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【 CASE REPORT 】

Pulmonary *Mycobacterium parascrofulaceum* Infection in a Patient with Chronic Progressive Pulmonary Aspergillosis: A Case Report and Literature Review

Hiroaki Ikegami[1], Kei Yamasaki[1], Toshinori Kawanami[1], Kazumasa Fukuda[2], Kentaro Akata[1], Midori Nakamura[1], Issei Ikushima[1], Yoko Fukuda[1], Shingo Noguchi[1] and Kazuhiro Yatera[1]

Abstract:

A 67-year-old man with a pulmonary cavity was admitted to our hospital. Mycobacterial culture of the bronchoalveolar lavage fluid sample obtained from the right upper pulmonary lesion tested positive for mycobacterium, and sequencing of the 16S rRNA genes, *hsp65*, and *rpoB* revealed that the cultured mycobacterium was *Mycobacterium parascrofulaceum*. Treatment with antimycobacterial agents was ineffective, and repeated culturing of bronchoscopic specimens revealed that the specimens were positive for *Aspergillus fumigatus*. Combination treatment of antimycobacterial agents and voriconazole improved the lung lesion. This is the first report of a patient with pulmonary *M. parascrofulaceum* infection complicated with chronic progressive pulmonary aspergillosis.

Key words: nontuberculous mycobacterium, *Mycobacterium parascrofulaceum*, chronic progressive pulmonary aspergillosis

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Introduction

The prevalence of pulmonary nontuberculous mycobacterial infections has been increasing in Japan, and the annual incidence rate per 100,000 person-years has increased from 4.7 in 2007 to 14.7 in 2017 (1). To date, 166 Mycobacterial types have been reported to be pathogenic to humans. Among these, the most common mycobacterial species are *Mycobacterium avium-intracellulare* complex (MAC), accounting for approximately 80% of mycobacterial isolates, followed by *M. chelonae/abscessus*, *M. fortuitum*, and *M. kansasii* (2).

M. parascrofulaceum, which belongs to Runyon group II (scotochromogens), was first reported in 2004 by Turenne et al. (3) and was observed in only 1 of the 4,069 clinical isolates in an epidemiological study (4). The precise identification of mycobacterial species is important because the clinical courses and treatments vary according to the species. To date, only a few cases of *M. parascrofulaceum* infection have been reported (3-11), and the infection has not yet been clinically characterized completely.

We herein report the case of a patient with pulmonary *M. parascrofulaceum* infection complicated with chronic progressive pulmonary aspergillosis (CPPA) and review previously reported cases of pulmonary *M. parascrofulaceum* infection.

Case Report

A 67-year-old Japanese man was referred to our hospital in July 2016 for the examination of a pulmonary cavitary lesion that appeared adjacent to a pulmonary consolidation in his right upper lung. He had a history of pulmonary tuberculosis that had been treated with typical anti-tuberculosis agents 20 years earlier. He had smoked previously (76 pack-years) and had suffered from chronic heart failure for 8 years after a myocardial infarction. Chest computed to-

[1]Department of Respiratory Medicine, University of Occupational and Environmental Health, Japan and [2]Department of Microbiology, University of Occupational and Environmental Health, Japan
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Correspondence to Dr. Kei Yamasaki, yamasaki@med.uoeh-u.ac.jp

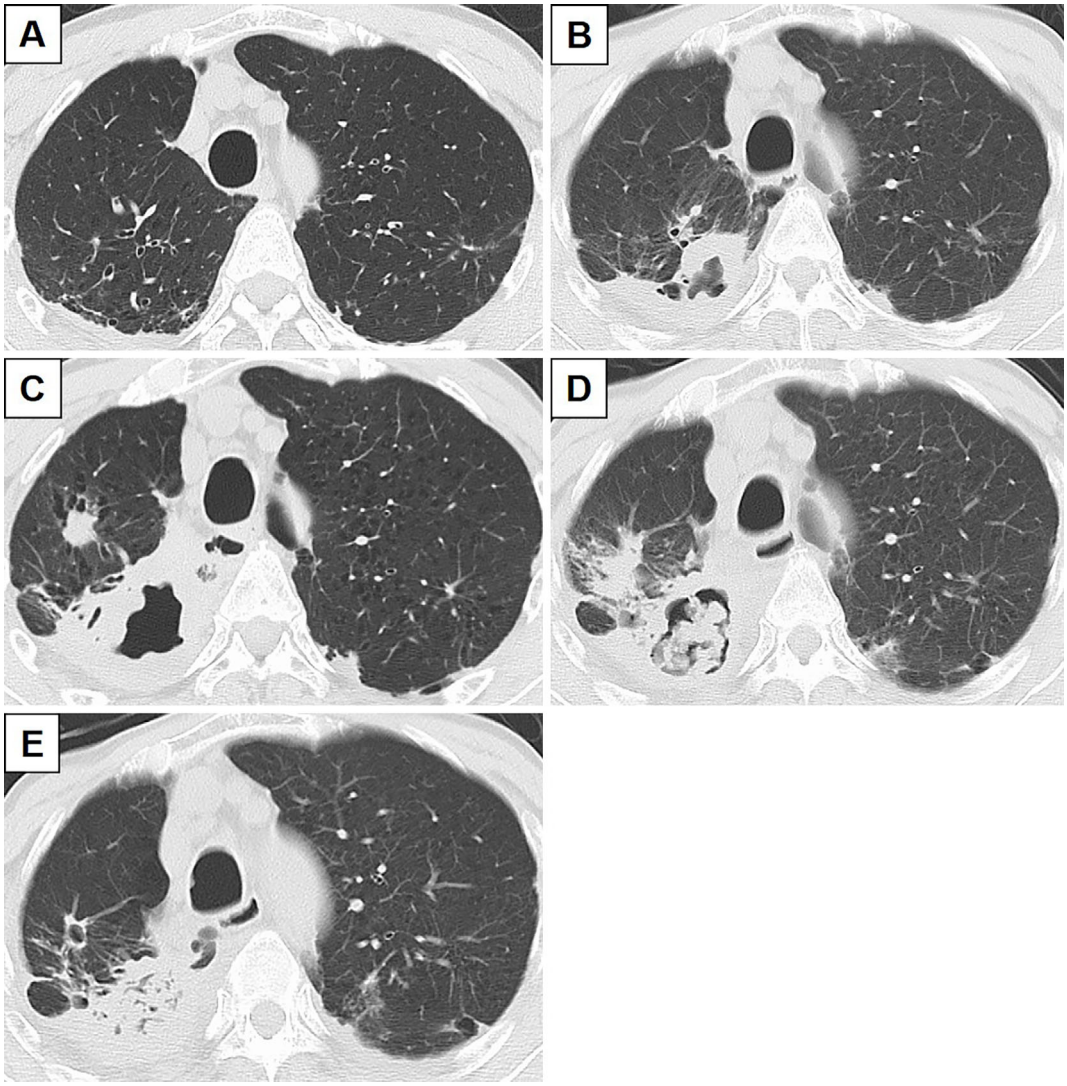

Figure 1. Chest computed tomography (CT). Chest CT 8 years before the patient's admission to our hospital showing a mild right subpleural consolidation lesion (A). Chest CT on July 2016 demonstrating a cavity and thicken peripheral lesion (B). After 9 months without antibiotic treatment, the cavity and thicken peripheral lesion were becoming larger and thicker (C). After 7 months of antimycobacterial treatment, the cavity and thick peripheral lesion had not improved, and a fungus ball appeared in the cavity (D). After 7 months of combination treatment, the fungus ball had almost disappeared.

mography (CT) revealed a consolidation in the right upper lung (S^2) (Fig. 1A) that had been unchanged for eight years. Although he was asymptomatic, he was referred to our hospital because the consolidation had worsened and a cavitary lesion had newly appeared adjacent to the consolidation.

Upon admission to our hospital, a physical examination revealed a height of 175 cm, body weight of 48 kg, body temperature of 37.5°C, heart rate of 92 bpm, blood pressure of 153/102 mmHg, and oxygen saturation of 95% (room air, rest). Chest auscultation demonstrated no abnormal findings in the lung fields. Laboratory findings on admission (Table 1) demonstrated low serum albumin levels and positive interferon-gamma releasing assay findings for *M. tuberculosis* (QuantiFeron TB Gold plus®; QIAGEN, Tokyo, Japan) and anti-MAC (anti-glycopeptidolipid core IgA) antibody (Capilia-MAC®; TAUNS, Izunokuni, Japan). A bronchoalveolar lavage fluid (BALF) sample was obtained from the right B^2 cavitary lesion, but no bacteria or fungi were found on culturing.

He was monitored with no medications for three months, and chest CT revealed that the cavity had grown and the consolidation worsened (Fig. 1B). A bronchoscopic examination was repeated, and the culture results of the BALF sample obtained from the same right B^2 cavitary lesion revealed the presence of oral bacteria and *Candida albicans*; however, acid-fast bacillus staining and qualitative real-time polymerase chain reaction (PCR) for *M. tuberculosis* and MAC were negative.

Four weeks after the second bronchoscopy, acid-fast bacilli were cultured from the BALF sample, but polymerase chain reaction (PCR) for *M. tuberculosis* and MAC, DNA-DNA hybridization (Kyokuto Pharmaceutical Industrial, To-

Table 1. Results of Peripheral Blood Analysis on Admission.

<Blood cell counts>		<Blood chemistry>		<Serology>	
WBC	5,600 /μL	TP	7.2 g/dL	CRP	0.26 mg/dL
Neutrophils	70.2 %	Alb	3.7 g/dL		
Lymphocytes	19.0 %	T-bil	0.3 mg/dL	CEA	2.6 ng/mL
Eosinophils	2.9 %	AST	20 IU/L	CYFRA	1.4 ng/mL
Monocytes	7.2 /μL	ALT	11 IU/L	Anti-MAC (Anti-glycopeptidolipid core IgA) antibody	(+)
Basophils	0.7 g/dL	LDH	205 IU/L	QFT (QuantiFeron Gold®)	(+)
RBC	371×10^4 /μL	ALP	359 IU/L	measurements A	2.68 IU/mL
Hb	12.5 g/dL	γ-GTP	28 IU/L	measurements M	>10 IU/mL
Ht	36.2 %	BUN	9 mg/dL	β-D-glucan	<6.0 pq/mL
Platelets	22.9×10^4 /μL	Cre	0.68 mg/dL	*Aspergillus*antigen	<4
				*Cryptococcus neoformans*antigen	(-)

WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, Ht: hematocrit, TP: total protein, Alb: albumin, T-bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, γ-GTP: gamma-glutamyl transferase, BUN: blood urea nitrogen, Cre: creatinine, CRP: C-reactive protein, CEA: carcinoembryonic antigen, CYFRA: cytokeratin 19 fragment

kyo, Japan), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker, Billerica, USA) of the cultured mycobacterial colony did not identify the pathogen (the score values of 0.00-1.59 were categorized as "no organism identification possible," the score value of *M. parascrofulaceum* 9032280 MVDb by MALDI-TOF MS was 1.24, indicating that the pathogen was unidentifiable).

For definitive mycobacterial species identification, we sequenced the 16S ribosomal RNA (rRNA) gene (Accession number LC487228). The comparison of the obtained 16S rRNA sequences with those in the publicly available database using the basic local alignment search tool (BLAST) revealed that the cultured isolate had 99.69% similarity (1,280/1,284 bp) with *M. parascrofulaceum* ATCC BAA-614 (NCBI Reference Sequence: NR_117220.1). A phylogenetic tree based on the 16S rRNA sequences of mycobacteria type strains also showed that the isolate was located near *M. parascrofulaceum* (Fig. 2) (12). For the further identification of the isolate, we sequenced the *hsp65* and *rpoB* genes. A comparison of the sequences of the *hsp65* and *rpoB* genes revealed 100% (439/439 bp) and 99.43% (350/352 bp) similarity with those in the *M. parascrofulaceum* ATCC BAA-614, respectively, using the BLAST. According to these findings, the patient was diagnosed with pulmonary NTM caused by *M. parascrofulaceum*. The detected mycobacteria were sensitive to RFP, levofloxacin, streptomycin, kanamycin, cycloserine, and enviomycin; however, the susceptibility to CAM and newly developed quinolones aside from levofloxacin was not tested.

The patient was asymptomatic and did not wish to begin antimycobacterial treatment until his chest CT findings worsened (Fig. 1C) (August 2017). As the imaging findings worsened, however, so did his condition, with the gradual exacerbation of his chronic productive cough. No standard treatment against *M. parascrofulaceum* has been established; therefore, MAC treatment agents, such as rifampicin (RFP), ethambutol (EB), and clarithromycin (CAM), were used for the treatment from August 2017. After treatment initiation, his cough and sputum ameliorated temporarily, and repeated mycobacterial smear tests showed positive findings, but no mycobacteria were cultured. Therefore, antimycobacterial treatments were partly effective against *M. parascrofulaceum*. Subsequently, the thickened wall of the cavitary lesion slightly thinned, but a fungus ball newly appeared in March 2018 (Fig. 1D). A BALF sample was obtained from the cavitary lesion, and culture results revealed the presence of *Aspergillus fumigatus* but not of NTM species. In addition, a serum anti-*Aspergillus* antibody test, which had been negative when performed in 2016, became positive, and the patient was diagnosed with CPPA.

Combination treatment of antimycobacterial agents and voriconazole was initiated, and chest CT taken in October 2018 revealed improvement in the cavity and fungus ball (Fig. 1E). After initiating voriconazole, RFP was discontinued because of its drug interactions, and we treated him with the other two drugs.

Discussion

The current case of our patient who experienced a coinfection of *M. parascrofulaceum* and *A. fumigatus* is the first such reported case. *M. parascrofulaceum*, which belongs to a group of pigmented NTM, was first reported in 2004 (3) and formerly called "MCRO 33" (GeneBank accession no. AF152559). It phenotypically resembles *M. simiae*; however, its most close genotypical relative is *M. scrofulaceum* (3). Although most *M. parascrofulaceum* isolates are reported from human samples, this bacillus was also reportedly detected in the hot springs at Yellowstone National Park (6). Similar to ordinary mycobacteria, *M. parascrofulaceum* may be able to survive in water, wet soil, house dust, and vegetation as well as in highly acidic environments and high temperatures (up to 56°C).

The pathogenesis of *M. parascrofulaceum* in humans is still unclear, and so far, only 10 cases of NTM caused by

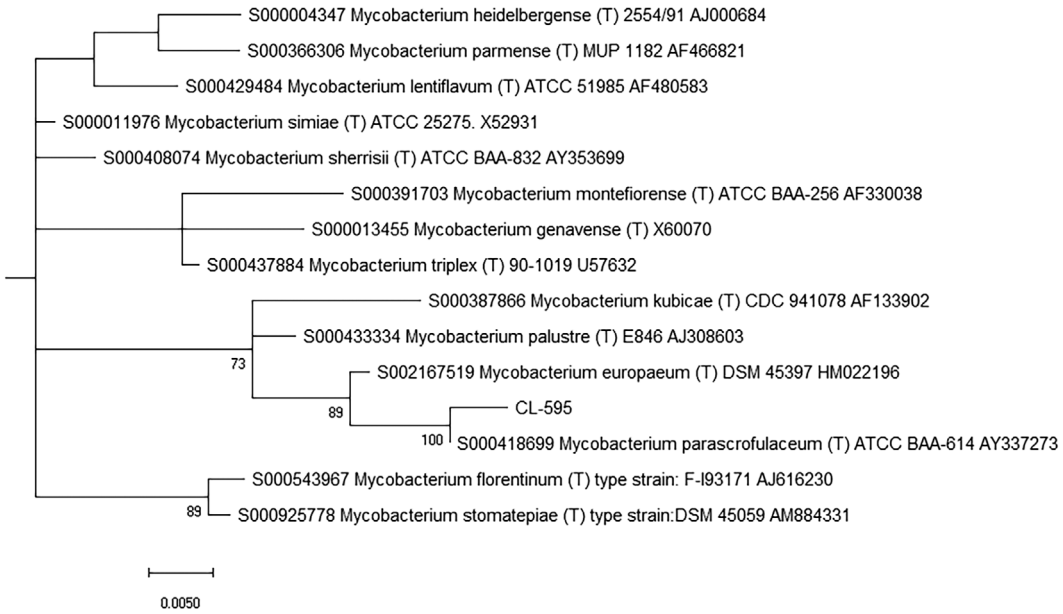

Figure 2. Phylogenetic analysis based on 16S rRNA sequences. The 16S rRNA gene sequences of our case, of similar Mycobacterial type strains (166 sequences) and of *Corynebacterium diphtheriae* were aligned using MUSCLE with the default settings. A sequence of *C. diphtheriae* was used as an outgroup. Using the resulting 1,353-position alignment, a phylogenetic tree was constructed using the maximum likelihood (ML) method with MEGA X software (12). The ML tree was constructed using the General Time Reversible model selected with the "Find Best DNA/Protein Models (ML)" tool and proportions of invariable sites and with 1,000 bootstrap replicates (values less than 70% were ignored). Our case was CL-595, and it was located next to the type strain of *Mycobacterium parascrofulaceum*. This figure was a cutaway of CL-595 and some of the bacteria around it.

M. parascrofulaceum have been reported, including that of our patient (Table 2). The lung is the most commonly infected area in patients with NTM infection, and the infection was found in the lungs in 7 of the 10 reported cases of *M. parascrofulaceum* infections. The other cases involved infection to the genital system (9) and the skin (10) (Table 2). Most reported patients had underlying diseases, such as acquired immune deficiency syndrome, old pulmonary tuberculosis, or chronic obstructive pulmonary disease. Among all of the patients, five had abnormal chest radiographic findings: three had a cavity, one had a consolidation, and one had lymphadenopathy (Table 2). With respect to radiographical findings or clinical symptoms, the clinical characteristic findings of *M. parascrofulaceum* infection are not distinguishable from those of other NTM infections. However, an extrapulmonary disease caused by *M. parascrofulaceum* manifests with skin rashes and diarrhea, depending on the infected site and the patient's underlying condition.

The microbiological criteria for the American Thoracic Society/Infectious Disease Society of America NTM definition (13) are generally used to diagnose pulmonary NTM infection, and a diagnosis requires the fulfillment of at least one of the following criteria: (a) positive culture results for at least two separate expectorated sputum samples, (b) positive culture results for one bronchial wash/lavage sample, and (c) positive findings on a transbronchial or other lung biopsy specimen with histopathological features of mycobacteria and one or more sputum or bronchial washing cultures positive for NTM. The BALF sample of our patient was culturally positive for mycobacteria, based on which the patient was diagnosed with pulmonary NTM. However, the identification of *M. parascrofulaceum* was challenging because of its genotypical closeness to *M. scrofulaceum*. To identify the mycobacterial species, molecular biological methods, a sequencing analysis of highly conserved genes (16S rRNA, *hsp65*, *rpoB*), and MALDI-TOF MS were used. MALDI-TOF MS was not useful in this patient because the resulting score values were low. This may be due to the fact that the data of this species have not yet been registered. In the phylogenetic tree constructed using 16S rRNA, the isolate was highly homologous to *M. parascrofulaceum*. Furthermore, in the tree diagram using 166 types of standard strains of *Mycobacterium*, the isolate was closest to *M. parascrofulaceum* and the sequences of *hsp65* and *rpoB* genes were also similar to that of *M. parascrofulaceum*; therefore, the patient was diagnosed with pulmonary *M. parascrofulaceum* infection.

Antimycobacterial agents are widely used for NTM treatment. CAM, RFP, and newly developed quinolones (especially moxifloxacin) are reportedly considered to be key drugs for pulmonary *M. parascrofulaceum* infection treatment (4, 7, 10). There have been no established treatments for *M. parascrofulaceum*. Therefore, after referencing previous reports, we treated him with the following agents used

Table 2. **Reported Cases of *Mycobacterium parascrofulaceum*.**

Case	Age (y)/ sex	Specimens isolated	Site	Comorbidity	Symptoms	X-ray	Treatment	Outcome
1	41/F	Sputum	Lung	Old TB	Cough	Cavity	CAM, EB, RFP	Improved
2	35/M	Sputum	Lung	AIDS	Chorea, fever, diarrhea	NA	EB, RFP	Died 1 month later
3	40/M	Blood	NA	AIDS	Fever	NA	Antimycobacterial drugs	Died 6 months later
4	67/M	Sputum	Lung	COPD carcinoma	NA	NA	NA	NA
5	63/M	Bronchial aspiration	Bronchus	Bronchiectasis	NA	Cavity	INH, EB, RFP	Died 4 months later
6	34/M	Lung lesion	Lung	AIDS (PCP)	NA	Lymphadenopathy	Operation and HAART	No recurrence
7	38/F	Vaginal discharge	Genital system	None	Lower abdominal pain	NA	Hysterectomy	NA
8	42/F	Skin	Skin	None	NA	NA	CAM, MFLX → CAM, AMK, EB	Improved
9	65/M	Sputum	Lung	Bronchiectasis	Hemosputum	Consolidation	NA	NA
Present case	67/M	BALF	Lung	Old TB	None	Cavity	CAM, EB, RFP	Worse (complication with CPPA)

HIV: human immunodeficiency virus, TB: tuberculosis, CAM: clarithromycin, EB: ethambutol, RFP: rifampicin, AIDS: acquired immune deficiency syndrome, NA: not analyzed, COPD: chronic obstructive pulmonary disease, INH: isoniazid, PCP: pneumocystis pneumonia, HAART: highly active anti-retroviral therapy, MFLX: moxifloxacin, AMK: amikacin, BALF: bronchoalveolar lavage fluid, CPPA: chronic progressive pulmonary aspergillosis

for treating MAC disease: CAM, RFP, and EB, despite the susceptibility test for *M. parascrofulaceum* showing no susceptibility to EB.

Based on the Japanese Domestic Guideline for Management of Deep-seated Mycoses 2014 (14), the patient's chest CT findings, the elevated levels of inflammatory markers (e.g., CRP), and the inefficacy of antibiotic treatments, we diagnosed the patient with clinical CPPA in March 2018. NTM is reportedly associated with the development of CPPA that includes chronic pulmonary aspergillosis (CPA) and chronic necrotizing pulmonary aspergillosis (CNPA). In a previous study by Jhun et al., 32 of 70 (45.7%) patients with CPA had previous or concurrent NTM diseases (16). Among patients with NTM, known risk factors for the development of CPA include old age, male gender, a low body mass index (<18.5 kg/m^2), chronic obstructive lung disease, systemic corticosteroids, *M. abscessus* complex (including *M. abscessus* and *M. massiliense*) as the etiologic organism, and a radiographically fibrocavitary form (17). Of these factors, our patient was male and had a low body mass index, chronic obstructive lung disease, and fibrocavitary form. Whether or not *M. parascrofulaceum* is a significant risk factor for CPPA is unclear; thus, the further accumulation of clinical information regarding this mycobacterial infection is necessary in order to elucidate its clinical significance in patients with CPPA.

The average duration from the diagnosis of pulmonary MAC disease to the diagnosis of CNPA is reportedly 36.0 months (18-72 months) (14). Similarly, the duration from the diagnosis of pulmonary *M. parascrofulaceum* to the diagnosis of CPPA was 16 months in our patient. The presence of coexisting CPPA is associated with mortality in patients with NTM; therefore, a regular evaluation is desirable.

This is the first report of a case involving coinfection of pulmonary *M. parascrofulaceum* and CPPA that was successfully treated with a combination of antimycobacterial and antifungal agents. Molecular methods targeting the 16S rRNA genes, *hsp65*, and *rpoB* were used in the diagnosis of this patient. The clinical characteristics of *M. parascrofulaceum* infection and those of coinfection with fungal infections should be elucidated in the future to ensure the early and precise diagnosis and appropriate and timely treatment.

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

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