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Original Article

# The additional role of Xpert MTB/RIF in the diagnosis of intrathoracic tuberculous lymphadenitis

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ABSTRACT

*Background:* Diagnosis of intrathoracic tuberculosis (TB) lymphadenitis remains a challenge because of difficulties in obtaining adequate tissue and the lack of a sensitive test. Recently, Xpert MTB/RIF assay is being used for rapid diagnosis of pulmonary TB, but it has not yet been widely validated in intrathoracic TB lymphadenitis. The aim of this study was to assess the additional role of Xpert MTB/RIF in diagnosing intrathoracic TB lymphadenitis using endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) specimen.

*Methods:* Consecutive patients who underwent Xpert MTB/RIF assay using EBUS-TBNA specimen from January 2012 and November 2013 at a tertiary referral hospital were recruited. Among them, the cases with malignant lymph nodes were excluded.

*Results:* Among 73 patients, 13 (17.8%) cases were diagnosed with intrathoracic TB lymphadenitis. In detail, 10 patients were diagnosed using conventional methods only (histology or AFB culture) and 3 patients were additionally diagnosed when adding Xpert MTB/RIF assay. The median time to diagnosis using Xpert MTB/RIF (1 day) was shorter than conventional methods (3 days for histology, 14 days for AFB culture). Rifampin resistance was not detected in any patients.

*Conclusion:* In patients with enlarged intrathoracic lymph nodes and low suspicion of malignancy, combination of conventional diagnostic methods with Xpert MTB/RIF could lead to additional and rapid diagnosis of intrathoracic TB lymphadenitis.



## 1. Introduction

Global efforts to control tuberculosis (TB) are mainly focused on pulmonary TB. However, World Health Organization reported that among 6.1 million notified TB cases, 0.8 million cases were extrapulmonary TB [1]. Unfortunately, diagnosis of extrapulmonary TB remains a challenge due to frequent atypical presentation, often simulating malignancy or inflammatory diseases. Mycobacterial load is generally low in non-respiratory specimens [2] Furthermore, it is difficult to obtain adequate tissue for histology and tissue microscopy is often negative.

TB lymphadenitis is one of the most common extrapulmonary TB [3]. Fine-needle aspiration is a useful technique in diagnosing peripheral TB lymphadenitis [4]. Unlike peripheral TB lymphadenitis, however, diagnosis of intrathoracic TB lymphadenitis is usually challenging. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) which has become the standard of care in lymph node staging and diagnosis of central lung cancer, has recently been proposed as a diagnostic tool for intrathoracic TB [5,6].

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA), a new assay enabling simultaneous detection of *Mycobacterium tuberculosis* and rifampin resistance, was endorsed by World Health Organization in 2010 [7]. It was initially developed and assessed for the detection of pulmonary TB using sputum. Diagnostic accuracy of Xpert MTB/RIF assay in extrapulmonary TB is reported to be highly heterogeneous, ranging from 25% to 96% [8]. However, a previous study from our institution showed that Xpert assay

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showed good sensitivity and specificity in the diagnosis of extrapulmonary TB in an intermediate TB burden country [9]. Although the use of Xpert MTB/RIF assay has been reported to be useful and accurate in the diagnosis of intrathoracic TB lymphadenitis [10], it has not yet been widely validated. The aim of this study was to assess the additional role of Xpert MTB/RIF assay in diagnosing intrathoracic TB lymphadenitis using EBUS-TBNA specimen, in a country with an intermediate TB burden.

## 2. Patients and methods

### 2.1. Study population

We retrospectively recruited all the patients who underwent Xpert MTB/RIF testing using EBUS-TBNA specimen from January 2012 and November 2013 at Seoul National University Hospital. Xpert MTB/RIF assay was requested by the clinician if TB lymphadenitis was suspected. Among them, the cases with malignant lymph nodes were excluded. The study was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. H-1304-084-481). Informed consent was waived by the Institutional Review Board. All patient records and information was anonymized and de-identified prior to analysis.

Xpert MTB/RIF assay was requested from EBUS-TBNA specimens in addition to acid-fast bacilli (AFB) smear and mycobacterial culture if intrathoracic TB lymphadenitis was clinically suspected. Intrathoracic TB lymphadenitis was suspected in patients with enlarged lymph nodes if 1) there were clinical signs suggestive of infection, or 2) no evidence of any malignancy. Lymph nodes larger than 1 cm short-axis diameter were considered enlarged.

### 2.2. EBUS-TBNA procedures and specimen processing

EBUS-TBNA was performed by four experienced pulmonology staffs using a real-time linear probe (BF-UC260F-OL8; Olympus, Tokyo, Japan). All procedures were performed under conscious sedation using midazolam and fentanyl. Lidocaine was used for topical anesthesia. The target lymph nodes were selected according to the pulmonologists' decision. When the target lymph nodes were visualized, 22-gauge needle (NA-201SX-4022; Olympus Medical Systems) was used for transbronchial aspiration. The aspirate was spread on to the glass slides and immediately fixed with 95% alcohol. Tissue cores were put into a formalin solution for pathologic evaluation. At least 2 nodal aspirations were done per nodal station usually until tissue core specimen was obtained. The remnants of each aspirate were collected in an aseptic tube and were sent for AFB smear, mycobacterial culture and Xpert MTB/RIF assay.

The process of AFB smear and culture in our institution is described in a previous study [9]. The Xpert MTB/RIF assay was performed following the manufacturer's instructions. Specimens were decontaminated with N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH, 1% final concentration) with the addition of phosphate-buffered saline, and were concentrated by centrifugation. Tissue samples were frozen with liquid nitrogen before decontamination. Tissue TB polymerase chain reaction (PCR) was performed formalin-fixed, paraffin-embedded (FFPE) lymph node tissues. In brief, DNA was extracted using automated Maxwell® 16 FFPE plus LEV DNA purification kit (Promega). The extracted DNA was amplified by PCR, then TB specific band which is located in 181 bp, was identified using HT DNA Labchip® kit (Perkinelmer).

### 2.3. Diagnosis of TB lymphadenitis

Patients were diagnosed with intrathoracic TB lymphadenitis if he or she met one of the following criteria: positive *M. tuberculosis* culture in aspirate or tissue samples, histological findings compatible with TB (chronic granulomatous inflammation with caseation necrosis), positive Xpert MTB/RIF assay, or positive TB-PCR assay in FFPE lymph node tissues.

Patients who met the prespecified criteria were classified as confirmed intrathoracic TB lymphadenitis and patients who were diagnosed by other methods were classified as possible intrathoracic TB lymphadenitis.

### 2.4. Statistical analysis

Data are presented as the mean ± SD (standard deviation) for continuous variables, or number (%) for categorical variables. The student's t-test was used to compare means, and chi-square test (or Fisher's exact test) for categorical data comparison. SPSS version 20.0 was used for the analysis and P values <0.05 were considered statistically significant.

## 3. Results

### 3.1. Diagnosis of intrathoracic TB lymphadenitis

Between August 2012 and November 2013, the Xpert MTB/RIF assay was requested in EBUS-TBNA samples of 93 patients with suspicion of intrathoracic TB. We excluded 20 patients who were diagnosed with malignant intrathoracic lymph nodes (Fig. 1). Their baseline characteristics are shown in Table 1. Among 73 patients, 17 (23.3%) patients were diagnosed with confirmed or possible intrathoracic TB lymphadenitis.

Fig. 2 shows the distribution of diagnostic criteria in 13 confirmed intrathoracic TB lymphadenitis. Most patients diagnosed by histologic diagnosis (7 patients) and positive Xpert MTB/RIF assay (7 patients). Five patients showed positive TB PCR assay in FFPE tissues and in four patients AFB culture was positive. Three patients (23%) were additionally diagnosed when adding Xpert MTB/RIF assay to other conventional methods. AFB smear was negative in all patients.

Four patients were diagnosed as possible intrathoracic TB lymphadenitis. Presence of TB was confirmed by pleural biopsy (n = 1), elevated pleural fluid adenosine deaminase levels (n = 1), endobronchial lesion biopsy (n = 1), percutaneous lung nodule biopsy (n = 1).

Patients with TB lymphadenitis were slightly younger (mean age 55.5 vs. 60.5 years; p = 0.26) and there were more female patients (64.7% vs. 48.2%; p = 0.36), but the differences were not statistically significant. Chest CT (computed tomography) was available in all patients. TB lymphadenitis patients were characterized by the decreased number of enlarged intrathoracic lymph nodes (3.5 ± 5.1 lymph nodes) compared to the patients without TB lymphadenitis (5.1 ± 2.8 lymph nodes) (p = 0.008). Also, central low attenuation was more frequently observed in the lymph nodes suggestive of necrosis (57.1% vs. 6.7%; p < 0.001). None of the patients had human immunodeficiency virus infection. Among 73 patients, serum interferon-gamma release assay (QuantiFERON—TB Gold) was tested in 10 patients. Six patients showed positive results and 5 patients were finally diagnosed with TB lymphadenitis.

### 3.2. Time to diagnosis

The median time between EBUS-TBNA and the diagnosis of intrathoracic TB lymphadenitis was 1 day (range, 0–1) for Xpert MTB/RIF assay, 2 days (range, 1–3) for pathologic diagnosis, 7 days (range, 5–8) for TB PCR assay in FFPE lymph node tissues and 18 days (range, 10–33) for mycobacterial culture. In total, median