

[CASE REPORT]

Pulmonary *Actinomyces graevenitzii* Infection Diagnosed by Bronchoscopy using Endobronchial Ultrasonography with a Guide Sheath

Daisuke Himeji, Sachika Hara, Takeshi Kawaguchi and Gen-ichi Tanaka

Abstract:

A 75-year-old man visited our hospital complaining of a low-grade fever, dry cough, and chest abnormal shadow. Chest computed tomography revealed a nodule with a cavity in the right upper lobe. Endobronchial ultrasonography (EBUS) of the lesion suggested that the lesion was benign. *Actinomyces graevenitzii* was cultured from the specimen obtained by bronchoscopy using endobronchial ultrasonography with a guide sheath technique and was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and 16S rRNA sequencing. The patient was treated with intravenous ampicillin; subsequently, his condition improved. We believe that careful observation of EBUS findings may be useful for the differential diagnosis.

Key words: pulmonary actinomycosis, *Actinomyces graevenitzii*, endobronchial ultrasonography with a guide sheath (EBUS-GS), bronchoscopy

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Introduction

Pulmonary actinomycosis is an indolent, slowly progressive infection caused by the anaerobic *Actinomyces* bacteria (1). Because the clinical and radiological findings of pulmonary actinomycosis mimic malignancy, it is frequently misdiagnosed, and adequate treatment is delayed. In addition, the identification of *Actinomyces* from clinical samples is difficult. Therefore, pulmonary actinomycosis is often diagnosed as a result of surgical operation. We herein report a case of pulmonary *A. graevenitzii* infection, which was identified by the anaerobic culture of samples obtained by bronchoscopy using endobronchial ultrasonography with a guide sheath (EBUS-GS).

Case Report

A 75-year-old man visited a regional hospital with a 10-day history of low-grade fever and dry cough. He had a smoking history of 40 cigarettes per day for 35 years. His

medical history included pneumonia and Guillain-Barré syndrome, and he had periodontitis. He underwent chest radiography, which revealed infiltrate in the right upper lobe. He was referred to Miyazaki Prefectural Miyazaki Hospital for an examination under suspicion of lung cancer. His vital signs were as follows: temperature, 35.6°C; blood pressure, 117/73 mmHg; pulse, 69/min; respiratory rate, 20/min with an O₂ saturation of 94% on room air. The results of a hemogram revealed a normal leucocyte count of 6,590/μL, and the renal and liver parameters were normal. The C-reactive protein level was 4.38 mg/dL. The tumor markers CEA, CYFRA, NSE, and proGRP were negative (Table). A sputum Gram stain was negative, while sputum and blood cultures revealed no growth. Chest radiograph revealed a nodule with a cavity in the right upper lobe, while chest computed tomography (CT) demonstrated a nodule with a cavity (Fig. 1).

We considered lung cancer, an acid-fast bacillus infection, and pulmonary actinomycosis as the differential diagnoses based on his smoking history and the presence of periodontitis. For the diagnosis, we performed bronchoscopy using

Table. Laboratory Findings on Admission.

Hematology		Biochemistry		Tumor marker	
WBC	6,590 /mm ³	TP	7.4 g/dL	CEA	1.4 ng/dL
Band	0.0 %	Alb	3.3 g/dL	CYFRA	1.2 ng/dL
Seg	65.0 %	T-Bil	0.4 mg/dL	proGRP	49.68 pg/mL
Eo	2.0 %	AST	23 U/L	NSE	10.14 ng/mL
Ba	0.0 %	ALT	11 U/L		
Mo	9.0 %	LDH	187 U/L	Bacteriology	
Ly	24.0 %	ALP	258 U/L	T-SPOT	(-)
Hb	13.2 g/dL	γ-GTP	28 U/L		
Ht	39.7 %	BUN	18.0 mg/dL	Serology	
Plt	33.4×10 ⁴ /mm ³	Cr	0.77 mg/dL	HBsAg	(-)
		CRP	4.38 mg/dL	HCVAb	(-)
		Glu	105 mg/dL		
Coagulation		HbA1c	6.3 %(NGSP)		
PT	11.2 s				
PT-INR	0.93				
APTT	41.0 s				
Fibrinogen	850 mg/dL				

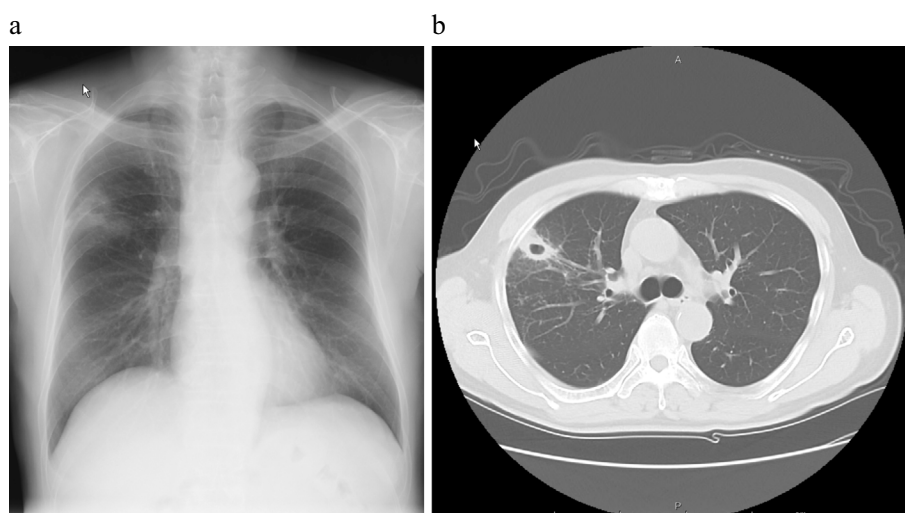


Figure 1. Chest X-ray and computed tomography (CT). **a:** The radiograph shows a nodule with a cavity in the right upper lung. **b:** The chest CT image shows a nodule with a cavity in the right upper lobe.

EBUS-GS, as described previously (2). After localizing the lesion using a radial probe, we obtained EBUS internal echo images (Fig. 2). The internal echo image of the lesion was homogeneous, prompting a designation of type Ib under Kurimoto's classification (2). After observing the EBUS findings, we performed bronchial brushing (BB), a trans-bronchial biopsy (TBB), and bronchial washing. After brushing, the brush was withdrawn, and the material's cells were transferred directly onto clean glass slides. Subsequently, we washed the brush in saline (BB washing). We then performed a TBB five times. After BB and the TBB, bronchial washings was obtained by lavage with 20 mL of normal saline. The bronchial washing was mixed with the BB washing, and 5 mL of the mixture was transferred immediately into a container for anaerobic culture.

Primary culturing of that mixture was performed with blood, chocolate, and Brucella agar with hemin and vitamin

K1 (Brucella HK agar). Brucella HK agar was incubated at 35°C under anaerobic conditions. After 96 hours of incubation, only molar-tooth-like colonies were observed on Brucella HK agar. The organism was a coryneform Gram-positive rod that did not produce catalase. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed for identification, and we identified the isolate as *A. graevenitzii*. For confirmation, we performed molecular identification by polymerase chain reaction (PCR) amplification and a sequencing analysis of the 16S rRNA gene using DNA extracted from the isolated organism. We performed the sequencing analysis using a GenBank BLAST search. The sequence of the 16S rRNA gene was 99.0% identical to that of the type strain of *A. graevenitzii*.

No other bacteria or fungi were detected in this specimen, and the results of the acid-fast staining and PCR tests of the

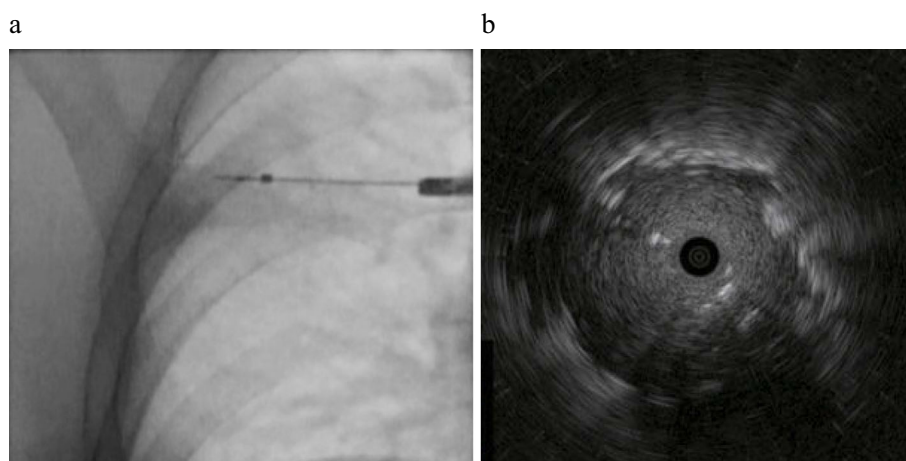


Figure 2. Fluoroscopy and endobronchial ultrasonography (EBUS) internal echo image. **a:** The probe is inserted into the lesion in the right upper lobe. **b:** EBUS demonstrates a hypoechoic homogeneous lesion.

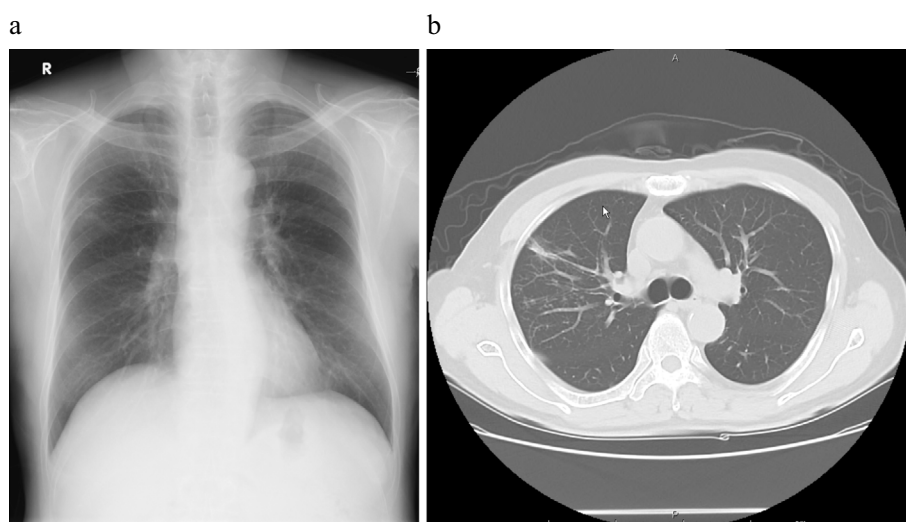


Figure 3. Chest X-ray and computed tomography after treatment. **a:** The radiograph shows remission of the nodule in the right upper lung. **b:** The nodule with a cavity in the right upper lobe has disappeared.

patient's sample for *Mycobacterium tuberculosis*, *M. avium*, and *M. intracellulare* were negative. The histological findings of the TBB specimen and the cytological findings of BB revealed only nonspecific inflammation, and the characteristic histology of pulmonary actinomycosis, such as granuloma or sulphur granules, was not observed. In addition, no microorganisms were detected by Gram or PAS stains. These results indicated that the pulmonary lesion of our patient was caused by *A. graevenitzii*.

The patient was treated with 9,000 mg ampicillin/sulbactam intravenously for 1 week. After the identification of *A. graevenitzii*, he was treated with 6,000 mg of ampicillin intravenously for 1 week. Subsequently, he was treated with 1,500 mg of ampicillin orally for 2 months. The clinical status gradually improved. The lung nodule disappeared in a dramatic response to the antibiotics (Fig. 3). We plan to continue treatment for the next 10 months.

Discussion

We encountered a case of pulmonary *A. graevenitzii* infection diagnosed by bronchoscopy using EBUS-GS and by molecular identification of the sample. In addition, we demonstrated that appropriate anaerobic culture of bronchial brushing samples and washing fluid were useful for the identification of the pathogen of pulmonary actinomycosis.

Pulmonary actinomycosis probably results from the aspiration of oropharyngeal or gastrointestinal secretions into the respiratory tract (3). Insufficient intraoral hygiene and associated dental disease may increase the risk (4). Pulmonary actinomycosis is rarely caused by distant hematogenous seeding (5). The patient in our case had periodontitis, so his poor oral hygiene may have been related to the pathogenesis of pulmonary actinomycosis infection.

Six species of this genus are considered pathogenic in humans: *A. israeli*, *A. naeslundii*, *A. odontolyticus*, *A. viscosus*, *A. meyeri*, and *A. gerencseriae* (6, 7). *A. graevenitzii* was first isolated in 1997 from respiratory and bone specimens (8), while cases of pulmonary infection caused by *A. graevenitzii* have been reported before (9, 10). However, little is known about the clinical features and pathogenesis of this bacterium. Because of its rarity, more studies on such cases are required to determine the pathogenesis of *A. graevenitzii*.

The diagnosis of pulmonary actinomycosis can be quite challenging. In one series, the diagnosis was suspected upon hospital admission in 7% of patients who later turned out to have the infection (11). Several reports found that 25-49% of cases of pulmonary actinomycosis were initially suspected of having lung malignancy at hospital admission (6, 12, 13). Even when the clinical suspicion of pulmonary actinomycosis is high, microbiological confirmation can be difficult. Medical intervention alone is sufficient for treatment; therefore, a careful diagnosis can avoid unnecessary surgery.

Bronchoscopy is a viable diagnostic method; however, the bacteriological identification of *Actinomyces* species from the samples obtained by bronchoscopy is reportedly very difficult. The sample should be handled anaerobically with caution, as the culture of *Actinomyces* species can be falsely negative if the sample is exposed to air for more than 20 minutes (6).

To diagnose pulmonary actinomycosis appropriately, it is important to consider pulmonary actinomycosis as a differential diagnosis of lung tumor-related shadow. In addition, to identify bacteria, we must ensure that we handle specimens appropriately and culture them under anaerobic conditions.

In the present case, we performed bronchoscopy using EBUS-GS. The utility of EBUS has been reported for the diagnosis of not only malignant lung diseases but also benign ones (14). Cases of actinomycosis of the lung diagnosed using EBUS have also been reported (15, 16). However, in those reports, EBUS was used only to confirm the lesions. Notably, we used EBUS not only for the identification of the lesion but also to observe its internal structure. Kurimoto et al. reported that the classification of ultrasonograms suggests the pathology and histology of the lesions (2). EBUS revealed that this lesion had a homogeneous pattern without vessels or bronchioles, which is a type Ib pattern under Kurimoto's classification. Type Ib is said to primarily reflect organized pneumonia or tuberculoma. The pathological feature of pulmonary actinomycosis is chronic inflammation comprising a granulomatous change (17); therefore, the EBUS findings of our patient may reflect the pathological features, particularly the granulomatous changes of the lesion. More studies reporting EBUS findings in cases of actinomycosis are necessary. In addition, a careful analysis of the EBUS findings will encourage physicians to consider the possibility of actinomycosis as the differential di-

agnosis, even if it had not been considered before bronchoscopy, thereby leading physicians to perform appropriate sampling and sample processing.

To our knowledge, this is the first report describing the usefulness of EBUS internal echo imaging in diagnosing pulmonary actinomycosis caused by *A. graevenitzii*, which was diagnosed using a molecular analysis of the sample obtained by bronchoscopy.

In conclusion, for the appropriate diagnosis of pulmonary actinomycosis, it is important that the samples be prepared and handled while considering the possibility of pulmonary actinomycosis. In addition, the careful observation of the EBUS findings may be useful for the differential diagnosis.

The authors state that they have no Conflict of Interest (COI).

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