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Role of Xpert MTB/RIF in differentiating tuberculosis from sarcoidosis in patients with mediastinal lymphadenopathy undergoing EBUS-TBNA: a study of 147 patients

Sahajal Dhooria[1], Nalini Gupta[2], Amanjit Bal[3], Inderpaul Singh Sehgal[1], Ashutosh N. Aggarwal[1], Sunil Sethi[4], Digambar Behera[1], Ritesh Agarwal[1]

[1] Department of Pulmonary Medicine, [2] Department of Cytology, [3] Department of Histopathology, [4] Department of Microbiology; Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

Abstract. *Background:* In patients with intrathoracic lymphadenopathy, differentiating tuberculosis from sarcoidosis is often difficult. We hypothesized that Xpert MTB/RIF assay, a semi-automated hemi-nested PCR would help in this regard. *Objective:* To evaluate the performance of Xpert MTB/RIF in the differential diagnosis of tuberculosis and sarcoidosis. *Methods:* This was a retrospective analysis of patients with intrathoracic lymphadenopathy who underwent endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (TBNA), and were diagnosed as either tuberculosis or sarcoidosis. The results of Xpert MTB/RIF assay, tuberculin skin test and endosonographic characteristics (heterogeneous echotexture and coagulation necrosis sign) of the lymph nodes were compared between the two groups. *Results:* During the study period, 465 EBUS procedures were performed and a diagnosis of sarcoidosis (n=94) or tuberculosis (n=53) was made in 147 patients. Xpert MTB/RIF was positive in 26 (49.1%) and two (2.1%) patients with tuberculosis and sarcoidosis, respectively. The sensitivity, specificity, positive and negative predictive values of Xpert MTB/RIF in the diagnosis of tuberculosis were 49.1 %, 97.9%, 92.9% and 77.3%, respectively. The presence of any of the four features namely positive Xpert MTB/RIF, positive tuberculin skin test, heterogeneous echotexture of the lymph nodes, or the presence of endosonographic coagulation necrosis sign yielded a sensitivity and negative predictive value of 83.0% and 88.0%, respectively in the diagnosis of tuberculosis versus sarcoidosis. *Conclusions:* Xpert MTB/RIF has good specificity and positive predictive value in the diagnosis of tuberculosis, and is a useful investigation in separating tuberculosis from sarcoidosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2016; 33: 258-266)*

Key words: tuberculosis, sarcoidosis, endobronchial ultrasound, endoscopic ultrasound, PCR, GeneXpert

Introduction

Tuberculosis and sarcoidosis are two disorders that share several clinical, radiological and pathological features (1, 2). In regions where tuberculosis is endemic and sarcoidosis is also prevalent, differentiating between the two disorders might be an arduous task (2-4). Mediastinal lymphadenopathy with or without pulmonary parenchymal opacities is a common presentation of both the diseases. As clinical and radiologic characteristics overlap, tissue diagnosis is

Received: 26 August 2015
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Correspondence: Dr Ritesh Agarwal MD, DM
Additional Professor
Dept. of Pulmonary Medicine
Postgraduate Institute of Medical Education
and Research, Sector-12
Chandigarh-160012, India
Tel. +91-172-2756825
Fax: +91-172-2748215
E-mail: agarwal.ritesh@outlook.in

of great importance (5). A cytological or histological specimen from intrathoracic lymph nodes can be obtained with various techniques such as CT-guided fine needle aspiration (FNA), conventional transbronchial needle aspiration (TBNA), endobronchial ultrasound (EBUS)-guided TBNA, endoscopic ultrasound (EUS) guided FNA, EUS with an echobronchoscope (EUS-B-FNA), and mediastinoscopy (6-11). Endobronchial ultrasound-guided TBNA has emerged as one of the preferred techniques for obtaining cytological specimen from mediastinal lymph nodes for the diagnosis of either disorder. (12, 13) Combining EBUS-TBNA with endobronchial biopsy (EBB) and transbronchial lung biopsy (TBLB) further increases the histopathological yield in sarcoidosis (14). Although, lymph node aspirates show the presence of granulomatous inflammation in both the disorders, certain features can help in differentiating between the two conditions.

For example, the growth of *Mycobacterium tuberculosis* on lymph node aspirate is confirmatory, for tubercolosis however the sensitivity of mycobacterial cultures in tuberculous lymphadenopathy is only about 50% (15). Morphological characteristics of the granuloma are also helpful (16). Presence of necrosis and demonstration of acid-fast bacilli in the tissue are two characteristics almost exclusively seen in tuberculosis as opposed to sarcoidosis, however they are not observed in all patients (17). Tuberculin skin test (TST) is a useful investigation as it has been shown to be negative in sarcoidosis even in high TB prevalence settings (18, 19). Thus, a combination of clinical, radiologic, immunologic and pathologic characteristics need to be considered while arriving at a diagnosis in the absence of microbiological confirmation, and any new method of differentiating between the two disorders is welcome.

As cultures for *M. tuberculosis* in tissue specimens have prolonged turn-around times, rapid molecular methods may act as effective alternatives (20-22). The Xpert MTB/RIF assay is a cartridge-based, semi-automated, hemi-nested, real time PCR, which permits rapid diagnosis of tuberculosis through detection of the DNA of *M. tuberculosis* and concurrent identification of the major mutation that confers rifampicin resistance (23). Recently, its use has been described in the diagnosis of tuberculous mediastinal lymphadenopathy by EBUS-TBNA (24). As almost 27% of the sarcoid tissue may show the presence of mycobacterial nucleic acids (25), the value of Xpert MTB/RIF (a method based on mycobacterial nucleic acid detection) in the differentiation of tuberculosis from sarcoidosis is uncertain. In this study, we describe the performance of Xpert MTB/RIF in the distinction of tuberculosis from sarcoidosis.

Methods

This was a retrospective study of data involving patients who had undergone EBUS-TBNA between 1st July 2013 and 28th February 2015 in the bronchoscopy suite of the Institute. The study protocol was approved by the Institutional Ethics Committee, and a written informed consent was obtained from all the patients.

Patients

Consecutive patients with enlarged mediastinal/hilar lymph nodes (≥1 cm in short axis) on computed tomography (CT) of the chest and who underwent EBUS-TBNA were enrolled in the study. Patients with any of the following were excluded: pregnancy, increased bleeding risk (prothrombin time ≥4 seconds prolonged compared to control, partial thromboplastin time >1.5 times control, platelet count <50,000/μL, serum creatinine >3 mg/dL), hypoxemia (pulse oximetric saturation <90 mm Hg on room air), failure to provide informed consent or lack of followup data.

Study protocol

After a thorough clinical evaluation, all patients underwent complete blood count, clotting profile, liver and renal function tests, angiotensin converting enzyme levels, serum calcium, sputum examination for acid-fast bacilli, chest radiograph and CT of the chest. The patients also underwent TST with five tuberculin unit purified protein derivative; the test was considered positive if the induration was ≥10 mm.

EBUS-TBNA procedure

EBUS procedures were performed by operators proficient in the technique. The International Association for the Study of Lung Cancer map was fol-

lowed for categorization of lymph node stations (26). The procedure was performed on an outpatient basis under moderate sedation and analgesia (intravenous midazolam and pentazocine in doses sufficient to maintain sedation and cough control). Intramuscular atropine (0.6 mg) and promethazine (25 mg) were administered as premedication followed by nebulized lignocaine (4% solution) immediately before the procedure. Lignocaine 10% solution was sprayed over the oropharynx along with instillation of 2% lignocaine over the vocal cords and the airways, as described previously (27). Pulse rate, respiratory rate, oxygen saturation by pulse oximetry and blood pressure were monitored throughout the procedure. The convex probe EBUS scope (BF-UC 180F; Olympus Medical Systems, Japan) with a 7.5 MHz transducer and a compatible endoscopic ultrasound scanner (EU-ME1; Olympus Medical Systems, Japan) were used.

Patients were placed in the supine position and EBUS-TBNA was performed by the oral route, in a customary fashion using the recommended, disposable, 21-gauge, Vizishot needle (NA-201SX-4021 Olympus Medical Systems, Japan) under endoscopic and sonographic visualization (10, 28). If the concerned lymph node was better accessible by the transesophageal route according to operator's discretion, an endoscopic ultrasound with bronchoscope-guided fine-needle aspiration (EUS-B-FNA) was performed, as described previously (29). The puncture was performed using the jabbing method under real-time ultrasound control. Continuous suction was applied with a dedicated 20 mL syringe while the catheter was moved back and forth for up to a maximum of 20 times. Slides were prepared from the TBNA specimen; half of them were air dried while the rest were immediately fixed in 95% alcohol. The residual blood in the suction syringe was discarded. A maximum of three aspirates were obtained from each lymph node. In patients with multiple enlarged lymph nodes, the largest lymph nodes were sequentially accessed. On-site cytological assessment for adequacy of the aspirate was unavailable. Aspirated material was obtained for cytological as well as for microbiological analysis including stain for acid-fast bacilli, mycobacterial cultures (mycobacterial growth indicator tube [MGIT]) and Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA). The samples for MGIT and Xpert MTB/RIF were collected in sterile tubes containing normal saline.

The Xpert MTB/RIF assay has a standardized technique in which sample reagent is added in a 2:1 ratio to untreated sample. The closed sample container is manually agitated at room temperature and then 2 mL of the sample is transferred to the test cartridge. Cartridges are then inserted into the test platform. After an automated cycle lasting about two hours, a print out of results is obtained.

The decision to perform an endobronchial biopsy (EBB) and transbronchial lung biopsy (TBLB) was left to the discretion of the bronchoscopist. In general, EBB was performed if there were endobronchial abnormalities and routinely in patients with clinical suspicion of sarcoidosis. A transbronchial lung biopsy (TBLB) was performed if there were any parenchymal abnormalities on CT scan and in all with a clinical diagnosis of sarcoidosis. A conventional flexible bronchoscope (BF-1T180, Olympus, Japan) along with standard biopsy forceps (FB19C, Olympus, Japan) were used for performing the biopsies. The EBB and TBLB specimen were subjected only to histopathological examination, Xpert MTB/RIF was not performed.

EBUS image features of lymph nodes

The endobronchial ultrasound image classification system proposed by Fujiwara et al. (30), modified for tuberculosis and sarcoidosis, was used to define the lymph node characteristics (31). The following endosonographic features of the lymph nodes were recorded: (1) echogenicity (homogeneous or heterogeneous): a node was labelled as heterogeneous if there were multiple small areas of varying echogenicity; and, (2) coagulation necrosis: defined on the presence of one or more large hypoechoic areas within a lymph node with absence of blood flow on Doppler (32).

Diagnosis of tuberculosis and sarcoidosis

A diagnosis of tuberculosis was based on the demonstration of all of the following: (a) consistent clinical and radiological presentation; (b) granulomatous inflammation or presence of acid-fast bacilli on microscopy or a positive culture for *Mycobacterium tuberculosis*; and, (c) clinicoradiological response to anti-tuberculosis treatment. A final diagnosis of sarcoidosis was made on the presence of all

of the following criteria: (a) consistent clinical and radiological presentation; (b) demonstration of non-necrotizing granulomas on either EBUS-TBNA, TBLB or EBB along with negative acid-fast bacilli and fungal stains; and no growth of mycobacteria on MGIT; and, (c) clinical and radiological response after treatment with glucocorticoids (33).

All patients were followed up clinically and radiologically for six months, and the diagnosis after six months was considered the final diagnosis.

Statistical analysis

Statistical analysis was performed using the commercial statistical package SPSS (Version 22 for MS-Windows). Data are expressed as median with interquartile range or number with percentage. Differences between continuous variables were compared using Mann-Whitney U test while differences between categorical data were compared using the chi-square test (or Fisher's exact test). The odds ratio (OR) were calculated for the diagnosis of tuberculosis over sarcoidosis with the use of different diagnostic characteristics. The performance characteristics of various combinations of Xpert MTB/RIF, TST and endosonographic signs are presented as sensitivity, specificity and predictive values. A Bayesian conditional probability plot was constructed for determining the post-test probability of tuberculosis with a positive or negative Xpert MTB/RIF results given a pre-test probability of disease. A p value <0.05 was considered statistically significant for all comparisons.

Results

A total of 465 EBUS procedures were performed during the study period. In six patients, EBUS was performed only for visualization; TBNA was not performed. Of the remaining, granulomatous inflammation (sarcoidosis or tuberculosis) was found on cytological examination in 220 patients on EBUS-TBNA, malignancy in 51, two patients had bronchogenic cysts, while one had aspergillosis. In 162 patients, the cytological examination did not reveal any pathology while 23 aspirates had inadequate material for analysis.

Xpert MTB/RIF was performed in 212 patients, 147 patients (94 sarcoidosis, 53 tuberculosis) had a definite histopathologic/cytologic diagnosis along with availability of the follow up data and were included in the study. The mean (SD) age of the 147 patients (63 [42.9%] women) was 41.5 (14.3) years (Table 1). Patients with tuberculosis were significantly younger than those with sarcoidosis and had a higher prevalence of smoking. A positive TST was seen in a larger proportion of patients with tuberculosis than sarcoidosis (49.1% vs. 2.1%, p<0.001). Sputum for acid-fast bacilli was negative in all the cases. TBLB was performed in 93 (63.3%) patients and EBB was performed in an equal number of patients.

All patients with sarcoidosis had non-necrotising granulomatous inflammation in one or more of the three (TBNA, EBB, or TBLB) specimens and responded to treatment with glucocorticoids. Of the 53 patients with a final diagnosis of tuberculosis, 27 were microbiologically confirmed (acid-fast bacilli on staining and/or *M. tuberculosis* culture) with or without granulomatous inflammation. Twenty-one and five patients had necrotizing and non-necrotising granulomatous inflammation, respectively without the presence of acid-fast bacilli or mycobacterial cultures. All patients improved with anti-tuberculosis treatment.

The overall size of lymph nodes on EBUS that were sampled by needle aspiration ranged from 5 to 39 mm (short axis). There was no difference in the sonographic size of the sampled lymph nodes in the two groups. A total of 369 lymph node stations (260 sarcoidosis, 109 tuberculosis) were aspirated in 147 patients (Table 1). On endosonography (Table 2), heterogeneous echotexture of lymph nodes was seen more often in tuberculosis than sarcoidosis (62.3% vs. 25.5%, p<0.001). The coagulation necrosis sign was also significantly more common with tuberculosis than sarcoidosis (18.9% vs. 3.2%, p=0.002).

Overall the Xpert MTB/RIF was positive in 49.1% (26/53) of the patients with tuberculosis; the positivity rate was 59.3% (16/27) in those with microbiologically proven disease. Two patients finally diagnosed with sarcoidosis also showed a positive result. Based on the positive result, the two patients were started initially on anti-tuberculosis treatment; however, there was no clinico-radiological response. Anti-tuberculosis treatment was stopped and the patients subsequently responded to the administration of steroids.

Table 1. Baseline characteristics and number of lymph nodes sampled at various stations of study patients

	Tuberculosis (n=53)	Sarcoidosis (n=94)	Total (n=147)	P value
Age (in years)	32 (25-49)	43.5 (33-55)	40 (30-53)	**<0.001**
Female gender, No. (%)	19 (35.8)	44 (46.8)	63 (42.9)	0.23
Smokers, No. (%)	16 (30.2)	9 (9.6)	25 (17.0)	**0.001**
Positive TST (induration ≥10 mm), No. (%)	26 (49.1)	2 (2.1)	28 (19.0)	**<0.001**
No. of lymph nodes sampled according to stations, No.				
4R	33	73	106	
4L	8	22	30	
7	42	89	131	
10R	3	4	7	
10L	0	2	2	
11R	5	7	12	
11L	16	60	76	
Others	2	3	5	
Short axis diameter on EBUS (mm)	16.4 (13.5-19.2)	15.6 (12.4-19.9)	15.9 (12.8-19.8)	0.45
Number of nodes sampled	2 (1.5-2.5)	3 (2-3)	3 (2-3)	**<0.001**
Number of passes per node	2 (2-3)	2 (1.7-2)	2 (1.7-2.5)	**<0.001**

All values are expressed as median (interquartile range) unless otherwise stated
TST- tuberculin skin test

Table 2. Comparison of positive Xpert MTB/RIF, tuberculin skin test (TST) positivity and endosonographic characteristics of lymph nodes between sarcoidosis and tuberculosis

	Tuberculosis (n=53)	Sarcoidosis (n=94)	Crude odds ratio (95% CI)	P value
Xpert MTB/RIF				
Positive	26 (49.1)	2 (2.1)	44.3 (9.9-198.7)	**<0.001**
Negative	27 (50.9)	92 (97.9)		
TST (induration ≥10 mm)				
Positive	26 (49.1)	2 (2.1)	44.3 (9.9-198.7)	**<0.001**
Negative	27 (50.9)	92 (97.9)		
Echogenicity				
Heterogeneous	33 (62.3)	24 (25.5)	4.8 (2.3-9.9)	**<0.001**
Homogeneous	20 (37.7)	70 (74.5)		
Coagulation necrosis sign				
Present	10 (18.9)	3 (3.2)	7.1 (1.8-26.9)	**0.002**
Absent	43 (81.1)	91 (96.8)		

Values in parentheses represent percentage unless otherwise stated
CI- confidence intervals.

Differential diagnosis

Among Xpert MTB/RIF, TST, endosonographic characteristics and the combinations thereof, a positive Xpert MTB/RIF had the highest specificity and positive predictive value for the diagnosis of tuberculosis similar to positive TST (Table 3). The presence of any of the four namely positive Xpert MTB/RIF, positive TST heterogeneous echotexture or coagulation necrosis sign had the best sensitivity and negative predictive value (Table 3). The post-test probability of a positive or negative Xpert MTB/RIF results depending on pre-test probability of tuberculosis is shown in Figure 1. In low TB prevalence areas (1-5%), Xpert MTB/RIF has a high negative predictive value (97-99.5%) while converse is true in high TB prevalence areas.

Three patients developed a pneumothorax after performing TBLB. Two of them underwent single time aspiration with resolution of the pneumothorax. Three patients had hypoxemia while performing EBUS-TBNA, which recovered after transient interruption of the procedure.

Table 3. Test characteristics of Xpert MTB/RIF, tuberculin skin test (TST), endosonographic lymph node features and their various combinations for the differential diagnosis of tuberculosis and sarcoidosis

	Sensitivity	Specificity	PPV	NPV
Xpert MTB/RIF	49.1 (36.1-62.1)	97.9 (92.6-99.4)	92.9 (77.4-98.0)	77.3 (69.0-83.9)
TST (induration ≥10 mm)	49.1 (36.1-62.1)	97.9 (92.6-99.4)	92.9 (77.4-98.0)	77.3 (69.0-83.9)
Heterogeneous echotexture	62.3 (48.8-74.1)	74.5 (64.8-82.2)	57.9 (45.0-69.8)	77.8 (68.2-85.1)
Coagulation necrosis sign	18.9 (10.6-31.4)	96.8 (91.0-98.9)	76.9 (49.7-91.8)	67.9 (59.6-75.2)
Positive Xpert MTB/RIF or positive TST (induration ≥10 mm) or heterogeneous echotexture or coagulation necrosis sign	83.0 (70.8-90.8)	70.2 (60.3-78.5)	61.1 (49.6-71.5)	88.0 (78.7-93.6)

Values in parenthesis represent the 95% confidence intervals
NPV-negative predictive value, PPV-positive predictive value

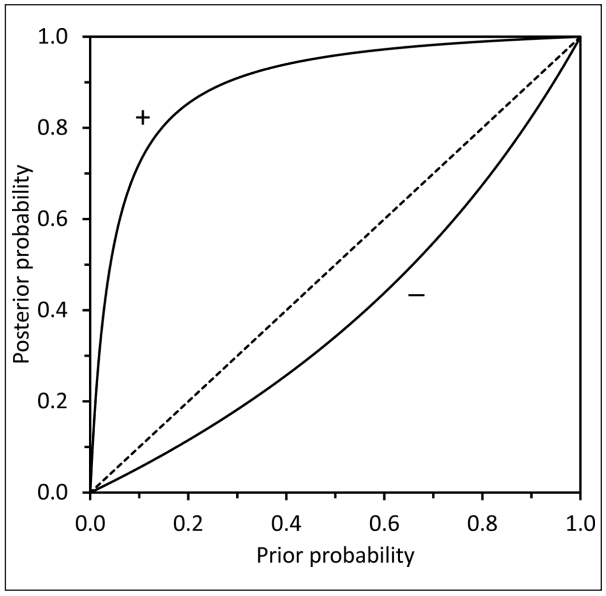

Fig. 1. Bayesian conditional probability plots for Xpert MTB/RIF in the diagnosis of tuberculous mediastinal lymphadenopathy. The curves depict the estimated post-test probability of tuberculosis in a patient, given a pre-test probability of disease and a positive (+) or negative (-) Xpert MTB/RIF result using estimates of sensitivity and specificity derived from the current study

Discussion

The results of this study demonstrate that Xpert MTB/RIF has high specificity (98%) and positive predictive value (93%) in differentiating tuberculosis from sarcoidosis in the diagnostic workup of patients with suspected granulomatous mediastinal lymphadenopathy. The sensitivity (49%) and negative predictive values (77%) are fairly low consistent with the findings of a recent meta-analysis, which also found a low sensitivity (77%) but good specificity (97%) of Xpert MTB/RIF for the detection of extrapulmonary tuberculosis (34). To the best of our knowledge, this is the first study that evaluates the performance of Xpert MTB/RIF in distinguishing tuberculosis from sarcoidosis in patients presenting with mediastinal lymphadenopathy.

The performance of Xpert MTB/RIF as a clinical test for pulmonary tuberculosis is well established, and the WHO has endorsed it for incorporation into national tuberculosis programs (35, 36). Several studies have also reported its performance in the diagnosis of tuberculous lymphadenopathy (24, 37-39). The present study demonstrates that Xpert MTB/RIF may be a useful adjunct to other modalities in the differentiation of tuberculosis from sarcoidosis in patients with granulomatous mediastinal lymphadenopathy. In a previous study, it was demonstrated that the presence of heterogeneous echotexture and/or coagulation necrosis sign significantly increases the odds for a diagnosis of tuberculosis over sarcoidosis in patients with mediastinal lymphadenopathy (31). The present study not only confirms the findings of the previous study on the utility of endosonographic characteristics, but also demonstrates the usefulness of Xpert MTB/RIF for identifying tuberculosis in this population. In contrast to the previous study, where various combinations of endosonographic features and positive TST offered the best sensitivity of 62% (31), we found that the presence of any of the four characteristics (positive Xpert MTB/RIF, positive TST, heterogeneous echotexture or coagulation necrosis sign) provides a much higher sensitivity (83%) in the diagnosis of tuberculosis. Conversely, if none of the four characteristics is present, in nine out of 10 cases, the diagnosis would be sarcoidosis.

An interesting observation from this study was that two sarcoidosis patients also had positive Xpert

MTB/RIF. The identification of mycobacterial DNA in sarcoidosis is not novel and the causal link between *M. tuberculosis* and sarcoidosis has been hotly debated (40). In a study on the detection of mycobacterial nucleic acids in sarcoidosis, the authors found *M. tuberculosis* DNA (by in-house qualitative *IS6110* PCR) in 37% of the sarcoid samples (41). A meta-analysis of 31 studies found that mycobacterial DNA is present in 0-72% of the sarcoid tissue in different studies, with a pooled prevalence of about 27% (25). The reason for these varying prevalence between different studies is an unresolved issue. Notably, most of the studies in the past had employed in-house PCR tests utilising amplification of different regions of the *M. tuberculosis* DNA, most frequently the IS6110 insertion sequence and the 65 kDa heat shock protein gene (41). In house PCRs are known to have a good sensitivity (96-100%) but suffer from poor specificity (50-81%) in the diagnosis of pulmonary and extrapulmonary tuberculosis (42, 43). Their poor specificity has been attributed to contamination of clinical samples as well as to presence of mycobacterial DNA in patients exposed to *M. tuberculosis* in the past (42, 44). Thus, with in-house PCRs it is difficult to determine whether the presence of mycobacterial DNA in sarcoid tissue is a true association or is due to the intrinsically poor specificity of the assay. In contrast to in-house PCRs, Xpert MTB/RIF assay involves the amplification of the 81-bp core region of the *rpoB* gene using real time hemi-nested PCR followed by five different nucleic acid hybridisation probes using the molecular beacon technology (45). Not only the *rpoB* gene is highly specific for *M. tuberculosis* complex, but also the automated technology reduces the risk of cross-contamination by minimizing the need for handling of PCR products (46). Thus, it is possible that a combination of semi-automated real time PCR with minimal human handling, utilization of multiple hybridization probes with molecular beacon technology, the use of the *rpoB* gene and a well standardized procedure might all be contributing together to the lower rates of detection of *M. tuberculosis* DNA in sarcoidosis by Xpert MTB/RIF.

The sensitivity of Xpert MTB/RIF was lower in the current study (49%) as compared to another recent study (73%) by Dhasmana et al (24). The possible reason is that in the study by Dhasmana et al., only culture positive tuberculosis patients were considered as the gold standard. As almost 50% of patients with tuberculous lymphadenopathy have a negative mycobacterial culture (15), we not only chose those tuberculosis patients who had microbiologically confirmed tuberculosis, but also those in whom the clinical, radiological and pathological data and the subsequent response to anti-tuberculosis treatment were consistent with tuberculosis, thus making the choice of patients more pragmatic. Further, in our study too, the sensitivity of Xpert MTB/RIF was higher (59%) in microbiologically confirmed cases compared to the entire tuberculosis study population.

This study is not without limitations. This is a single centre retrospective study with multiple operators performing the procedures. The clinician had access to all the data while observing the endosonographic characteristics and while assigning the final diagnosis. On the other hand, the cytologist, histopathologist and the microbiologist were all blinded to the clinicoradiological data, thus ensuring that no bias crept in while assigning the tissue diagnosis.

In conclusion, the Xpert MTB/RIF assay has high specificity and positive predictive value for the diagnosis of tuberculosis, thus making it a useful investigation in the discrimination of tuberculosis from sarcoidosis.

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