Bronchoscopy in suspected pulmonary TB with negative induced-sputum smear and MTD® Gen-probe testing

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Received 6 August 2010; accepted 3 March 2011
Available online 21 March 2011

KEYWORDS
Tuberculin skin test (TST); Nucleic acid amplification (NAA); Tuberculosis (TB); Bronchoscopy

Summary
Introduction: In our institution, patients with suspected pulmonary TB undergo multiple induced-sputum sampling for microscopy, culture and nucleic acid amplification (NAA) with the MTD® Gen-probe assay. Those with negative induced-sputum results still suspected with TB are then referred for bronchoscopy. We sought to determine the diagnostic yield of bronchoscopy in these patients with negative initial induced-sputum results both via smear and NAA testing.

Methods: We identified 30 consecutive cases of suspected pulmonary TB between 2001 and 2007, who had undergone a diagnostic bronchoscopy after negative results on induced-sputum smears and the MTD® Gen-probe on at least 2 samples.

Results: The cohort (M = 20 & F = 10) had a median age of 37 (range 16–85 yrs); were predominantly foreign born (27/30); HIV-negative (29/30) individuals with strongly positive TST’s (mean 18 ± 5 mm). Induced-sputum cultures were negative for M-TB in all patients after a full 60-day incubation period. BAL was culture positive for M-TB in 3/30 cases (10%) with 2 strains being pan-sensitive and the third being INH resistant. BAL microscopy with acid-fast smear (n = 30) and BAL Gen-probe (n = 23) were negative in all cases. A third of the patients (9/27, 33%) with negative bronchoscopy results were treated for culture negative TB. Treatment for latent TB was initiated in 5/27 (18%) individuals whereas 13/27 (48%) received no further treatment.
Introduction

Tuberculosis is a worldwide public health crisis of immense proportions and the majority of the world’s TB burden is carried by developing countries with limited resources. The diagnosis of pulmonary TB can be obtained from microscopy and culture of a number of different sources including regular sputum, induced sputum, gastric washings and bronchoscopy. The sensitivity, specificity and diagnostic yield of each of these tests vary widely between studies.1–7 Sputum microscopy and culture are relatively easy to perform but can be negative in a substantial proportion of pulmonary TB patients with reported sensitivities ranging from 25 to 45%.1,8,9 Sputum induction with hypertonic saline requires additional resource allocation and manpower training, but has been shown to increase the diagnostic yield of sputum examination in several studies.10–12 Several studies have confirmed the higher diagnostic yield of bronchoscopy over sputum examination in the diagnosis of pulmonary TB both from higher microscopy and higher culture positivity results.13–15 Clinicians often face a real dilemma when a patient suspected with active pulmonary TB has multiple negative induced-sputum results. They have a choice to either proceed with empiric treatment for pulmonary TB or to perform an invasive test like bronchoscopy in order to confirm the diagnosis. The question then is whether bronchoscopy offers any additional diagnostic yield in patients suspected with active pulmonary TB and with multiple negative induced-sputum results. The existing literature on this subject reports varying diagnostic yields for bronchoscopy depending on the study design and patient population studied. In addition, none of these studies have included patients who had undergone NAA testing on sputum samples prior to bronchoscopy. Higher diagnostic yields are obtained from patients undergoing bronchoscopy because of an inability to expectorate any sputum. This is in contrast to patients who underwent bronchoscopy after an adequate sputum sample was determined to be smear negative.13,16,17 Other studies however have shown no additional yield of bronchoscopy over sputum induction.18,19 We performed this study to answer the clinical question as to whether bronchoscopy provides additional diagnostic yield after multiple negative induced-sputum smear and NAA testing.

Patient population

In our center, patients suspected of pulmonary TB were first evaluated with repeated induced-sputum collections (see below for technique) for acid-fast sputum usually on at least three consecutive days. Nucleic acid amplification (NAA) testing specific for M-TB is performed using the MTD® Gen-probe test on at least 2 of these samples. Bronchoscopy is often considered while awaiting sputum culture results if a clinical suspicion for TB persists even after 2 or more induced-sputum samples were negative by smear and the MTD® Gen-probe test. We identified suspected pulmonary TB patients from 2001 to 2008 who underwent a bronchoscopy after at least 2 negative induced-sputum smear and NAA tests. The charts of these consecutive patients were abstracted for relevant data including demographics, co-morbidities and bronchoscopy/sputum results. Data about PPD skin testing and the QuantIFERON®-TB Gold test were also obtained from the charts when available.

PPD skin test

The PPD skin test was performed using either Tubersol or Aprisol at a dose of 5 TU injected intradermally into the forearm. The reaction was read after a period of 48 h measuring induration.

Amplified mycobacterium tuberculosis MTD® Gen-probe test

This test was performed according to the manufacturer’s recommendations. The test was performed both on sputum samples and on BAL specimens.20,21

QuantIFERON®-TB Gold™ test

The QuantIFERON®-TB Gold™ test is an interferon-gamma release assay that detects T-cell immune responses to antigens specific to M-TB. The details about this test and its methodology are detailed elsewhere.22

Induced-sputum technique

Each induced-sputum collection was performed using 10% hypertonic saline nebulized through a mouthpiece using a DeVilbiss ultrasonic nebulizer for at least 20 min. Morning samples were obtained on consecutive days whenever possible. Each sample was then centrifuged and concentrated according to standard published methods and subjected to acid fast smear examination, culture and MTD® Gen-probe testing. The MTD® Gen-probe test was performed on at least 2 of the induced-sputum samples as per established protocols described in detail elsewhere.20,21 All sputum testing was performed either at the Mayo Clinic or at the Olmsted County TB Clinic. Both facilities have CLIA certified labs with standardized and rigorous procedures for sputum induction.

Conclusion: Bronchoscopy provided diagnostic confirmation of pulmonary TB in 10% of subjects at least 2 negative induced-sputum samples by smear microscopy and NAA testing. © 2011 Elsevier Ltd. All rights reserved.

Methods

This was a retrospective study that was approved by the institutional review board at the Mayo Clinic, Rochester, Minnesota, USA. Only patients who had consented to have their medical charts available for research studies were included in this study. The data were analyzed using JMP 7.0 (SAS Institute, Cary, North Carolina).
Bronchoscopic technique

Bronchoscopies were performed by fellows in pulmonary-critical care medicine under the supervision of experienced faculty members at the Mayo Clinic. Bronchoalveolar lavage specimens were obtained by wedging the bronchoscope into the radiologically involved lung segment and instilling 20 cc aliquots of saline solution followed by a 5 ml air chaser. Repeat aliquots were instilled and aspirated until a total of 35–40 cc of fluid return was obtained. Transbronchial biopsies were obtained under fluoroscopic guidance from the involved lung segment whenever possible. The use of lidocaine was minimized as much as possible because of its known antimicrobial properties.23

Results

The study cohort identified 20 males and 10 females (Table 1) with a median age of 37 (range 16–85 yrs). Cough was the most common symptom (n = 18, 60%) followed by weight loss (n = 6, 20%), hemoptysis (n = 4, 13%) and chest pain (n = 4, 13%). Cases were predominantly foreign born (27/30); HIV negative (29/30) individuals with strongly positive TST’s (mean 18 mm). QuantiFERON®-TB Gold serum testing was performed in 14 patients and was positive in 13 of these patients. Previous treatment for M-TB was recorded in 8 patients (4 for LTBI and 4 for active TB). Chest X-ray and CT results predominantly showed unilateral upper lobe disease (20/30) (Fig. 1).

Prior to bronchoscopy, the numbers of induced-sputum samples collected on each patient were as follows: 2 samples in 6/30 patients (20%), 3 collections in 22/30 patients (73%) and 6 collections in 2 patients (7%). The MTD® Gen-probe NAA test was performed in all patients on at least 2 induced-sputum samples and these were all negative (69 tests, 30 patients). Induced-sputum cultures were negative for M-TB culture after a full 60-day incubation period in all patients (92 samples, 30 patients), confirming sputum negativity.

Bronchoscopy was performed in all patients within a few days after negative induced-sputum smear and NAA test results. (Table 2) BAL collections were culture positive for M-TB in 3/30 cases (10%). BAL microscopy (n = 30) and BAL Gen-probe NAA tests (n = 23) were negative in all cases. Transbronchial biopsies were obtained in 14 patients with one biopsy each showing fibrosis and organizing pneumonia. (Table 3) Normal biopsies were obtained in 10/14 cases. Non-caseating granulomas were obtained in 2/14 patients with concurrent positive BAL culture results in one of these two patients. All biopsies simultaneously underwent AFB culture with no positive results.

Gastric washings with culture and NAA testing were performed in two patients (2 samples each) and were negative in both cases.

Table 1 Demographic data (n = 30).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, IQR)</td>
<td>37 years (IQR, 22–63 years) Range: 16–85 years</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>n = 20 (67%)</td>
</tr>
<tr>
<td>Female</td>
<td>n = 10 (33%)</td>
</tr>
<tr>
<td>Previous TB treatment</td>
<td></td>
</tr>
<tr>
<td>Active TB</td>
<td>n = 4 (13%)</td>
</tr>
<tr>
<td>Latent TB</td>
<td>n = 4 (13%)</td>
</tr>
<tr>
<td>None</td>
<td>n = 22 (69%)</td>
</tr>
<tr>
<td>BCG status</td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>n = 3 (10%)</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>n = 2 (7%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>n = 25 (83%)</td>
</tr>
<tr>
<td>Tuberculin skin test (mm)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>n = 23 (mean 18 mm)</td>
</tr>
<tr>
<td>Unknown</td>
<td>n = 7 (positive by history in 4 of these individuals)</td>
</tr>
<tr>
<td>QuantiFERON®-TB Gold</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>n = 13 (43%)</td>
</tr>
<tr>
<td>Negative</td>
<td>n = 1 (3%)</td>
</tr>
<tr>
<td>Not done</td>
<td>n = 16 (53%)</td>
</tr>
</tbody>
</table>

Table 2 Results of bronchoscopy (n = 30).

<table>
<thead>
<tr>
<th>Bronchoscopy results for M-Tb</th>
<th>Smear (n = 30)</th>
<th>Gen-probe (n = 23)</th>
<th>Culture (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>23</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 3 Normal biopsies were obtained in 10/14 cases. Non-caseating granulomas were obtained in 2/14 patients with concurrent positive BAL culture results in one of these two patients. All biopsies simultaneously underwent AFB culture with no positive results.

Gastric washings with culture and NAA testing were performed in two patients (2 samples each) and were negative in both cases.

Pan-sensitive organisms were isolated from BAL fluid in 2/3 cases and Isoniazid resistance was detected in the third case. All three individuals received treatment for culture positive TB (Table 4). The QuantiFERON®-TB Gold test was positive in two of these patients and was not done in the 3rd patient. Treatment for culture negative TB was started in a third of the patients (9/27, 33%) with negative
bronchoscopy results. After completion of this treatment, 4 showed signs of radiological improvement, 3 had stable radiological disease and 1 patient was lost to follow up. Treatment for latent TB was initiated in 5/27 (18%) individuals whereas 13/27 (48%) received no TB treatment and were treated with antibiotics with improvement in 7/13 and stability in 4/13 individuals.

**Discussion**

The diagnosis of pulmonary TB in patients with negative expectorated sputum results remains challenging due to the variable diagnostic yields of any single test. Sputum induction, gastric lavage and bronchoscopy have all been reported to increase diagnostic yield and have useful and often complimentary roles in the diagnosis of pulmonary TB.

We report the diagnostic yield of bronchoscopy in patients with suspected pulmonary TB and with negative results on smear and NAA test results on at least 2 induced-sputum specimens. All patients in our study underwent induced-sputum collection on at least 2 occasions and a majority underwent 3 or more sputum inductions (73%). Culture of the BAL fluid obtained at bronchoscopy successfully identified 3 cases (10%, 3/30) of pulmonary TB that would have been otherwise missed by smear and NAA testing of multiple induced-sputum samples. In addition all patients had negative culture results on the induced-sputum samples after full 60-day incubation, thus confirming true sputum negativity.

The role of Bronchoscopy in the diagnosis of pulmonary TB has been addressed before with considerably different yields reported across studies. These differences in yield from bronchoscopy reflect differences in study design and the patient population being studied. In some of these reports, patients with negative expectorated sputum results, did not undergo sputum induction as the next step, but proceeded directly to bronchoscopy. Not surprisingly, these studies reported a high diagnostic yield for bronchoscopy of between 32.5% and 90%. Other studies focussed on patients who were sputum negative, but also included those who were unable to expectorate any sputum and again reported relatively high diagnostic yields for bronchoscopy.

Induced-sputum has been widely recognized to increase diagnostic yields over spontaneously expectorated sputum. The question of whether bronchoscopy provides any additional diagnostic yield over induced-sputum has yielded conflicting reports. Schoch et al. compared the yield between regular sputum, induced-sputum and bronchoscopy in the diagnosis of TB in an immigrant population with suspected pulmonary TB. They found that bronchoscopy had a significantly higher diagnostic yield when compared to multiple induced-sputum samples. They also found that the diagnostic yield of any one modality was low and varied from 36% to 63%, emphasizing the complimentary nature of each of these tests. Brown et al. on the other hand performed bronchoscopies in 21 patients with negative induced-sputum results and did not find any additional yield of performing this procedure. McWilliams also reported similar results with induced-sputum yielding 26/27 culture positive cases whereas BAL yielded only 14/27. Anderson similarly compared induced-sputum (without NAA testing) with bronchoscopy in patients with negative sputum results and reported a better yield from induced-sputum.

Several explanations have been proposed by these authors and others to explain the lower yield from bronchoscopy. They speculated that lower yields from bronchoscopy may have resulted from the inexperience of the operators performing the procedure. The use of lidocaine during bronchoscopy has also been implicated in these regards. Lidocaine has well described antibacterial and antifungal properties. In addition, Lidocaine has been shown to have specific inhibitory effects on growth of

<table>
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<tr>
<th>Transbronchial biopsy pathology</th>
<th>MTD( ^{\circ} ) Gen-probe on BAL</th>
<th>BAL culture</th>
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<tbody>
<tr>
<td>Non-caseating granuloma (n = 2)</td>
<td>Positive (1/2)</td>
<td></td>
</tr>
<tr>
<td>Fibrosis (n = 1)</td>
<td>Negative (1/1)</td>
<td></td>
</tr>
<tr>
<td>Organizing pneumonia (n = 1)</td>
<td>Negative (1/1)</td>
<td></td>
</tr>
<tr>
<td>Normal (n = 10)</td>
<td>Positive (10/10)</td>
<td></td>
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<table>
<thead>
<tr>
<th>Group</th>
<th>Number of patients</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture positive TB (pan-sensitive)</td>
<td>2</td>
<td>2 months with 4 drugs</td>
</tr>
<tr>
<td>Culture positive TB (INH-resistance)</td>
<td>1</td>
<td>4 months with 2 drugs</td>
</tr>
<tr>
<td>Latent TB</td>
<td>5</td>
<td>Initially started on 2 months</td>
</tr>
<tr>
<td>Culture negative TB</td>
<td>9</td>
<td>with 4 drugs, then switched to</td>
</tr>
<tr>
<td>No treatment</td>
<td>13</td>
<td>6 months with 3 drugs (INH dropped)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 months of INH</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 months with 4 drug</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No treatment</td>
</tr>
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Mycobacterium tuberculosis and non-tuberculous mycobacteria in culture. As little as 1 ml of 2% lidocaine instilled in the tracheobronchial tree was shown to reduce the culture positivity rate for M. tuberculosis by 50%.\textsuperscript{33} Limiting the amount of lidocaine used during bronchoscopy has its practical limitations due to patient comfort related issues, but may be responsible for a substantial decrease in the yield from this procedure.

Our study differs from previous studies in several respects. Firstly, every patient underwent sputum induction on multiple occasions as per the protocol in our institution. Attempts were made to collect at least 3 induced-sputum samples on separate days.

Secondly, in each patient, at least 2 of these induced-sputum samples also underwent concurrent NAA testing using the MTD\textsuperscript{10} Gen-probe test. (Gen.-probe Inc. San Diego, CA). NAA tests are very sensitive and specific (>90%) in smear positive cases and at least moderately sensitive (50–80%) in smear negative cases. The incorporation of routine NAA testing on multiple induced-sputum samples in our clinical practice has resulted in a quicker turnaround time and lower false negative rate for induced-sputum samples.\textsuperscript{34} Thus the individuals referred for bronchoscopy in our practice were more likely to be truly sputum ‘negative’. This is corroborated by the fact that all induced-sputum samples were culture negative at the end of the 60-day incubation period.

Thirdly, patients were considered for bronchoscopy only after multiple induced-sputum samples were negative by microscopy and NAA testing. This step-wise approach incorporating universal sputum induction and NAA testing likely reduced the yield of bronchoscopy in our patients when compared to other published studies on this subject.\textsuperscript{13,26}

The decision to proceed with bronchoscopy for the purposes of confirming TB by smear and/or culture confirmation is influenced by several factors. Drug susceptibility testing can obviously be performed only on culture confirmed cases of M-TB. The global spread of MDR and XDR-TB challenge the existing paradigm of empiric standard multidrug treatment in sputum negative cases of suspected pulmonary TB. This is especially true of patients coming from areas known to harbor high rates of drug resistance. In Olmsted County, Minnesota (where the study was performed), the numbers of new TB cases have been increasing over the past decade with a majority of the new cases occurring in foreign-born individuals (78%).\textsuperscript{35} Similar statistics have been reported for the state of Minnesota as a whole.\textsuperscript{36} The majority of drug resistance has been reported in foreign born individuals with prevalence rates around 13% for one or more 1st line agents and around 10% for isoniazid resistance.\textsuperscript{35–37} In 2006 a single case of XDR-TB was also reported in the state. Patients co-infected with both HIV and TB have higher rates of drug resistant TB and drug susceptibility testing is very important in these patients.\textsuperscript{38,39}

Our study reaffirms the diagnostic utility of bronchoscopy in culture confirmation of TB even after negative results on multiple induced-sputum samples.\textsuperscript{40} Bronchoscopy is also useful in the identification of alternative diagnoses including other granulomatous lung diseases and malignancy. One note of caution is that rigorous decontamination procedures and strict protocols need to be in place in order to avoid nosocomial transmission of TB and other infectious agents via contaminated bronchoscopy equipment.\textsuperscript{41–43}

Although bronchoscopy may be useful in resource rich and low TB endemicity areas, much of worldwide TB occurs in developing countries where resources are severely limited. Given the high pre-test probability for TB, a high proportion of patients receive treatment for TB after negative sputum results due to diagnostic resource constraints.\textsuperscript{44} Alternate sputum based methods using real time PCR that can simultaneously diagnose TB and identify drug resistant strains appear to be a promising new avenue in settings where the role of bronchoscopy is limited by cost and logistical constraints.\textsuperscript{45}

In summary, our study shows that bronchoscopy is useful in obtaining culture confirmation of pulmonary TB in about 10% of patients with negative results on multiple induced-sputum samples by microscopy and NAA testing. In resource poor settings, the role of bronchoscopy in TB diagnosis is likely to be limited due to availability, cost and logistical challenges.

Conflict of interest

The authors report no conflicts of interest.

References


