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ORIGINAL ARTICLE

Clinical usefulness of routine AFB culture and MTB PCR of EBUS-TBNA needle rinse fluid

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ABSTRACT

Background and objective: **We evaluated the usefulness of acid-fast bacilli (AFB) culture and *Mycobacterium tuberculosis* (MTB) polymerase chain reaction (PCR) of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) needle rinse fluid for diagnosing tuberculous lymphadenitis.**

Methods: **EBUS-TBNA needle rinse fluid was routinely used for AFB culture and MTB PCR. The patients were categorized according to the pre-procedural diagnosis (Group A, suspected/histology-confirmed lung cancer; Group B, extrapulmonary malignancy; and Group C, other benign diseases).**

Results: **Of the 4672 subjects, 104 (2.2%) were diagnosed with tuberculous lymphadenitis; 1.0%, 4.6% and 12.7% of Group A, B and C, respectively. Tuberculous lymphadenitis was diagnosed in 0.2%, 1.0% and 4.5% Group A, B and C patients, respectively, by histopathology. On addition of AFB culture to histopathology, tuberculous lymphadenitis was diagnosed in 1.0%, 4.4% and 10.3% of Group A, B and C patients, respectively ($P < 0.001$, $P = 0.001$ and $P = 0.005$, respectively). On addition of MTB PCR to histopathology, tuberculous lymphadenitis was diagnosed in 0.4%, 1.9% and 8.8%, respectively (Group C; $P = 0.029$).**

Conclusion: **Routine AFB culture of needle rinse fluid was useful to increase the diagnostic yield of tuberculous lymphadenitis for all subjects who underwent EBUS-TBNA regardless of pre-procedural diagnosis in an intermediate tuberculosis (TB)-burden country. However, MTB PCR was only useful in subjects with pre-procedural diagnosis of benign pulmonary diseases.**

SUMMARY AT A GLANCE

The routine acid-fast bacilli (AFB) culture of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) needle rinse fluid is useful to increase the diagnostic yield of tuberculous lymphadenitis in an intermediate tuberculosis (TB)-burden country.

Key words: endobronchial ultrasound-guided transbronchial needle aspiration, *Mycobacterium tuberculosis*, polymerase chain reaction, rinse fluid, tuberculous lymphadenitis.

INTRODUCTION

Extrapulmonary tuberculosis (TB) accounts for 15% of the 6.3 million TB cases that were recorded worldwide in 2016, ranging in prevalence from 8% in the Western Pacific region to 24% in the Eastern Mediterranean region.[1] The most common site of extrapulmonary TB is the lymph nodes, including the intrathoracic lymph nodes.[2] Unfortunately, diagnosis of intrathoracic tuberculous lymphadenitis is usually challenging because of the difficulty of the approach and the lack of specific clinical and radiologic characteristics; it often simulates malignancy or other inflammatory diseases.[3–5]

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive intervention that has been demonstrated as a superior diagnostic yield compared to conventional TBNA, and shows similar results to mediastinoscopy for lymph node staging in patients with primary lung cancer.[6–9] The indications for EBUS-TBNA have recently been expanded beyond malignant diseases to benign diseases, such as sarcoidosis, tuberculous lymphadenitis and fungal infections.[10–13] Several reports have suggested that acid-fast bacilli (AFB) culture and *Mycobacterium tuberculosis* (MTB) polymerase chain reaction (PCR) can increase the diagnostic yield when patients undergo EBUS-TBNA for evaluation of suspected

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intrathoracic tuberculous lymphadenitis.[12,14,15] However, previous reports included small numbers of cases and required additional tissue samples for specialized testing, such as culture or MTB PCR. Moreover, they did not consider differences in diagnostic yield according to the pre-procedural diagnosis.

Therefore, this study investigated the role of AFB culture and MTB PCR assay of EBUS-TBNA rinse fluid in diagnosing tuberculous lymphadenitis, according to the pre-procedural diagnosis, in an intermediate TB-burden country.

METHODS

Study patients

We retrospectively reviewed the EBUS-TBNA registry data at Samsung Medical Center (a 1979-bed referral hospital), Seoul, South Korea, between August 2009 and December 2016. Among patients who underwent EBUS-TBNA, the patients who received AFB culture and MTB PCR of the EBUS-TBNA needle rinse fluid were included in the study. This study was approved by the Institutional Review Board (IRB) of Samsung Medical Center (IRB no. 2018-01-019). The requirement for informed consent was waived by the IRB due to the retrospective nature of the study. All patient records and data were anonymized and de-identified prior to analysis.

EBUS-TBNA procedures and specimen processing

Details of EBUS-TBNA procedure were described in our previous reports.[8,16] The target lymph nodes were selected by the pulmonologist based on imaging studies including chest computed tomography (CT) and positron emission tomography (PET)-CT if available.[17] If a station had multiple lymph nodes on EBUS, we selected nodes based on size and ^{18}F-fluorodeoxyglucose uptake. All lymph nodes were classified using the International Association for the Study of Lung Cancer lymph node map.[18] We did not use rapid on-site cytopathological examination.

The core tissue was spread on filter paper to absorb excess blood and fixed in 10% (v/v) formalin; the tissue coagulum clot was sent for histological examination.[19] After obtaining the core tissue, 1 cc of sterile normal saline was flushed through the needle to obtain a rinse fluid sample. All rinse fluid samples were collected in an aseptic tube and sent for AFB stain and culture, and MTB PCR assay. Decontaminated samples were inoculated into a mycobacterial growth indicator tube (MGIT 960 system; Becton Dickinson, Sparks, MD, USA) for AFB culture, and also onto 3% Ogawa agar (Shinyang, Seoul, Korea), and were incubated for 6 weeks. Positive cultures were subjected to AFB staining to confirm the presence of AFB and exclude contamination. The MTB PCR assay was performed with 100-μL aliquots of the decontaminated samples using the COBAS TaqMan MTB Test (Roche Diagnostics, Basel, Switzerland), a real-time PCR assay targeting part of the 16S rRNA gene for detection of MTB complex DNA, according to the manufacturer's instructions.[20,21]

Diagnosis of TB lymphadenitis

Tuberculous lymphadenitis was defined as histological findings compatible with TB (chronic granulomatous inflammation with caseation necrosis) or a positive AFB culture or MTB PCR assay of rinse fluid.[11,22,23]

Pre-procedure diagnosis

The patients were divided into three groups according to the pre-procedural diagnosis at the discretion of pulmonologists performing EBUS-TBNA based on a clinical setting and combined results of imaging studies such as chest CT and PET-CT scans: patients with suspected or histologically confirmed primary lung cancer (Group A), patients with an extrapulmonary malignancy (Group B) and patients with other benign pulmonary diseases (Group C).

Statistical analysis

All data are presented as means ± SD for continuous variables, and as numbers and percentages for categorical variables. Continuous and categorical variables were analysed by one-way analysis of variance and Pearson's chi-square or Fisher's exact tests, respectively. If there was a difference, post hoc analysis was performed. The effect of the method used to diagnose tuberculous lymphadenitis was evaluated by the chi-square test and the number needed to test (NNT) to find one case of tuberculous lymphadenitis. SPSS software (ver. 23.0; SPSS Inc., Chicago, IL, USA) was used for the analysis, and P-values of <0.05 were considered significant.

RESULTS

Baseline characteristics

A total of 4795 patients underwent EBUS-TBNA between August 2009 and December 2016. After excluding patients who did not receive MTB PCR, AFB culture or biopsy (n = 123), 4672 patients who underwent EBUS-TBNA with AFB culture and MTB PCR of the EBUS-TBNA needle rinse fluid were included in the study. The patients were further classified into Group A (n = 3863, 82.7%), Group B (n = 478, 10.2%) and Group C (n = 331, 7.1%) according to the pre-procedural diagnosis (Fig. 1). The baseline characteristics of the patients are summarized in Table 1. The mean age of all patients was 64.2 ± 11.3 years, and there were 3273 (70.1%) male patients. In Group A, 1150 (29.8%) patients had histologically confirmed primary lung cancer and 2713 (70.2%) were suspected to have primary lung cancer. In addition, 147 (3.8%) and 316 (8.2%) patients had a history of chemotherapy and TB treatment, respectively, at the time of EBUS-TBNA. The most frequent origin of malignancy in Group B was the gastrointestinal organs (35.1%). Eight (1.7%) and 127 (26.6%) patients had a history of chemotherapy and TB treatment, respectively. The most common pre-procedural diagnosis in Group C was suspected sarcoidosis (59.5%), followed by suspected tuberculous lymphadenitis (26.3%). Fourteen (4.2%) patients had a history of TB treatment; however, there was no previous history of chemotherapy. The mean number of lesions examined per patient was 2.4 ± 1.1. A total of 114 (2.4%) patients were clinically

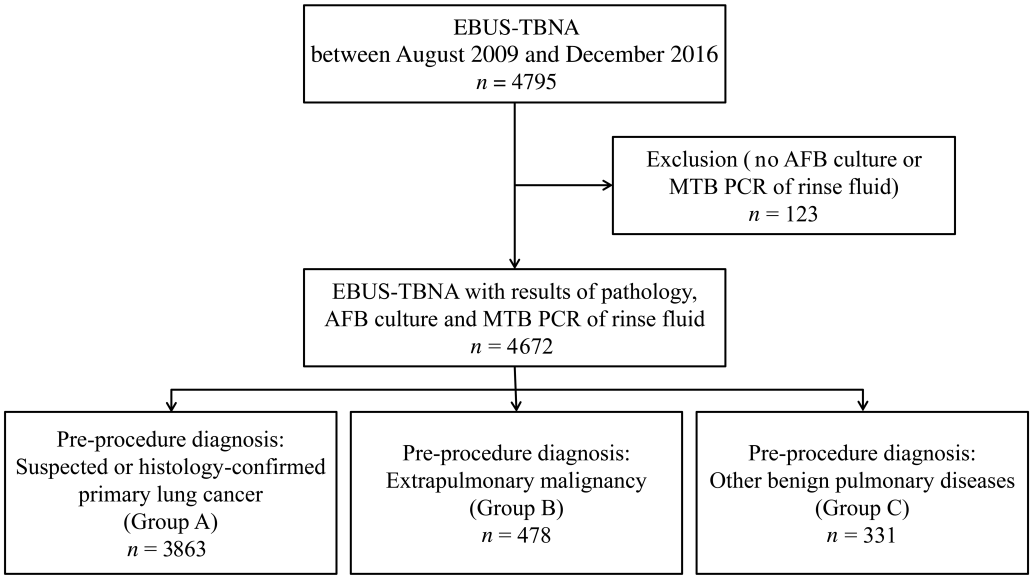


Figure 1 Flow chart of the study. AFB, acid-fast bacilli; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; MTB, *Mycobacterium tuberculosis*; PCR, polymerase chain reaction.

diagnosed with active pulmonary TB at the time of EBUS-TBNA. PET-CT was performed in 73.1% (3414/4672) of patients before EBUS-TBNA procedures.

A total of 10 662 lymph nodes and 381 other lesions, including lung parenchymal lesions and pleural seeding nodules, were examined by EBUS-TBNA in 4672 patients (Table 2). At least one tissue core was obtained from 10 851 of 11 043 (98.3%) lesions.

Diagnosis of intrathoracic TB lymphadenitis

Of the 4672 total patients, 104 (2.2%) were diagnosed with tuberculous lymphadenitis (Table 3). In Groups A, B, and C, 1.0% (40/3863), 4.6% (22/478) and 12.7% (42/331) of the patients were diagnosed with tuberculous lymphadenitis, respectively. Final diagnosis of Group C included sarcoidosis (n = 166), TB lymphadenitis (n = 42), reactive changes (n = 34), cancer metastasis (n = 29), anthracofibrosis (n = 28), no definite diagnosis (n = 17), other infection-related lymphadenitis (n = 8) and lymphoma (n = 7). Patients with tuberculous lymphadenitis were younger (57.6 ± 17.6 vs 64.4 ± 11.0 years, $P < 0.001$), less likely to be male (50.0% vs 70.5%, $P < 0.001$) and concurrently diagnosed with pulmonary TB more frequently (45.2% vs 1.5%, $P < 0.001$) than those without tuberculous

Table 1 Baseline and clinical characteristics of the study subjects

	Total (n = 4672)	Group A[†] (n = 3863)	Group B[‡] (n = 478)	Group C[§] (n = 331)	P-value
Age (years)	64.2 ± 11.3	65.6 ± 9.8	61.2 ± 13.0	52.5 ± 15.6	<0.001[¶,††,‡‡]
Gender, male	3273 (70.1)	2812 (72.8)	288 (60.3)	173 (52.3)	<0.001[¶,††,‡‡]
History of TB treatment	169 (3.6)	147 (3.8)	8 (1.7)	14 (4.2)	0.052
History of chemotherapy	443 (9.5)	316 (8.2)	127 (26.6)	0 (0)	<0.001[¶,††,‡‡]
Number of lesions examined per patient	2.4 ± 1.1	2.5 ± 1.1	1.9 ± 1.0	2.0 ± 0.9	<0.001[¶,††]
Mediastinal lymph nodes	1.9 ± 1.0	2.0 ± 1.0	1.4 ± 0.9	1.6 ± 0.8	<0.001[¶,††]
Hilar, interlobar and lobar lymph nodes	0.4 ± 0.6	0.4 ± 0.6	0.4 ± 0.6	0.4 ± 0.6	0.769
Diagnosis of tuberculous lymphadenitis	104 (2.2)	40 (1.0)	22 (4.6)	42 (12.7)	<0.001[¶,††,‡‡]
Concurrent diagnosis of pulmonary TB	114 (2.4)	62 (1.6)	13 (2.7)	39 (11.8)	<0.001[††,‡‡]

[†]Patients with a pre-procedural diagnosis of suspected primary lung cancer (n = 2713) and histologically confirmed primary lung cancer (n = 1150) were included.

[‡]Gastrointestinal malignancy (n = 168), metastasis of unknown origin (n = 111), breast cancer (n = 64), lymphoma (n = 40), nasopharyngeal cancer (n = 31), urinary cancer (n = 23), gynaecological cancer (n = 15), thymic carcinoma (n = 12), thyroid cancer (n = 8) and other disease (n = 6).

[§]Patients with a pre-procedural diagnosis of suspected sarcoidosis (n = 197), suspected tuberculous lymphadenitis (n = 87), fever of unknown origin (n = 18) and other disease (n = 29) were included.

[¶]$P < 0.05$ in the post hoc analysis between Groups A and B.

[††]$P < 0.05$ in the post hoc analysis between Groups A and C.

[‡‡]$P < 0.05$ in the post hoc analysis between Groups B and C.

TB, tuberculosis.

Table 2 Lymph node characteristics

	Total (n = 11 043)	Group A (n = 9517)	Group B (n = 878)	Group C (n = 648)	P-value
Station					
1R	87 (0.8)	70 (0.7)	16 (1.8)	1 (0.2)	<0.001†,‡
1L	13 (0.1)	7 (0.1)	6 (0.7)	0 (0)	0.001†
2R	676 (6.1)	596 (6.3)	60 (6.8)	20 (3.1)	0.003‡,§
2L	67 (0.6)	49 (0.5)	14 (1.6)	4 (0.6)	0.002†
3	42 (0.4)	35 (0.4)	7 (0.8)	0 (0)	0.037
4R	3017 (27.3)	2567 (27.0)	241 (27.4)	209 (32.3)	0.014§
4L	1871 (16.9)	1726 (18.1)	96 (10.9)	49 (7.6)	<0.001†,§
5	56 (0.5)	49 (0.5)	6 (0.7)	1 (0.2)	0.345
7	3102 (28.1)	2639 (27.7)	232 (26.4)	231 (35.6)	<0.001‡,§
8	72 (0.7)	69 (0.7)	3 (0.3)	0 (0)	0.027
9	15 (0.1)	14 (0.1)	1 (0.1)	0 (0)	1.000
10R	81 (0.7)	69 (0.7)	9 (1.0)	3 (0.5)	0.427
10L	55 (0.5)	51 (0.5)	4 (0.5)	0 (0)	0.150
11R	888 (8.0)	719 (7.6)	104 (11.8)	65 (10.0)	<0.001†
11L	608 (5.5)	510 (5.4)	44 (5.0)	54 (8.3)	0.005‡,§
12R	5 (0.0)	4 (0.0)	1 (0.1)	0 (0)	0.525
12L	7 (0.1)	5 (0.1)	2 (0.2)	0 (0)	0.157
Others¶	381 (3.5)	338 (3.6)	32 (3.6)	11 (1.7)	0.041§
Size of lymph node (mm)					
Short-axis diameter	11.7 ± 7.4	11.3 ± 7.4	13.5 ± 7.2	14.9 ± 6.3	<0.001†,‡,§
Long-axis diameter	16.9 ± 10.5	16.2 ± 10.4	19.3 ± 10.5	22.8 ± 10.7	<0.001†,‡,§
Number of needle passes per lesion	2.0 ± 0.9	1.9 ± 0.9	2.2 ± 1.1	2.3 ± 1.1	<0.001†,§
Number of tissue cores obtained per lesion	1.6 ± 0.7	1.6 ± 0.7	1.8 ± 0.8	1.8 ± 0.8	<0.001†,§

†P < 0.05 in the post hoc analysis between Groups A and B.
‡P < 0.05 in the post hoc analysis between Groups B and C.
§P < 0.05 in the post hoc analysis between Groups A and C.
¶Lung parenchymal lesions and pleural seeding nodules were included.

lymphadenitis. However, no significant differences were detected in terms of history of TB treatment or chemotherapy.

Figure 2 shows the impact of method used for diagnosing tuberculous lymphadenitis as a Venn diagram, according to the pre-procedural diagnosis. AFB culture was the most effective single test to diagnose tuberculous lymphadenitis. Of the 79 patients with culture-proven disease, positive AFB stain results, a positive culture on solid medium, and a positive culture on liquid medium were seen in 1 (1.3%), 39 (49.4%) and 62 (78.5%) patients, respectively. Of the 104 patients

Table 3 Comparison of clinical characteristics according to tuberculous lymphadenitis diagnostic status

	Tuberculous lymphadenitis (n = 104)	No tuberculous lymphadenitis (n = 4568)	P-value†
Age (years)	57.6 ± 17.6	64.4 ± 11.0	<0.001
Gender, male	52 (50.0)	3221 (70.5)	<0.001
History of TB treatment	5 (4.8)	164 (3.6)	0.427
History of chemotherapy	5 (4.8)	438 (9.6)	0.100
Number of lesions examined per patient	2.0 ± 1.0	2.4 ± 1.1	<0.001
Mediastinal lymph nodes	1.7 ± 0.8	1.9 ± 1.0	0.006
Hilar, interlobar and lobar lymph nodes	0.3 ± 0.5	0.4 ± 0.6	0.080
Concurrent diagnosis of pulmonary TB	47 (45.2)	67 (1.5)	<0.001
Size of lymph node (mm)			
Short-axis diameter	13.4 ± 6.3	13.2 ± 8.4	0.801
Long-axis diameter	18.8 ± 9.3	18.8 ± 11.7	0.993
Number of needle passes per lesion	2.6 ± 1.3	2.1 ± 1.0	<0.001
Number of tissue cores obtained per lesion	2.0 ± 0.9	1.8 ± 0.8	0.001

†Continuous variables and categorical variables were compared using Student's t-test and the Pearson's chi-square or Fisher's exact tests, respectively.
TB, tuberculosis.

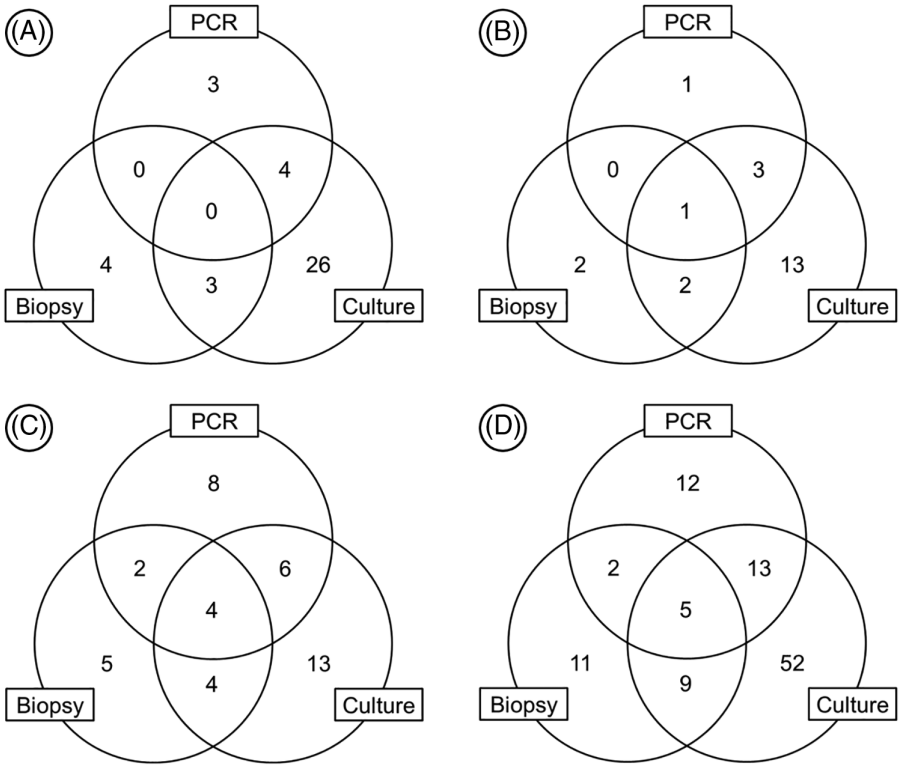


Figure 2 Venn diagram of the impact of tuberculous lymphadenitis diagnostic method (histopathology, AFB culture and MTB PCR) in each patient group. (A) Of the 3863 patients with suspected or histologically confirmed primary lung cancer (Group A), 40 were diagnosed with tuberculous lymphadenitis. (B) Of the 478 patients with extrapulmonary malignancy (Group B), 22 were diagnosed with tuberculous lymphadenitis. (C) Of the 331 patients with other benign pulmonary diseases (Group C), 42 were diagnosed with TB lymphadenitis. (D) Of the total 4672 patients, 104 were diagnosed with tuberculous lymphadenitis. AFB, acid-fast bacilli; MTB, *Mycobacterium tuberculosis*; PCR, polymerase chain reaction; TB, tuberculosis.

with tuberculous lymphadenitis, 12 (11.5%) were diagnosed with tuberculous lymphadenitis by MTB PCR alone, 11 (10.6%) were diagnosed by biopsy only and 52 (50.0%) were diagnosed by AFB culture only. Only five patients (4.8%) had positive histopathological, AFB culture and MTB PCR results.

Usefulness of AFB culture and MTB PCR

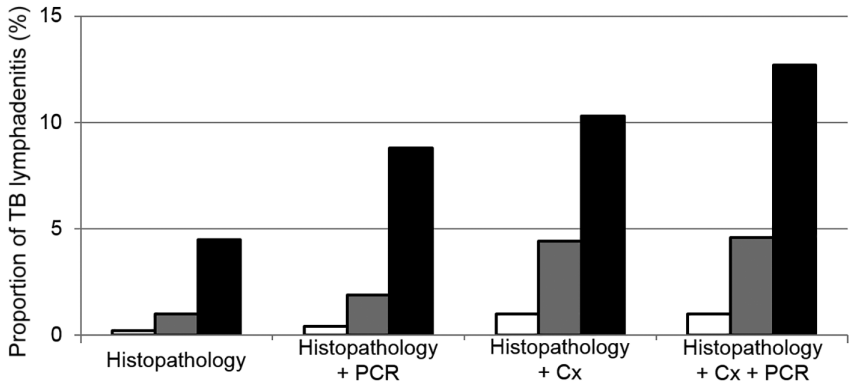
Figure 3 shows the effect of the method used for diagnosing tuberculous lymphadenitis when histopathology, AFB culture and the MTB PCR assay were added sequentially. Tuberculous lymphadenitis was diagnosed in 0.2% (7/3863) of Group A, 1.0% (5/478) of Group B and 4.5% (15/331) of Group C patients by histopathology only. On adding AFB culture of rinse fluid to histopathological analysis, tuberculous lymphadenitis was diagnosed in 1.0% (37/3863), 4.4% (21/478) and 10.3% (34/331) of the Group A, B and C patients, respectively ($P < 0.001$, $P = 0.001$ and $P = 0.005$, respectively). NNT values of Groups A, B, and C were 129, 30 and 18, respectively. On addition of the MTB PCR assay to histopathological analysis, tuberculous lymphadenitis was diagnosed in 0.4% (14/3863), 1.9% (9/478) and 8.8% (29/331) of the patients in Groups A, B and C, respectively ($P = 0.126$, $P = 0.281$ and $P = 0.029$, respectively). The addition of AFB culture to histopathology resulted in a lower NNT than did addition of the MTB PCR assay across all three groups (129 vs 552 in Group A, 30 vs 120 in Group B and 18 vs 24 in Group C). Finally, on adding the MTB PCR assay to the combined histopathology and AFB culture results, tuberculous lymphadenitis was diagnosed in 1.0% (40/3863), 4.6% (22/478) and 12.7% (42/331) of the patients in Groups A, B and C, respectively. NNT values of Groups A, B and C were 1288, 478 and 42, respectively.

DISCUSSION

This study investigated the usefulness of routine AFB culture and MTB PCR assay of EBUS-TBNA needle rinse fluid for diagnosing tuberculous lymphadenitis according to the pre-procedural diagnosis in an intermediate TB-burden country. This study revealed that the addition of rinse fluid AFB culture to histopathology significantly increases the diagnosis yield of tuberculous lymphadenitis compared to histopathology only. However, MTB PCR of the needle rinse fluid was only useful in the subjects with pre-procedural diagnosis of benign pulmonary diseases.

Many studies have investigated the efficacy of EBUS-TBNA to diagnose tuberculous lymphadenitis; however, most studies included patients who were suspected of having tuberculous lymphadenitis.[3,11,12,14,24–27] The strength of our study was that a large number of consecutive patients were included and analysed according to the pre-procedural diagnosis. Importantly, the usefulness of AFB culture and MTB PCR assay of EBUS-TBNA needle rinse fluid differs according to the pre-procedural diagnosis. These results indicate that the pre-procedural diagnosis should be considered to determine which combination of tests (histopathology only, histopathology plus AFB culture or histopathology plus AFB culture plus MTB PCR) are appropriate for diagnosing tuberculous lymphadenitis.

This study also showed how AFB culture and MTB PCR assay increase the diagnostic yield when each or both are added to histopathology to diagnose



	Histopathology	Histopathology + PCR	NNT for 'Histopathology' vs 'Histopathology + PCR'	Histopathology + Cx	NNT for 'Histopathology' vs 'Histopathology + Cx'	Histopathology + Cx + PCR	NNT for 'Histopathology + Cx' vs 'Histopathology + Cx + PCR'	*P*-value for 'Histopathology' vs 'Histopathology + PCR'	*P*-value for 'Histopathology' vs 'Histopathology + Cx'	*P*-value for 'Histopathology + Cx' vs 'Histopathology + Cx + PCR'
Group A	0.2% (7/3863)	0.4% (14/3863)	552	1.0% (37/3863)	129	1.0% (40/3863)	1288	0.126	<0.001	0.820
Group B	1.0% (5/478)	1.9% (9/478)	120	4.4% (21/478)	30	4.6% (22/478)	478	0.281	0.001	0.876
Group C	4.5% (15/331)	8.8% (29/331)	24	10.3% (34/331)	18	12.7% (42/331)	42	0.029	0.005	0.329

Figure 3 Effect of tuberculous lymphadenitis diagnostic method. □, Group A (patients with suspected or histology-confirmed primary lung cancer; ▩, Group B (patients with extrapulmonary malignancy; ■, Group C (patients with other benign pulmonary diseases). Cx, positive AFB culture of EBUS needle rinse fluid; Histopathology, histopathological findings compatible with tuberculous lymphadenitis (chronic granulomatous inflammation with caseous necrosis); PCR, positive MTB PCR assay of EBUS needle rinse fluid. AFB, acid-fast bacilli; EBUS, endobronchial ultrasound; MTB, *Mycobacterium tuberculosis*; NNT, number needed to test; PCR, polymerase chain reaction; TB, tuberculosis.

tuberculous lymphadenitis. Our results demonstrate that the addition of AFB culture to histopathology resulted in a lower NNT than did addition of the MTB PCR assay across all three groups and this result might be related to most of the culture-proven cases being positive in liquid medium (78.5%). AFB culture using liquid medium could be better able to detect MTB than the MTB PCR assay, particularly when almost all specimens have a lower bacterial burden, such as that associated with the rinse fluid from an EBUS-TBNA needle.[28–30]

In this study, among MTB culture-positive cases, MTB PCR positivity was observed in 12.1% (4/33), 21.0% (4/19), 37.0% (10/27) and 22.8% (18/79), respectively, in Group A, B and C patients, and total patients (Fig. 2). These relatively low positive rates of COBAS TaqMan MTB PCR assay could be partly explained by the paucibacillary specimens in this study. Tortoli *et al.* reported that the sensitivities of COBAS TaqMan MTB PCR were 100% and 49% in smear-positive and smear-negative specimens, respectively. In addition, the sensitivity of respiratory specimens was 81%, but those of pleural fluid and cerebrospinal fluid (paucibacillary specimens) were 9% and 25%, respectively.[31] Cho *et al.* also reported similarly that the diagnostic sensitivities of COBAS TaqMan MTB PCR for smear-positive, smear-negative, respiratory and non-respiratory specimens were 99%, 49%, 71% and 33%, respectively.[32] In our study, 96.5% of specimens had EBUS-TBNA needle rinse fluid from intrathoracic lymph nodes (paucibacillary specimens) and only 2 patients (1.9%) had smear-positive specimens in 104 patients with intrathoracic TB lymphadenitis. Boonsarngsuk *et al.* reported that the sensitivity of MTB PCR (Anyplex MTB/NTM Real-Time Detection Kit, Seegene, South Korea) using EBUS-TBNA needle rinse fluid was 56.2%, which was higher than ours (32/104, 30.8%).[15] However, the smear-positive rate (12.5% (2/16) vs 1.9% (2/104)) was higher in their study than in our study.[15]

In the present study, AFB culture and the MTB PCR assay were performed on rinse fluid from the EBUS-TBNA needle. Obtaining a sufficient amount of core tissue is very important for histopathological diagnosis and molecular testing of patients with lung cancer.[33,34] Furthermore, it is difficult to distinguish tuberculous lymphadenitis from metastatic lymphadenopathy using imaging studies alone, because there are few specific findings of tuberculous lymphadenitis on such studies.[3,11,12,14,25] In addition, molecular testing and histopathological examination are necessary for an accurate diagnosis of tuberculous lymphadenitis.[35,36] Acquiring additional tissue core for AFB culture and MTB PCR is more difficult than acquiring rinse fluid from the EBUS-TBNA needle which is easy and quick, especially in prolonged procedural cases with multiple lymph node stations.[15,26]

This study had several limitations. First, selection bias may have affected our findings due to the retrospective nature of the study. However, selection bias would be minimal because we analysed the data according to a prospectively collected pre-procedural diagnosis in almost all patients (97.4% (4672/4795)) who underwent EBUS-TBNA during the study period. Second, this study was conducted at a single centre in an intermediate TB-burden country in which an annual TB incidence is 70 per 100 000 individuals and extrapulmonary TB accounts for 20% of TB.[1] Because the incidence of TB and proportion of extrapulmonary TB vary significantly by region and country,[1] our data should be interpreted conservatively. We did not

conduct a formal cost–benefit analysis of MTB PCR and the results may vary among intermediate TB-burden countries due to different medical costs. Third, we used the rinse fluid from an EBUS-TBNA needle, not the core tissue obtained by EBUS-TBNA. Therefore, we were unaware of the superiority of rinse fluid and core tissue to diagnose tuberculous lymphadenitis. Analysis of rinse fluid is advantageous because it does not require additional puncture or procedure time.

In conclusion, routine AFB culture of needle rinse fluid was useful to increase the diagnostic yield of tuberculous lymphadenitis for all subjects who underwent EBUS-TBNA regardless of pre-procedural diagnosis in an intermediate TB-burden country. However, MTB PCR was only useful in the subjects with pre-procedural diagnosis of benign pulmonary diseases.

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Abbreviations: AFB, acid-fast bacilli; CT, computed tomography; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; IRB, Institutional Review Board; MTB, *Mycobacterium tuberculosis*; NNT, number needed to test; PET, positron emission tomography; TB, tuberculosis.

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