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
sarcoidosis showed a good diagnostic yield and low complication rates.\textsuperscript{6,8,10}

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.\textsuperscript{11} Recently, EBUS-TBNA needle rinse fluid has been becoming an emerging diagnostic material for tuberculosis.\textsuperscript{6,12} 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{6} 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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).\textsuperscript{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-
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 culture 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-TBNA needle rinse fluid, 1 (1.5%) had a positive result. Among the 6031 patients whose work-up included a histopathologic examination, 7 (0.1%) had a positive result.

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

|                             | Positive fungal test (n=41) | Negative fungal test (n=6031) |
|-----------------------------|-----------------------------|------------------------------|
|                             | All (n=41)                  | Suspected fungal contamination (n=32) | Clinical fungal infection (n=9) | p value | p value |
| Age (yr)                    | 67 (58–75)                  | 69 (60–75)                   | 64 (55–67)                   | 0.277   | 0.235   |
| Sex, male                   | 26 (63.4)                   | 23 (71.9)                    | 3 (33.3)                     | 0.084   | 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) | 0.147   |
| Other cancer                | 5 (12.2)                    | 4 (12.5)                     | 1 (11.1)                     | 521 (8.6) | 0.147   |
| Other benign disease        | 5 (2.4)                     | 3 (9.4)                      | 2 (22.2)                     | 353 (5.9) | 0.147   |
| Underlying disease          |                             |                              |                              | 0.277   | 0.235   |
| 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 |
| Primary TB treatment        | 3 (7.3)                     | 2 (6.2)                      | 1 (11.1)                     | 1,000   | 281 (4.7) | 0.666   |
| Chemotherapy                | 4 (9.8)                     | 3 (8.4)                      | 1 (11.1)                     | 1,000   | 656 (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, 10^3/μL                | 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)

| Examined lesion                      | 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.
Fungal Culture of EBUS-TBNA Core Tissue and Needle Rinse Fluid

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. 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. 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. Liquid media have high sensitivity for the detection of mycobacteria, even in specimens with a low mycobacterial burden. However, fungal culture shows an extremely low sensitivity when the specimens have a low fungal burden.

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. 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. 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, 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. 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.
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, coccidiomycosis, 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,
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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