

Performance of cytology, acid-fast bacilli smear, gene Xpert and mycobacterial cultures in endobronchial ultrasound-transbronchial needle aspiration aspirate in diagnosing mediastinal tuberculous lymphadenitis

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ABSTRACT

Background: Diagnosis of isolated mediastinal tuberculosis (TB) can be challenging. Endobronchial ultrasound (EBUS) increases the diagnostic yield by direct sonographic visualization of mediastinal and hilar lymph nodes. With the advent of molecular techniques such as Gene Xpert, their addition to the cytology and cultures increases the diagnostic yield and detection of rifampicin resistance (RR) which helps change the effective therapeutic regimen immediately. **Materials and Methods:** Prospective analysis of all patients undergoing EBUS-guided transbronchial needle aspiration (EBUS-TBNA) with a clinical possibility of TB in isolated mediastinal lymphadenopathy patients at a tertiary care referral center between June 2016 and January 2018. All patients had at least five passes from each node of which two passes from each lymph node sampled in 2 ml of saline for culture and Gene Xpert for microbiologic, pathologic, and molecular analysis as per hospital protocol. **Results:** Out of 60 patients, 44 were diagnosed to have mediastinal tuberculous lymphadenitis, 8 sarcoidosis, 2 malignancies, and 6 reactive lymphadenitis. TBNA cytology was positive in 40/44 patients (90.9%), out of which 18 patients were culture positive with the sensitivity of 100%, specificity 47.6%, positive predictive value (PPV) 45%, and negative predictive value (NPV) 100%, (P value 0.011). TBNA acid-fast bacilli (AFB) smear was positive in 20/44 patients (45.45%) out of which 12 were culture positive, with sensitivity of 67%, specificity 80.95%, PPV 60%, NPV 85% (P value 0.011). TBNA Gene Xpert was positive in 30/44 patients (68.2%), out of which 6 (13.63%) showed RR-TB and two were cytology negative. Sixteen patients where culture positive with sensitivity of 88.89%, specificity 66.67%, PPV 53.33%, NPV 93.33% (P value of 0.005). TBNA AFB culture was positive in 18/44 patients (40.9%). **Conclusion:** EBUS-TBNA is an effective and safe diagnostic tool for intrathoracic TB, especially for mediastinal tuberculous lymphadenitis. The combination of various tests increases the diagnostic yield. Mediastinal nodal aspirates traditionally believed to be paucibacillary can still be captured by Gene Xpert.

KEY WORDS: Cytology, endobronchial ultrasonography, gene Xpert, mediastinal tuberculous lymphadenitis, transbronchial needle aspiration, tuberculous lymphadenopathy

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INTRODUCTION

In India, where tuberculosis (TB) is endemic, mediastinal lymphadenopathy secondary to TB is common. TB continues to rise and the diagnosis of both disease and drug resistance can be challenging. Endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA) is increasingly be used to evaluate such cases.^[1] In 2017, TB caused an estimated 1.3 million deaths in HIV-negative people and there were an additional 300,000 deaths from TB among HIV-positive people. Globally, the best estimate is that 10.0 million people developed TB disease. The best estimate is that, worldwide in 2017, 558,000 people developed TB that was resistant to rifampicin (RR-TB), the most effective first-line drug, and of these, 82% had multidrug-resistant TB (MDR-TB).^[2] Three countries accounted for almost half of the world's cases of MDR/RR-TB: India (24%), China (13%), and the Russian Federation (10%). Globally, 3.5% of new TB cases and 18% of previously treated cases had MDR/RR-TB. Among cases of MDR-TB in 2017, 8.5% were estimated to have extensively drug-resistant TB.^[3] The sites of extrapulmonary TB include lymph nodes, pleura, meninges, bone, military, or disseminated (less common), gastrointestinal, genitourinary, and other sites (rare). Diagnostic parameters such as an acid-fast smear and culture of specimens from extrapulmonary sites are often not as sensitive as those from pulmonary TB. Tuberculous adenitis is a common cause of lymphadenopathy. The most common site is cervical lymphadenitis. This is referred to as "scrofula." Tuberculous adenitis has also been found at intrathoracic, intra-abdominal (mesenteric lymph nodes or paraaortic), and occasionally, axillary, inguinal, and intramammary sites. India has incidence rates of 4.4 cases per 1000. In recent years, lymphadenitis is common between 20 and 40 years of age and exhibits female predominance.^[4] Without parenchymal lung abnormalities, inaccessible isolated thoracic lymphadenopathy often poses a diagnostic challenge due to the low diagnostic yield of sputum studies.^[2,5] TBNA is an effective and safe technique in the diagnosis of intrathoracic lymphadenopathy.^[6-8] EBUS can improve the diagnostic yield of TBNA by enabling visualization of the lymph nodes beyond the tracheal or bronchial wall, and with the advent of the convex probe, it allows real-time sampling of the mediastinal and hilar lymph nodes.^[9]

The standard procedures such as a smear for acid-fast bacilli (AFB), microbiological culture, and pathologic examination still play a key role.^[10] Various techniques are available for obtaining pathologic material from intrathoracic masses, including computed tomographically guided percutaneous needle aspiration, conventional TBNA, EBUS-guided TBNA, transesophageal US-guided needle aspiration, mediastinoscopy, and surgery. EBUS-guided TBNA is an important minimally invasive technique for the diagnosis of mediastinal, paratracheal, and peribronchial lesions.^[11,12] Compared

with conventional TBNA, EBUS-TBNA offers direct visualization of the target lesion and real-time sampling, yields more nodal stations, and improves the diagnostic sensitivity.^[12] EBUS guided TBNA is less invasive than mediastinoscopy^[12] and is safe and well-tolerated for elderly patients.^[13] Recently, several studies have evaluated the potential benefits of EBUS-TBNA for diagnosis of TB.^[10,14,15] Pulmonary TB is often accompanied by intrathoracic tuberculous lymphadenopathy (TBLA), which is not a rare manifestation.^[10] The lack of a specific clinical or radiologic presentation leads to diagnostic challenges for intrathoracic TBLA.^[14,15] The microbiologic or pathologic evidence is often crucial. EBUS guided TBNA provides a new sampling technique for intrathoracic lymph nodes and peribronchial tissues. The polymerase chain reaction-based Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is a cartridge-based, an automated diagnostic test that can identify *Mycobacterium* TB (MTB) and resistance to rifampicin on biological samples in <2 h. The assay has high sensitivity and specificity for the identification of both MTB and RR in expectorated samples of lower airways secretions. However, relatively few performance data have been published to date on nonrespiratory specimens.^[16-22] We previously demonstrated the usefulness of EBUS-TBNA in the investigation of intrathoracic lymphadenopathy, with the improved yield for TB when combined with cytological analysis.^[15] Mediastinal nodal aspirates traditionally believed to be paucibacillary, can still be captured by Gene Xpert. Gene Xpert may be generating an increased yield through improved sensitivity compared with culture. This study was undertaken to assess the diagnostic yield of various tests from the nodal aspirate.

MATERIALS AND METHODS

This is a prospective study conducted at the Department of Pulmonology, Fortis Flt. Lt. Rajan Dhall Hospital, Vasant Kunj, New Delhi, tertiary health-care center from March 2016 and January 2018 after the institutional ethics committee approval.

This study included 60 patients who had undergone EBUS-guided lymph node aspiration for suspected isolated mediastinal TBLA, due to mediastinal or hilar lymphadenopathy on contrast-enhanced computed tomography (CT) chest in the absence of parenchymal abnormality and were either sputum or BAL smear negative. Results from the procedures were recorded, including microscopy for AFB, mycobacterial cultures, molecular test including Gene Xpert, and pathological cytologic examination.

Diagnostic criteria

Patients were considered to have had a diagnosis of mediastinal tuberculous lymphadenitis if the EBUS TBNA aspirate met one or more of the following criteria: .

- I. A positive culture result for MTB
- II. A positive Gene Xpert result with a high clinical index of suspicion;
- III. A positive AFB smear
- IV. Culture-negative, but a high clinical index of suspicion and demonstration of supportive histological changes like necrotizing granulomatous inflammation
- V. The detection of caseating granulomatous reaction and/or direct identification of MTB by Ziehl–Neelsen stain and/or growth in BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 medium and Gene Xpert positive were considered as TBLA.

Exclusion criteria

Mediastinal tuberculous lymphadenitis where the specific alternate diagnosis was obtained at EBUS-TBNA such as sarcoidosis, malignancy, and reactive lymphadenitis.

Performance of Endobronchial ultrasound-transbronchial needle aspiration

The EBUS-TBNA procedure was performed under general anesthesia. A 7.5-MHz OLYMPUS BF TYPE UC180F convex-probe bronchoscope was used. Lymph nodes were classified using the International Association for the Study of Lung Cancer (IASLC) classification and a short axis > 1 cm on CT was included in the study. The dimensions of the lymph nodes seen on the convex probe-EBUS were recorded from archived US images. All TBNA specimens were obtained using a dedicated, disposable, 21-gauge, Vizishot needle (NA-201SX-4021 Olympus Medical Systems, Singapore) using the jabbing the method under real-time ultrasound control and power Doppler mode to avoid vascular injury. The same needle was used for all stations as the aim of EBUS-TBNA in this study was only diagnostic. We used 21G instead of 22G due to the proposed better preservation of the histological structure of the samples with the larger needle. Continuous suction was applied with a dedicated 20 ml syringe while the catheter was moved back and forth up to a maximum of 20 times. A maximum of five aspirates was obtained from each location. The specimens obtained were immediately smeared on slides and fixed in 95% ethanol and flushed in 0.9%, 2 ml normal saline for pathological and microbiological assessment respectively. There was not a pathologist on-site and the Ziehl–Neelsen smear reading was done in the microbiology laboratory after the bronchoscopy finished, but on the same day. The specimens were stained with Ziehl–Neelsen stain and cultured with BACTEC MGIT 960 media. Cytopathological findings revealing caseating granulomatous reaction or detection of AFB on smear or Gene Xpert or growth of mycobacterium TB in the BACTEC system were considered to be TBLA. Results from the procedures were recorded, including microscopy for AFB, mycobacterial cultures, and molecular analysis for Gene Xpert and pathologic cytological examination. All TBLA cases received anti-TB treatment and followed for at least 6 months.

Statistical testing was conducted with the statistical package for the social science system version SPSS 17.0

of IBM SPSS Statistics, developed by IBM, India. For all statistical tests, a $P < 0.05$ will be taken to indicate a significant difference.

RESULTS

From June 2016 to January 2018, 60 EBUS-TBNA procedures where specimens were sent for microbiologic, pathologic, and molecular analysis were performed. No procedural complications occurred. No patients were known to be infected with the human immunodeficiency virus. The mean age distribution was 45.26yrs. The predominant group with males 83% and females 17%.

Out of 60 patients 44 were diagnosed to be mediastinal tuberculous lymphadenitis, 8 sarcoidosis, 2 malignancies, and 6 reactive lymphadenitis. TBNA cytology was positive in 40/44 patients (90.9%), out of which 18 patients were culture positive with the sensitivity of 100%, specificity 47.6%, positive predictive value PPV 45%, and negative predictive value (NPV) 100%, (P value 0.011). TBNA AFB smear was positive in 20/44 patients (45.45%) out of which 12 were culture positive, with sensitivity of 67%, specificity 80.95%, PPV 60%, NPV 85% (P value 0.011). TBNA Gene Xpert was positive in 30/44 patients (68.2%), out of which 6 (13.63%) showed RR-TB and two were cytology negative. Sixteen patients where culture positive with sensitivity of 88.89%, specificity 66.67%, PPV 53.33%, NPV 93.33% (P value of 0.005). TBNA AFB culture was positive in 18/44 patients (40.9%).

Of the 60 patients enrolled, the majority were in the age group 21–40 (40%). This was followed by 41–60 years and >60 years with a percentage of frequency 33.3% and 23.3%, respectively. The mean age distribution was 45.26 years. Predominant are males with 83% and females 17% [Table 1]. Forty four of the 60 enrolled patients were diagnosed to have TB. Gene Xpert was positive in 30 patients, with 68.18% and 6 patients showing RR, i.e., 13.8% and showed sensitivity of 88.89%, specificity of 66.67% and PPV of 55.53% and NPV of 93.33%, with P value of 0.005 which is statistically significant. AFB smear was positive in a total of 20 patients where AFB culture was positive in 6 patients and showed with the Sensitivity of 67%, Specificity of 80.95%, PPV of 60%, and NPV of 85% with a P value of 0.011 which is statistically significant. Cytology showed necrotizing granulomatous inflammation in 40 patients with 90.9% and showed the sensitivity of 100%, specificity of 47.61%, and PPV of

Table 1: Demographic distribution in present study

Age	Sex		Total (%)	χ^2	P
	Female (%)	Male (%)			
<20	2 (20.0)	0	2 (3.3)	5.7	0.127
21–40	2 (20.0)	22 (44.0)	24 (40.0)		
41–60	4 (40.0)	16 (32.0)	20 (33.3)		
>60	2 (20.0)	12 (24.0)	14 (23.3)		
Total	10 (100.0)	50 (100.0)	60 (100.0)		

45%, and NPV of 100% with a *P* value of 0.011 which is statistically significant [Table 2].

DISCUSSION

EBUS-TBNA is a minimally invasive technique that has become the procedure of choice for evaluation of mediastinal/hilar lymphadenopathy due to its excellent diagnostic accuracy in the staging of lung cancer and evaluation of numerous other processes.^[23,24] This procedure has an excellent safety profile and cost advantages in comparison to surgical mediastinoscopy.^[25] Multiple studies have demonstrated diagnostic sensitivity is equivalent to surgical mediastinoscopy.^[24,26] Our findings demonstrate excellent efficacy and safety of EBUS-TBNA for the diagnosis of suspected isolated mediastinal tuberculous lymphadenitis

In our study, patients with isolated mediastinal lymphadenopathy suspicious of tuberculous mediastinal lymphadenitis who are either sputum or BAL smear-negative, out of 60 patients 16 patients had an alternate diagnosis with 8 sarcoidosis, 2 malignancies, and 6 reactive lymphadenitis. Sensitivity, specificity, PPV, and NPV were calculated using positive mycobacterial culture as the reference gold standard. TBNA cytology was positive in 40/44 patients (90.9%), TBNA AFB smear was positive in 20/44 patients (45.45%), TBNA Gene Xpert was positive in 30/44 patients (68.2%), TBNA AFB culture was positive in 18/44 patients (40.9%).

Table 2: Transbronchial needle aspiration gene Xpert, transbronchial needle aspiration AFB smear, and transbronchial needle aspiration cytology in present study

	TBNA AFB culture		Total (%)	χ^2	<i>P</i>
	Positive (%)	Negative (%)			
TBNA gene Xpert					
Positive	16 (88.9)	14 (33.3)	30 (50.0)	7.77	0.005
Negative	2 (11.1)	28 (66.7)	30 (50.0)		
Total	18 (100.0)	42 (100.0)	60 (100.0)		
Sensitivity (%)		88.89			
Specificity (%)		66.67			
PPV (%)		53.33			
NPV (%)		93.33			
TBNA smear					
Positive	12 (66.7)	8 (19.0)	20 (33.3)	6.42	0.011
Negative	6 (33.3)	34 (81.0)	40 (66.7)		
Total	18 (100.0)	42 (100.0)	60 (100.0)		
Sensitivity (%)		67			
Specificity (%)		80.95 or 81			
PPV (%)		60			
NPV (%)		85			
TBNA cytology					
Positive	18 (100.0)	22 (52.4)	40 (66.7)	6.42	0.011
Negative	0 (0.0)	20 (47.6)	20 (33.3)		
Total	18 (100.0)	42 (100.0)	60 (100.0)		
Sensitivity (%)		100			
Specificity (%)		47.61			
PPV (%)		60			
NPV (%)		85			

If *P* < 0.05, there is a statistically significant association between two variables. TBNA: Transbronchial needle aspiration, PPV: Positive predictive value, NPV: Negative predictive value

Vallandramam *et al.* which was published as an abstract in Interventional Pulmonology Poster in CHEST 2015 had similar results with the EBUS-TBNA aspirate showed necrotizing granulomatous inflammation on cytology in 20 patients (90.9%), smear for AFB was positive in 14 patients (63.63%), Gene Xpert was positive in 15 patients (68.1%) and mycobacterial cultures positive in 9 patients (41%).

Geake *et al.* in a study conducted in 2014, in 159 patients who underwent EBUS-TBNA and had tissue referred for mycobacterial culture, of which 158 were included in the final analysis. Thirty-nine were ultimately diagnosed with TB (25%). The sensitivity of EBUS-TBNA for microbiologically confirmed tuberculous mediastinal lymphadenitis was 62% (24/39 cases), specificity was 100%. NPV and diagnostic accuracy for microbiologic diagnosis were 89% and 91%, respectively. For a composite clinicopathologic diagnosis of TB NPV and accuracy were 98% and 98%, respectively. The sensitivity for Nucleic acid amplification test was 38%. They have concluded that EBUS-TBNA is a safe and well-tolerated procedure in the assessment of patients with suspected, isolated mediastinal lymphadenitis and demonstrates good sensitivity for a microbiologic diagnosis of isolated mediastinal lymphadenitis. When culture and histological results are combined with high clinical suspicion, EBUS-TBNA demonstrates excellent diagnostic accuracy and NPV for the diagnosis of mediastinal TB lymphadenitis.^[27]

In a country like India where TB is endemic, high clinical suspicion warrants the addition of tests like Gene Xpert increases the diagnostic yield and detection of RR. As in our study, 6 patients were diagnosed to be MDR/RR- TB which helped us to change the regimen and everyone had combined isoniazid resistance. All of them received MDR-TB regimen was being followed till now. The rest of the patients diagnosed with TBNA with or without empiric treatment improved clinically after the follow-up.

Transbronchial needle aspiration Gene Xpert

TBNA Gene Xpert was positive in 30 patients (68.2%), out of which 6 (13.63%) showed RR-TB [Table 3]. Eight patients were culture positive and 4 of the cytology negative were Xpert positive which shows its addition can increase diagnostic yield. Sensitivity was 88.89%, Specificity 66.67%, PPV 53.33%, and NPV 93.33%, with a *P* value of 0.005 which is statistically significant. In Ghariani A *et al.*^[28] lymph node aspirate showed that the sensitivity and specificity of the Xpert assay were 87.5% and 73.3% respectively. In Dhasmana DJ *et al.*^[29] the GeneXpert Sensitivity 66.7%, specificity 96.3%, PPV 88.9% and NPV 86.5%. Our study showed greater sensitivity and NPV [Figure 1].

Transbronchial needle aspiration acid-fast bacilli smear

TBNA AFB smear was positive in 20 patients (45.45%) out of which 12 were culture positive with Sensitivity of 67%,

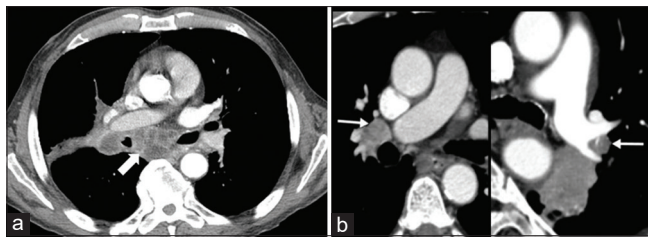


Figure 1: CECT chest imaging of patients demonstrating (a) a 38-year-old male with necrotic subcarinal lymph node, (b) a 44-year-old male with right and left hilar nodes. In both cases, EBUS-TBNA demonstrated culture-positive and Gene Xpert positive tuberculous lymphadenitis

Table 3: Transbronchial needle aspiration AFB culture in present study

EBUS TBNA	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Gene Xpert	88.89	66.67	53.33	93.33
AFB smear	67	80.95	60	85
Cytology	100	47.61	45	100

TBNA AFB Culture was positive in 18 patients out of 44 patients. EBUS: Endobronchial ultrasound, TBNA: Transbronchial needle aspiration, PPV: Positive predictive value, NPV: Negative predictive value

specificity 80.95%, PPV 60%, NPV 85%, and the *P* value was 0.011 which is statistically significant [Table 3].

Transbronchial needle aspiration cytology

TBNA cytology has the highest positivity in 40 patients (90.9%), out of which 18 patients were culture positive with the sensitivity of 100%, specificity of 47.6%, PPV of 45%, and NPV of 100%. *P* value showed 0.011 which is statistically significant [Table 3]. Dhasmana DJ *et al.*^[29] cytology showed the sensitivity of 94.6%, specificity 55.6%, PPV 49.3%, and NPV 95.7%. Cytology remained with the highest diagnostic value and our study showed greater sensitivity and NPV [Figure 2].

Transbronchial needle aspiration acid-fast bacilli culture

TBNA AFB culture was positive in 18/44 patients (40.9%) [Table 2]. This result is consistent with prior reports of increased culture prevalence and the difficulty in distinguishing pulmonary TB based on symptoms, signs, and radiological features. The performance of Löwenstein–Jensen medium in our study was similar to that reported by Muyoyeta *et al.* In their report, only <50% of smear-positive sputum specimens yielded TB with Löwenstein–Jensen medium, and nearly 50% had a negative result.^[30]

CONCLUSION

EBUS-TBNA is a safe and well-tolerated procedure in the assessment of patients with suspected isolated mediastinal lymphadenitis. Combination of tests increases the diagnostic yield as mediastinal tuberculous lymphadenitis is paucibacillary. Gene Xpert can pick up even cytology negative patients, which is very rare giving a definitive diagnosis. Gene Xpert which detects RR can help change

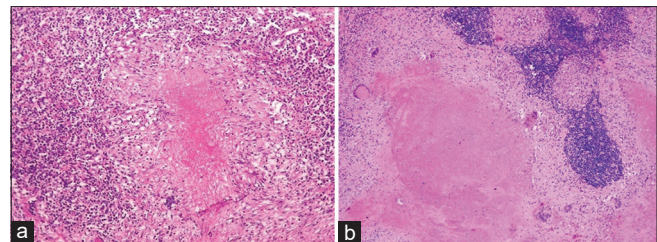


Figure 2: Cytology showing caseous necrosis

the effective therapeutic regimen. We suggest EBUS-TBNA should be considered the procedure of choice for patients in whom TB is suspected with the addition of Gene Xpert.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Vallandramam PR, Srinivasan A, Sivaramakrishnan M, Yadav P. Diagnostic value of cytology, gene Xpert, and mycobacterial cultures in EBUS-TBNA Aspirate. *Chest* 2015;148:804A.
- Chang SC, Lee PY, Perng RP. Clinical role of bronchoscopy in adults with intrathoracic tuberculous lymphadenopathy. *Chest* 1988;93:314-7.
- World Health Organization. Global Tuberculosis Report. World Health Organization; 2018.
- Lazarus AA, Thilagar B. Tuberculous Lymphadenitis 0011-5029; 2007.
- Watanabe K, Inoue Y, Shimoda T, Watanabe T, Hayashi T, Tsutsumi T, *et al.* Diagnostic usefulness of transbronchial aspiration and bronchial lavage for pulmonary tuberculosis. *Kekkaku* 1990;65:227-30.
- Holty JE, Kuschner WG, Gould MK. Accuracy of transbronchial needle aspiration for mediastinal staging of non-small cell lung cancer: A meta-analysis. *Thorax* 2005;60:949-55.
- Detterbeck FC, Jantz MA, Wallace M, Vansteenkiste J, Silvestri GA; American College of Chest Physicians. Invasive mediastinal staging of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). *Chest* 2007;132:202S-205S.
- Medford AR, Agrawal S, Free CM, Bennett JA. A prospective study of conventional transbronchial needle aspiration: Performance and cost utility. *Respiration* 2010;79:482-9.
- Burgers JA, Herth F, Becker HD. Endobronchial ultrasound. *Lung Cancer* 2001;34 Suppl 2:S109-13.
- Ren S, Zhang Z, Jiang H, Wu C, Liu J, Liang L, *et al.* Combination of endobronchial ultrasound-guided transbronchial needle aspiration with standard bronchoscopic techniques enhanced the diagnosis yields of pulmonary tuberculosis patients with lymphadenopathy. *Panminerva Med* 2013;55:363-70.
- Figueiredo VR, Jacomelli M, Rodrigues AJ, Canzian M, Cardoso PF, Jatene FB. Current status and clinical applicability of endobronchial ultrasound-guided transbronchial needle aspiration. *J Bras Pneumol*. 2013;39:226-37.
- Medford AR, Bennett JA, Free CM, Agrawal S. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA): Applications in chest disease. *Respirology* 2010;15:71-9.
- Evison M, Crosbie PA, Martin J, Bishop P, Doran H, Joseph L, *et al.* EBUS-TBNA in elderly patients with lung cancer: Safety and performance outcomes. *J Thorac Oncol* 2014;9:370-6.
- Sun J, Teng J, Yang H, Li Z, Zhang J, Zhao H, *et al.* Endobronchial ultrasound-guided transbronchial needle aspiration in diagnosing intrathoracic tuberculosis. *Ann Thorac Surg*. 2013;96:2021-7.
- Navani N, Molyneaux PL, Breen RA, Connell DW, Jepson A, Nankivell M, *et al.* Utility of endobronchial ultrasound-guided transbronchial needle aspiration in patients with tuberculous intrathoracic lymphadenopathy: A multicentre study. *Thorax* 2011;66:889-93.
- El-Hajj HH, Marras SA, Tyagi S, Kramer FR, Alland D. Detection of

- rifampin resistance in *Mycobacterium tuberculosis* in a single tube with molecular beacons. *J Clin Microbiol* 2001;39:4131-7.
17. Piatek AS, Tyagi S, Pol AC, Telenti A, Miller LP, Kramer FR, *et al.* Molecular beacon sequence analysis for detecting drug resistance in *Mycobacterium tuberculosis*. *Nat Biotechnol* 1998;16:359-63.
18. Hillemann D, Rüscho-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *J Clin Microbiol* 2011;49:1202-5.
19. Ligthelm LJ, Nicol MP, Hoek KG, Jacobson R, van Helden PD, Marais BJ, *et al.* Xpert MTB/RIF for rapid diagnosis of tuberculous lymphadenitis from fine-needle-aspiration biopsy specimens. *J Clin Microbiol* 2011;49:3967-70.
20. Moure R, Muñoz L, Torres M, Santin M, Martín R, Alcaide F. Rapid detection of *Mycobacterium tuberculosis* complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. *J Clin Microbiol* 2011;49:1137-9.
21. Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, *et al.* Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. *Eur Respir J*. 2012 Aug;40(2):442-7.
22. Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. *J Clin Microbiol* 2011;49:4138-41.
23. Varela-Lema L, Fernández-Villar A, Ruano-Ravina A. Effectiveness and safety of endobronchial ultrasound-transbronchial needle aspiration: A systematic review. *Eur Respir J* 2009;33:1156-64.
24. Yasufuku K, Pierre A, Darling G, de Perrot M, Waddell T, Johnston M, *et al.* A prospective controlled trial of endobronchial ultrasound-guided transbronchial needle aspiration compared with mediastinoscopy for mediastinal lymph node staging of lung cancer. *J Thorac Cardiovasc Surg* 2011;142:1393-4000.
25. Steinfort DP, Liew D, Conron M, Hutchinson AF, Irving LB. Cost-benefit of minimally invasive staging of non-small cell lung cancer: A decision tree sensitivity analysis. *J Thorac Oncol* 2010;5:1564-70.
26. Ernst A, Anantham D, Eberhardt R, Krasnik M, Herth FJ. Diagnosis of mediastinal adenopathy-real-time endobronchial ultrasound guided needle aspiration versus mediastinoscopy. *J Thorac Oncol* 2008;3:577-82.
27. Geake J, Hammerschlag G, Nguyen P, Wallbridge P, Jenkin GA, Korman TM, *et al.* Utility of EBUS-TBNA for diagnosis of mediastinal tuberculous lymphadenitis: A multicentre Australian experience. *J Thorac Dis* 2015;7:439-48.
28. Ghariani A, Jaouadi T, Smaoui S, Mehiri E, Marouane C, Kammoun S, *et al.* Diagnosis of lymph node tuberculosis using the GeneXpert MTB/RIF in Tunisia. *International Journal of Mycobacteriology*. 2015 Dec;4(4):270-275.
29. Dhasmana DJ, Ross C, Bradley CJ, Connell DW, George PM, Singanayagam A, *et al.* Performance of Xpert MTB/RIF in the diagnosis of tuberculous mediastinal lymphadenopathy by endobronchial ultrasound. *Ann Am Thorac Soc*. 2014 Mar;11(3):392-6.
30. Muyoyeta M, Schaap JA, De Haas P, Mwanza W, Muvwimi MW, Godfrey-Faussett P, *et al.* Comparison of four culture systems for *Mycobacterium tuberculosis* in the Zambian National Reference Laboratory. *Int J Tuberc Lung Dis* 2009;13:460-5.