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Clinical Usefulness of Fungal Culture of EBUS-TBNA Needle Rinse Fluid and Core Tissue

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Purpose: The diagnosis of pulmonary fungal infections is challenging due to the difficulty of obtaining sufficient specimens. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) needle rinse fluid has become an emerging diagnostic material. This study evaluated the role of routine fungal culture from EBUS-TBNA needle rinse fluid, in addition to histopathologic examination and fungal culture of EBUS-TBNA core tissue, in the diagnosis of pulmonary fungal infections.

Materials and Methods: Among patients who underwent EBUS-TBNA, those with results for at least one of three tests (histopathologic examination, fungal culture of EBUS-TBNA core tissue or needle rinse fluid) were included. Patients with a positive test were divided into two groups (clinical fungal infection and suspected fungal contamination) according to their clinical assessment and therapeutic response to antifungal.

Results: Of 6072 patients, 41 (0.7%) had positive fungal tests and 9 (22%) were diagnosed as clinical fungal infection. Of the 5222 patients who were evaluated using a fungal culture from EBUS-TBNA needle rinse fluid, 35 (0.7%) had positive results. However, only 4 out of 35 (11.4%) were classified as clinical fungal infection. Positive results were determined in 4 of the 68 (5.9%) evaluated by a fungal culture of EBUS-TBNA core tissue, and all were diagnosed as clinical fungal infection.

Conclusion: Routine fungal culture of EBUS-TBNA needle rinse fluid is not useful due to the low incidence of fungal infection and high rate of contamination. However, fungal culture of EBUS-TBNA core tissue and needle rinse fluid should be considered in patients with clinically suspected fungal infection.

Key Words: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), fungus, infection, rinse fluid

INTRODUCTION

Fungal infections are an important consideration in the differential diagnosis of pulmonary granulomatous inflammation, especially in immunocompromised patients.[1,2] The rapid recognition of fungal infections and their appropriate treatment are essential to reduce morbidity and mortality. However, no method has proven sufficiently sensitive and specific to allow proper diagnosis.[3]

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive intervention that is increasingly used to obtain diagnostic materials from peribronchial and mediastinal lymph nodes, as well as lung parenchymal lesions.[4] The indications for EBUS-TBNA have recently been expanded beyond malignant diseases to include benign diseases, such as sarcoidosis, tuberculous lymphadenitis, and fungal infections.[5-8] In addition, several studies of EBUS-TBNA in benign diseases mainly suspected tuberculosis and

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sarcoidosis showed a good diagnostic yield and low complication rates.[6,9,10]

The material collected by EBUS-TBNA can be analyzed using several modalities, including cytology, flow cytometry, cell block preparation for immunohistochemical or molecular studies, and culture or other forms of microbiological testing.[11] Recently, EBUS-TBNA needle rinse fluid has been becoming an emerging diagnostic material for tuberculosis.[8,12] EBUS-TBNA needle rinse fluid is an additional sample that can be easily obtained by flushing sterile normal saline through the needle after obtaining the core tissue, and can be acquired quickly within a few seconds. However, its utility in the evaluation of fungal infections has yet to be determined.

Therefore, this study assessed the role of routine fungal culture of EBUS-TBNA needle rinse fluid in addition to histopathologic examination and fungal culture of EBUS-TBNA core tissue in the diagnosis of pulmonary fungal infections.

MATERIALS AND METHODS

Study population

We retrospectively reviewed the prospectively collected EBUS-TBNA registry data at Samsung Medical Center (a 1979-bed referral hospital, in Seoul, South Korea) between November 2009 and December 2017. Patients who underwent at least one of three tests for fungal examination (histopathologic examination or fungal culture of EBUS-TBNA core tissue or fungal culture of EBUS-TBNA needle rinse fluid) were included in the study.

This study was approved by the Institutional Review Board of Samsung Medical Center (IRB no.2019-02-048). The requirement for informed consent was waived 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 the EBUS-TBNA procedure were described in our previous reports.[13,14] Briefly, EBUS-TBNA was performed using a convex probe-EBUS bronchoscope (BF-UC260F-OL8; Olympus, Tokyo, Japan) and a 22-gauge needle (NA-201SX-4022; Olympus) under moderate sedation with intravenous midazolam and fentanyl. Three passes per lesion were attempted, and at least two passes when core tissue was obtained.

The core tissue was blotted on filter paper to remove excess blood and fixed in 10% (v/v) formalin, and the tissue coagulum clot was sent for histological examination.[15] Rapid on-site cytopathological evaluation was not available. After the core tissues had been obtained, 1 cc of sterile normal saline was flushed through the needle to obtain a rinse fluid sample.[8] All rinse fluid samples were collected into an aseptic tube and sent for microbiological examination including fungal culture.

Diagnosis of fungal infection

Eligible patients were grouped according to the results of fungal tests. Patients with a positive fungal test were defined as those having positive results on any of the three fungal tests, and all others were defined as those with negative fungal test. If a fungal infection was suspected based on the initial standard stains (hematoxylin and eosin) on histopathologic examination of EBUS-TBNA core tissue, Grocott methenamine silver staining or periodic acid-Schiff was added to optimize the identification of infectious agents.[16] A fungal culture of EBUS-TBNA needle rinse fluid was routinely performed in most patients during study period. A fungal culture of EBUS-TBNA core tissue was performed only when a fungal infection was strongly suspected. The rinse fluid and tissue specimens were inoculated on Sabouraud dextrose agar and cultured at a temperature of 30°C for 3 weeks. Species were identified according to macro- and micro-morphological criteria.[17] If the morphological criteria were not typical for a specific species, molecular identification was performed by sequencing the D1/D2 region of 28S rDNA and internal transcribed spacer regions.

Patients with a positive fungal test were further divided into two subgroups (clinical fungal infection vs. suspected fungal contamination) according to the clinical decision and therapeutic response to antifungal therapy. If an attending physician considered a patient with a positive fungal test as highly suspected to have a fungal infection and therefore initiated antifungal treatment, the patient was assigned to the clinical fungal infection group.[18] For patients who had a positive fungal test, if the attending physician considered the test result indicative of fungal contamination and the clinical course did not worsen without antifungal treatment, the patient was assigned to the suspected fungal contamination group.

Data collection

Data on baseline characteristics were collected, including age, gender, pre-procedure diagnosis, factors associated with immune status, and location and size of the lesion. The pre-procedure diagnosis was prospectively recorded and classified as suspected or histologically confirmed primary lung cancer, other cancer, or other benign disease according to the opinion of the pulmonologists performing the EBUS-TBNA and based on the clinical presentation of the patient, as well as the results of imaging studies.[8]

Data on the factors associated with immune status were also collected, and included previous cancer diagnosis, diabetes, immunosuppressive agent use, and absolute neutrophil count within the week before the procedure. Immunosuppressive agent use was defined as the use of any of the following within 6 months before the procedure: anti-cancer chemotherapy, corticosteroid (of ≥5 mg of prednisolone or its equivalent dose, for at least 1 month) and T-cell mediated immunosuppressants (e.g., azathioprine, tacrolimus, or mycophenolate for at least 1 month).[19]

Lymph node station was defined according to the lymph node map of the International Association for the Study of Lung Cancer.[20]

Statistical analysis

Data are presented as the median and interquartile range for continuous variables, and as numbers and percentages for categorical variables. Continuous and categorical variables were analyzed using the Mann-Whitney U test and Pearson's chi-squared or Fisher's exact test, respectively. The numbers and proportions of positive results of histopathologic examinations and fungal cultures of the EBUS-TBNA needle rinse fluid and core tissue are illustrated in Venn diagrams displaying total patients, and patients divided into the suspected fungal contamination and clinical fungal infection groups. A two-sided *p*-value ≤0.05 was considered to indicate significance in all statistical analyses. All analyses were performed using SPSS statistical software (version 23.0; IBM Corp., Armonk, NY, USA).

RESULTS

Baseline characteristics

Between November 2009 and December 2017, 6082 patients underwent EBUS-TBNA. After excluding patients who did not have fungal culture or histopathologic examination results (n=10), 6072 patients were included in the study. All of these patients underwent EBUS-TBNA with fungal culture of the needle rinse fluid, a histopathologic examination or fungal culture of the core tissue (Fig. 1).

Baseline characteristics of the patients are presented in Table 1. A positive fungal test was determined in 41 (0.7%) and a negative fungal test in 6031 (99.3%). There was no statistical difference in baseline characteristics between the two groups, including previous cancer diagnosis, diabetes, immunosuppressive agent use, and absolute neutrophil count. Among the 41 patients with a positive fungal test, 32 had suspected fungal contamination and 9 had a clinically confirmed fungal infection. There was no statistical difference in baseline characteristics between these two groups.

The characteristics of the examined lesions are presented in Table 2. A total of 13927 lymph nodes, 683 lung parenchymal lesions, and 12 pleural lesions were examined in the 6072 patients. The median short-axis diameter was 10 mm, and the median long-axis diameter was 14 mm. A median of two needle passes was performed, and a median of two tissue cores per lesion were obtained.

Diagnostic yield by test

The diagnostic yield of each test for fungal infection is shown as a Venn diagram in Fig. 2. The proportion of positive results for each fungal test in the study population as a whole is shown in Fig. 2A. A fungal culture of EBUS-TBNA needle rinse fluid was performed in 5222 of the 6072 patients in the study; 35 (0.7%) had a positive result. Fungal culture of EBUS-TBNA core tissue was performed in 68 patients who had a high index of clinical suspicion for fungal infection (i.e., fever, travel history to the endemic area, increased inflammatory markers, or ra-

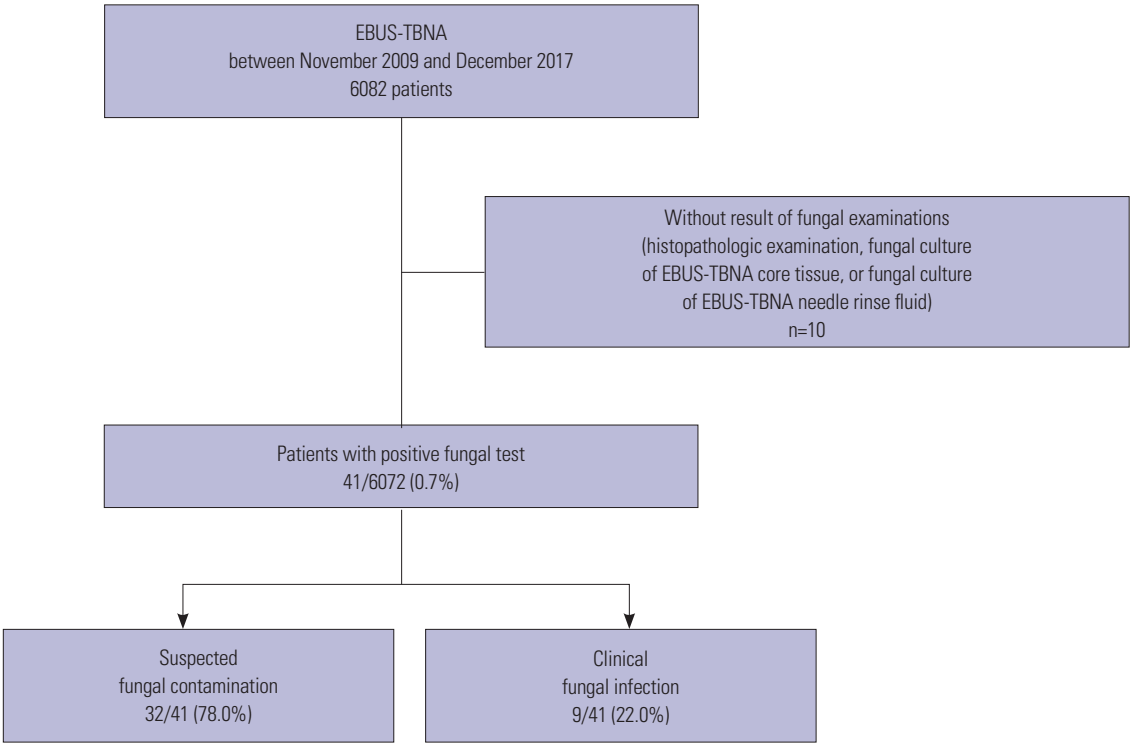

Fig. 1. Flowchart of the study population. EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration.

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Table 1. Characteristics of the Study Patients (n=6072)

	Positive fungal test (n=41)				Negative fungal test (n=6031)	*p* value†
	All (n=41)	Suspected fungal contamination (n=32)	Clinical fungal infection (n=9)	*p* value*		
Age (yr)	67 (58–75)	69 (60–75)	64 (55–67)	0.277	66 (58–72)	0.235
Sex, male	26 (63.4)	23 (71.9)	3 (33.3)	0.084	4,202 (69.7)	0.485
Pre-procedure diagnosis				0.582		0.147
Primary lung cancer	31 (85.4)	25 (78.1)	6 (66.7)		5,157 (85.5)	
Other cancer	5 (12.2)	4 (12.5)	1 (11.1)		521 (8.6)	
Other benign disease	5 (2.4)	3 (9.4)	2 (22.2)		353 (5.9)	
Underlying disease						
Previous cancer diagnosis	8 (19.5)	7 (21.9)	1 (11.1)	0.807	1,202 (19.9)	>0.999
Diabetes	5 (12.2)	4 (12.5)	1 (11.1)	1.000	679 (11.3)	>0.999
Previous TB treatment	3 (7.3)	2 (6.2)	1 (11.1)	1.000	281 (4.7)	0.666
Immunosuppressive agent used	5 (12.2)	3 (9.4)	2 (22.2)	0.643	731 (12.1)	>0.999
Chemotherapy	4 (9.8)	3 (9.4)	1 (11.1)	1.000	568 (9.4)	>0.999
Corticosteroid	2 (4.9)	1 (3.1)	1 (11.1)	0.915	302 (5.0)	>0.999
T-cell immunosuppressant	0 (0)	0 (0)	0 (0)	-	18 (0.3)	>0.999
ANC, $\times 10^3/\mu L$	4.4 (3.2–6.0)	4.5 (3.3–6.1)	4.4 (3.2–5.6)	0.614	4.3 (3.2–5.8)	0.629

TB, tuberculosis; ANC, absolute neutrophil count.
*Between patients with suspected fungal contamination (n=32) and those with clinical fungal infection (n=9), †Between patients with positive fungal test (n=41) and those with negative fungal test (n=6031).

Table 2. Characteristics of the Examined Lesions (n=14667)

	No. (%) or median (IQR)
Examined site	
Lymph nodes	13972
Lung parenchymal lesions	683
Pleural lesions	12
Size of lesion, mm	
Lymph nodes	
Short-axis diameter	10 (7–13)
Long-axis diameter	14 (10–19)
Lung parenchymal lesions	
Short-axis diameter	23 (15–34)
Long-axis diameter	32 (21–46)
Pleural lesions	
Short-axis diameter	14 (9–29)
Long-axis diameter	25 (18–41)
Number of needle passes per lesion	2 (1–2)
Number of tissue cores obtained per lesion	2 (1–2)

IQR, interquartile range.

diologic findings). Of 68 patients, 4 (5.9%) had a positive result. Among the 6048 patients whose work-up included a histopathologic examination, 7 (0.1%) had a positive result.

Fig. 2B and C show the proportion of patients who had a positive fungal test, and their distribution in the suspected fungal contamination and clinical fungal infection groups. Among the 35 patients with a positive EBUS-TBNA needle rinse fluid culture, most (n=31, 88.6%) were assigned to the suspected fungal contamination group. One patient was assigned to the clinical fungal infection group, based only on a fungal culture of the EBUS-TBNA rinse fluid. This case is summarized in Fig. 3. In contrast, all 4 (100%) with a positive fungal culture of EBUS-TBNA core tissue, as well as 6 of the 7 (85.7%) who had a positive result on histopathologic examination, were assigned to the clinical fungal infection group. Among patients with a positive result on histopathologic examination, only one had a suspected fungal contamination. This case is summarized in Fig. 4. Of the 32 patients in the suspected fungal contamination group, the most commonly detected fungus was *Candida* (*Candida*, n=25; *Aspergillus*, n=2, *Paecilomyces*, n=1; *Trichosporon*, n=1; and unidentified molds, n=3). Of the 9 patients with clinically confirmed fungal infection, the most common fungus was *Aspergillus* (*Aspergillus*, n=7; *Candida*, n=1; and *Coccidioides*, n=1).

DISCUSSION

In this study, we investigated whether routine fungal culture of EBUS-TBNA needle rinse fluid can play an additional role in the diagnosis of fungal infection with histopathologic examination or fungal culture of EBUS-TBNA core tissue. The study population consisted of a large number (6702) of consecutive patients who underwent EBUS-TBNA. Within this group, 41 (0.7%) had positive fungal results on histopathologic examination of either the fungal culture of EBUS-TBNA rinse fluid or the fungal culture of the core tissue. However, only 9 of these 41 patients were diagnosed with a clinical fungal infection. Among the 68 patients who had a fungal culture of EBUS-TB-

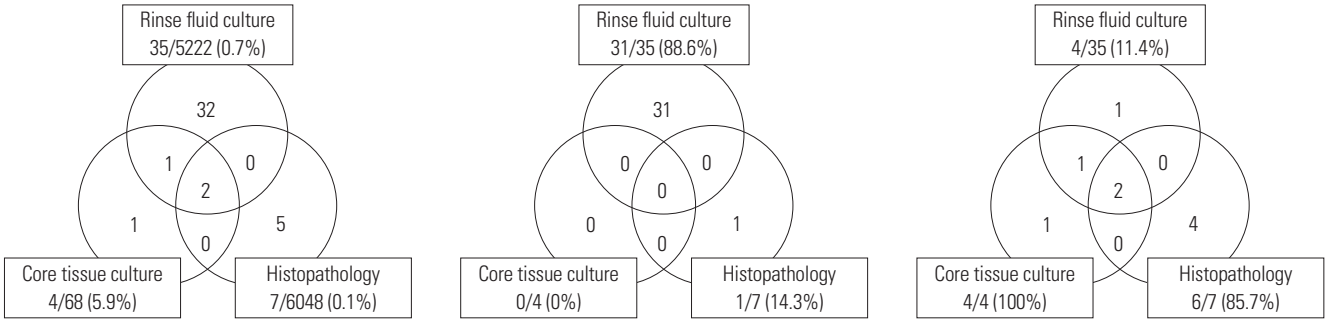

Fig. 2. Venn diagram of the impact of a fungal test (fungal culture of EBUS-TBNA needle rinse fluid, fungal culture of EBUS-TBNA core tissue, and histopathologic examination). (A) Fungal culture of EBUS-TBNA needle rinse fluid was performed in 5222 of the 6072 patients enrolled in the study, and positive results were determined in 35 (0.7%). A positive result was determined in 4 of the 68 (5.9%) tested by fungal culture of EBUS-TBNA core tissue. Histopathologic examination was performed in 6048 patients and fungal invasion was detected in 7 (0.1%). (B) The results of 32 patients with suspected fungal contamination. Among the 35 patients with positive results for a fungal culture from EBUS-TBNA needle rinse fluid, most (n=31, 88.6%) were diagnosed with suspected fungal contamination. Four patients had positive results from the fungal culture of EBUS-TBNA core tissue, but no patient was considered as suspected fungal contamination. Only 1 patient with suspected fungal contamination had a positive result on the histopathologic examination. (C) The results of 9 patients with clinical fungal infection. Four of the 35 (11.4%) with a positive fungal test from the culture of EBUS-TBNA needle rinse fluid were diagnosed with clinical fungal infection. Only 1 patient with a clinical fungal infection was diagnosed by EBUS-TBNA needle rinse fluid culture alone. By contrast, all 4 (100%) with a positive fungal culture of EBUS-TBNA core tissue were diagnosed with a clinical fungal infection. Of the 7 patients with positive results on the histopathologic examination, 6 (85.7%) were diagnosed with a clinical fungal infection. EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration.

NA core tissue based on a suspicion of fungal infection, 4 had a positive result and were subsequently diagnosed with a clinical fungal infection. Fungal culture of EBUS-TBNA needle rinse fluid was performed in 5222 patients, 35 of whom had a positive result; however, only 4 of those 35 patients had a clinical fungal infection. Only 1 patient was diagnosed with a fungal infection by fungal culture of EBUS-TBNA needle rinse fluid (Fig. 4).

EBUS-TBNA needle rinse fluid can be quickly and easily collected by flushing 1 cc of sterile normal saline through the needle after EBUS-TBNA core tissue has been obtained.[8,12] Since most patients undergo EBUS-TBNA for cancer evaluation, it is important to obtain a sufficient amount of core tissue for histopathologic examination and molecular testing to allow a proper diagnosis and treatment. In these patients, it is typically difficult to obtain additional core tissue for the surveillance of infectious disease. Routine mycobacterial culture of EBUS-TBNA needle rinse fluid is effective for tuberculosis surveillance.[8,12] By contrast, our study showed that this material is not effective for the diagnosis of clinical fungal disease. The difference in utility between fungal and mycobacterial testing using EBUS-TBNA needle rinse fluid can be explained as follows. First, EBUS-TBNA needle rinse fluid may have a low fungal burden and low sensitivity for fungal culture. In our previous study, only 1.9% of the patients with intrathoracic tuberculous lymphadenitis had a positive smear from the EBUS-TBNA needle rinse fluid, which showed that the mycobacterial burden of EBUS-TBNA needle rinse fluid is low.[8] Liquid media have high sensitivity for the detection of mycobacteria, even in specimens with a low mycobacterial burden.[21] However, fungal culture shows an extremely low sensitivity when the specimens have a low fungal burden.[22] Second, unlike *Mycobacterium tuberculosis*, fungi may be present in the airway as colonizers, and the fungal burden in the airway may increase in patients treated with antibiotics.[23] Therefore, clinically distinguishing between fungal infection and contamination is necessary in patients with a positive fungal culture of EBUS-TBNA needle rinse fluid. Finally, tuberculosis is relatively common in South Korea, with an incidence of 77/100000 per year,[24] whereas fungal diseases are not endemic in the country.

In the present study, all patients with positive results for the fungal culture of EBUS-TBNA core tissue were diagnosed with a clinical fungal infection. In addition to mycobacterial culture, EBUS-TBNA core tissue used for fungal culture allows for the diagnosis of granulomatous inflammation related to intrathoracic lymphadenopathy.[7,25] Although histopathologic examination provides more rapid results compared to culture-based methods, it is not sufficient on its own to identify the pathogen.[26,27] The addition of a fungal culture of EBUS-TBNA core tissue to the routine histopathologic examination may facilitate the diagnosis of fungal infections.[26]

The role of fungal culture of EBUS-TBNA core tissue in the diagnosis of fungal infection has been investigated in a few studies. Harris, et al.[28] analyzed 85 EBUS-TBNA specimens and concluded that routine microbiologic tests, including fungal culture of EBUS-TBNA core tissue, are not sufficiently sensitive to rule out infectious causes of adenopathy. However, that study included only two cases of fungal infection (both were histoplasmosis). Based on a case review of seven patients, Egressy and colleagues found that EBUS-TBNA can play a role in the diagnosis of subacute pulmonary histoplasmosis.[29] Although that study demonstrated the utility of EBUS-TBNA in supporting the

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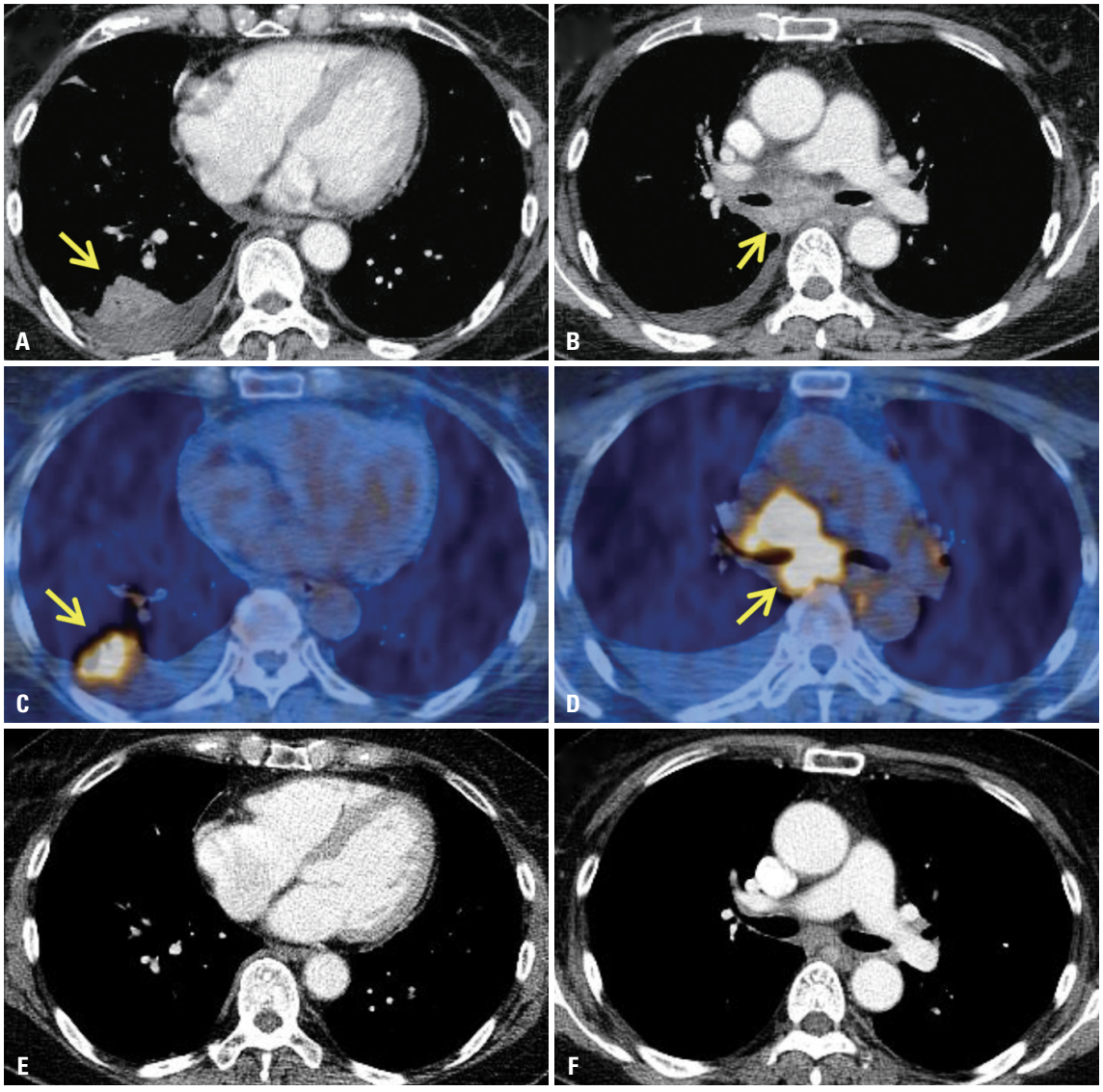

Fig. 3. A representative case of clinical fungal infection diagnosed solely by fungal culture of the EBUS-TBNA needle rinse fluid. A 65-year-old woman without any underlying disease and no history of smoking was referred to our hospital with suspected lung cancer. She had a history of travel to Arizona for 3 weeks. After 2 weeks of hospitalization due to fever and cough, her symptoms did not improve. Chest CT revealed (A) a 43-mm sub-pleural mass (yellow arrow) in the right lower lobe and pleural effusion, as well as (B) suspected metastatic mediastinal LN (yellow arrow). (C and D) Both the pleural mass and the mediastinal LN showed increased metabolic activity on the integrated PET/CT images (yellow arrows). EBUS-TBNA of the subcarinal and right lower paratracheal LNs was performed, with two core tissues obtained for each lesion. On the histopathologic examination, there was no evidence of malignancy, fungal invasion, or granulomatous inflammation. Fungal culture for EBUS-TBNA needle rinse fluid identified Coccidioides, and the patient was treated with fluconazole (450 mg daily). Four months after the initiation of treatment, chest CT showed that the (E) subpleural mass, pleural effusion, and (F) mediastinal LN had disappeared. EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; CT, computed tomography; LN, lymph node; PET, positron emission tomography.

pathologic diagnosis of histoplasmosis, fungal culture of EBUS-TBNA core tissue was negative in all seven cases. By contrast, in the study of Berger and colleagues, the efficacy of fungal stain and fungal culture was 35% and 15%, respectively, in 20 patients with caseating granulomas who were from an area where fungal infection is endemic.[7]

Our study had several limitations. First, histoplasmosis, coccidiodomycosis, and penicillosis may present as mediastinal lymphadenopathy in endemic areas, such as the United States and China.[30-32] However, this study was conducted in South Korea, where these fungal infections are not endemic. One patient was diagnosed based on the fungal culture of EBUS-TBNA needle rinse fluid. She had traveled to Arizona, where coccidioidomycosis is endemic (Fig. 3). Therefore, whether routine fungal culture of EBUS-TBNA needle rinse fluid would be useful in areas where fungal infections are endemic should be determined. Second, the fungal culture of EBUS-TBNA core tissue was processed only in patients with a suspected fungal infection. Therefore, our study does not allow any conclusions to be drawn on the usefulness of routine fungal culture of core tissue. Third,

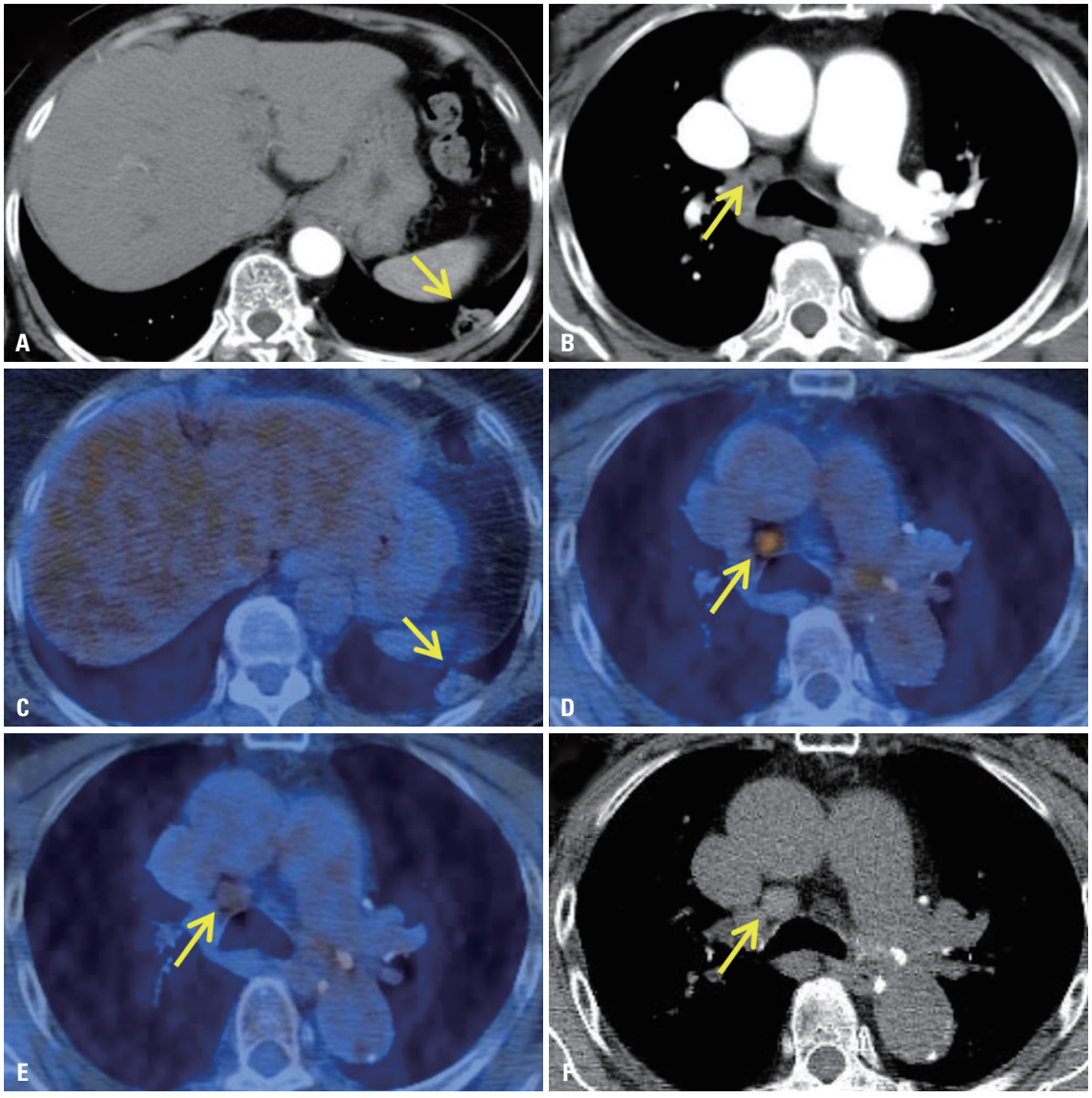

Fig. 4. A representative case of suspected fungal contamination in a patient with a positive histopathologic examination. A 70-year-old woman with diabetes and hypertension, and no history of smoking, was referred to our hospital with a lung nodule in the left lower lobe detected on routine medical examination. The chest CT showed (A) a 24-mm nodule (yellow arrow) in the left lower lobe and (B) enlarged right lower paratracheal LN (yellow arrow). (C) On integrated PET/CT, the lung nodule had faint FDG uptake (SUVmax=1.5; yellow arrow) and (D) the right lower paratracheal LN showed high FDG uptake (SUVmax=3.8; yellow arrow). EBUS-TBNA of the subcarinal and right lower paratracheal LN was performed. Although there was no evidence of malignancy, fungal invasion was detected in the small tissue fragments of the right lower paratracheal LN obtained by EBUS-TBNA. The patient underwent wedge resection of the nodule in the left lower lobe without mediastinal LN dissection. Four and 6 years after the lung cancer surgery, (E) integrated PET/CT and (F) chest CT were performed, respectively. There was no evidence of cancer recurrence or progressive fungal infection without anti-fungal therapy (yellow arrow). Therefore, the diagnosis was suspected fungal contamination. CT, computed tomography; LN, lymph node; FDG, ^{18}F-fluorodeoxyglucose; SUVmax, maximum standardized uptake value; PET, positron emission tomography; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration.

two patients underwent both EBUS-TBNA for intrathoracic lymph node and lung parenchymal lesion in this study. In these two patients, the needle rinse fluids were collected in the one bottle from lymph nodes and lung parenchymal lesion. Therefore, the site of infection could not be definitely described in these patients.

In conclusion, routine fungal culture of EBUS-TBNA needle rinse fluid was not useful in our study population, due to the low incidence of fungal infection and high proportion of contamination in a country where fungal infection is not endemic. Fungal culture of EBUS-TBNA core tissue and needle rinse fluid should be considered, in addition to histopathologic examination, in cases where fungal infection is clinically suspected.

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