6) |
| Unilateral infiltrates | 103 (66.8) |
| Cavitary lesion | 4 (2.6) |
| Hilar prominence | 2 (1.3) |
| Pleural effusion | 26 (16.8) |
| Risk factors for TB | |
| Diabetes | 28 (18.2) |
| Solid cancer, on chemotherapy | 8 (5.2) |
| Autoimmune disorder, on systemic steroids | 9 (5.8) |
| ESRD | 3 (1.9) |
| Sputum status | |
| Sputum scarce | 96 (62.3) |
| Sputum smear-negative for AFB | 58 (37.7) |
| Final diagnosis | |
| Pulmonary TB | 72 (46.7) |
| Culture proven | 34 (47.2) |
| Treated on clinical grounds | 38 (52.8) |
| Alternate diagnosis | 82 (53.3) |
| Mycobacteria other than tuberculosis | 2 (2.4) |
| Pneumonia (16 culture proven, rest diagnosed on clinical grounds) | 39 (47.5) |
| Aspergillosis | 5 (6.0) |
| Lung cancer (histopathologically proven) | 17 (20.8) |
| Others* | 19 (23.1) |

*Others: Hypersensitivity pneumonitis (1), sarcoidosis (2), solitary pulmonary nodule (3), bronchiectasis (4), undiagnosed (9).

TB: Tuberculosis, ESRD: End-stage renal disease, AFB: Acid fast bacilli.

**Figure 1:** Details of patients inclusion in study.
Idrees, et al.: Diagnostic performance of MTBDRplus in bronchoalveolar lavage

The sensitivity and PPV of MTBDRplus for BAL have been found to be comparatively lower than Xpert MTB/RIF assay.[12-15] Published data report that diagnostic accuracy of MTBDRplus and Xpert MTB/RIF assay in sputum is similar.[22,23] However, head-to-head comparison of these assays in BAL has not been reported yet.

An advantage of MTBDRplus is that it additionally detects isoniazid resistance along with rifampicin resistance. In our study, the agreement of rifampicin and isoniazid resistance detection between conventional DST and MTBDRplus was 100% making it a reliable modality to start treatment before the availability of culture results. In our study, there was no discrepancy found for rifampicin and isoniazid resistance with the phenotypical assay. This could be because of low number of samples, as it is well known that although the rifampicin has a good correlation with the phenotypic DST, the correlation decreases in isoniazid since there could be missense mutations or mutations in representative resistance genes (katG and inhA) that cannot be detected by the molecular assay. More MDR and non-MDR and smear-negative patients should be included in the future studies for an accurate evaluation of MTBDRplus.

### Table 2: Frequency table for *Mycobacterium tuberculosis* detection by acid-fast bacilli smear and MTBDRplus among 72 samples included in the study. *Mycobacterium tuberculosis* culture was taken as gold standard

| MTBDRplus | BAL specimens of pulmonary TB patients (n=72) |  |
|---|---|---|
| MTB culture positive (n=34) | MTB culture negative (n=38) |  |
| Smear positive | Smear negative | Smear positive | Smear negative |
| Positive | 11 | 12 | 0 | 6 |
| Negative | 0 | 11 | 0 | 32 |
| Total | 11 | 23 | 0 | 38 |

MTB: *Mycobacterium tuberculosis*, TB: Tuberculosis, BAL: Bronchoalveolar lavage

### Table 3: Diagnostic performance of MTBDRplus in bronchoalveolar lavage using culture as a reference standard (n=72)

| 95% CI | Sensitivity % | Specificity % | PPV % | NPV % |
|---|---|---|---|---|
| Smear microscopy performed on BAL | 32.4 (17.41-50.53) | 100 (96.94-100.00) | 100 (71.33-100.00) | 83.9 (76.85-89.52) |
| MTBDRplus performed on BAL | 67.6 (49.47-82.59) | 84.2 (68.75-93.98) | 79.31 (63.96-89.23) | 74.42 (63.71-82.82) |

BAL: Bronchoalveolar lavage, PPV: Positive predictive value, NPV: Negative predictive value, CI: Confidence interval

### Table 4: Drug susceptibility pattern of culture-positive patients

| Drug | Culture positive for AFB (n=34) |  |
|---|---|---|
| MTBDRplus positive (n=23) | MTBDRplus negative (n=11) | Total (n=34) |
| Sensitive | Resistant | Sensitive | Resistant | Sensitive (%) | Resistant (%) |
| Ethambutol (2 µg) | 21 | 2 | 10 | 1 | 31 (91.2) | 3 (8.8) |
| Isoniazid (0.2 µg) | 21 | 2 | 8 | 3 | 29 (85.3) | 5 (14.7) |
| Rifampicin | 21 | 2 | 8 | 3 | 29 (85.3) | 5 (14.7) |
| Pyrazinamide | 21 | 2 | 8 | 3 | 29 (85.3) | 5 (14.7) |
| Streptomycin (2 µg) | 18 | 5 | 7 | 4 | 25 (73.5) | 9 (26.5) |

MDR (%): 2 (5.8) 3 (8.8)

MDR: Multidrug resistant, AFB: Acid-fast bacilli

detected 4 weeks earlier with MTBDRplus than conventional phenotypic testing. Of the 23 culture positive and MTBDRplus cases on which the results of susceptibility testing were known, the agreement of rifampicin and isoniazid resistance detection between conventional DST and MTBDRplus was 100%. Two out of 23 isoniazid-resistant MTB strains and 2/23 rifampicin-resistant strains were accurately detected by MTBDRplus.

**Discussion**

Collection of respiratory secretions by bronchoscopy is performed for pulmonary TB diagnosis in patients who cannot produce sputum or those who are sputum AFB smear negative with clinical suspicion of TB. No previous study has evaluated the performance of MTBDRplus in BAL samples for TB diagnosis in a high-burden country.

In this study, MTBDRplus clearly outperformed smear, detecting 12 out of 23 smear-negative cases 4 weeks before the culture results. Furthermore, our data showing sensitivity and specificity of 79.41% and 94.74%, respectively, for MTBDRplus in BAL suggest that this test will be valuable for MTB detection.
One of the limitations of our study is the low sample size of culture-positive TB cases. The low numbers may have contributed to a higher sensitivity and specificity of resistance detection by MTBDRPlus as compared to previous studies.[24,25]

MTBDRplus also detected six cases that were culture negative. This phenomenon has been observed in previous studies with both Xpert MTB/RIF assay and MTBDRplus[22,23] and may represent either cross-contamination (either during specimen collection or in the laboratory) or due to detection of DNA from nonviable bacteria in previously treated patients. Although treated patients were excluded, a transcription error in the patient file may have led to a failure to detect such cases. An earlier study has reported two such cases which were initially culture negative but became culture positive during treatment follow-up.[22] In our study, out of these six patients, four were treated with ATT and three successfully completed the treatment. It is possible that these cases may have represented “partially treated” TB as in our setup, many antibiotics including fluoroquinolones have already been used by physicians before the patient is referred to a pulmonary specialist or a diagnosis of TB is considered.

**Conclusion**

Our study indicates that MTBDRplus detected TB more rapidly and accurately than smear microscopy with significant accuracy for isoniazid and rifampicin resistance. Its use in clinical practice would lead to rapid detection and effective management of TB in sputum scarce or smear-negative patients. In cases where the LiPA is positive and culture is negative, the management should be based on clinical decision-making and follow-up cultures.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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