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

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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-
mography (CT) revealed a consolidation in the right upper lung (S2) (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, Shizuoka, Japan). A bronchoalveolar lavage fluid (BALF) sample was obtained from the right B2 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 B2 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 IN-
DUSTRIAL CO., LTD., Tokyo, Japan), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; BRUKER, Billerica, MA, 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 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 (1280/1284 bp) with M. parascrofulaceum 9032280 MVDb by MALDI-TOF MS was 1.24, indicating that the pathogen was unidentifiable.

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
M. parascrofulaceum was found in the lungs in 7 of the 10 reported cases of infected area in patients with NTM infection, and the infection and (c) positive findings on a transbronchial or other lung at least two separate expectorated sputum samples, (b) positive findings 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 (12) are generally used to diagnose pulmonary NTM infection. The other cases involved infections to the genital system (9) and the skin (10) (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 (12) 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 closest 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 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 (15). The ML tree was constructed using the General Time Reversible model selected with the “Find Best DNA/Protein Models (ML)” tool and proportions of invariant sites and with 1000 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.
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 (13), 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/m²), 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 antymycobacterial 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).

### 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    | NA    | CAM, EB  | Improved|
| 2    | 35/M    |     | Sputum            | Lung | AIDS        | NA       | 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      | Cavity   | CAM, EB, RFP | NA |

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, CPA: chronic progressive pulmonary aspergillosis

References

1. Namkoong H, Kurashima A, Morimoto K, et al. Epidemiology of Pulmonary Nontuberculous Mycobacterial Disease, Japan. Emerg Infect Dis 22 (6): 1116-1117, 2016.
2. Prevots DR, Shaw PA, Strickland D, et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. Am J Respir Crit Care Med 182 (7): 970-976, 2010.
3. Turenne CY, Cook VJ, Burdz TV, et al. *Mycobacterium parascrofulaceum* sp. nov., novel slowly growing, scotochromogenic clinical isolates related to *Mycobacterium simiae*. Int J Syst Evol Micro-
4. Liu G, Wang GR, Yu X, et al. Bacteriological characterization of a Mycobacterium parascrofulaceum strain isolated from a Chinese pneumonia patient. Int J Infect Dis 25: 82-87, 2014.
5. Tortoli E, Chianura L, Fabbro L, et al. Infections due to the newly described species Mycobacterium parascrofulaceum. J Clin Microbiol 43 (8): 4286-4287, 2005.
6. Santos R, Fernandes J, Fernandes N, Oliveira F, Cadete M. Mycobacterium parascrofulaceum in acidic hot springs in Yellowstone National Park. Appl Environ Microbiol 73 (15): 5071-5073, 2007.
7. Teruya H, Tateyama M, Hibiya K, et al. Pulmonary Mycobacterium parascrofulaceum infection as an immune reconstitution inflammatory syndrome in an AIDS patient. Intern Med 49 (16): 1817-1821, 2010.
8. Hibiya K, Tateyama M, Teruya H, et al. Immunopathological characteristics of immune reconstitution inflammatory syndrome caused by Mycobacterium parascrofulaceum infection in a patient with AIDS. Pathol Res Pract 207 (4): 262-270, 2011.
9. Shojaei H, Hashemi A, Heidarieh P, Daei-Naser A. Chronic pelvic pain due to Mycobacterium parascrofulaceum in an Iranian patient: first report of isolation and molecular characterization from Asia. Braz J Infect Dis 15 (2): 186-187, 2011.
10. Zong W, Zhang X, Wang H, et al. The first case of cutaneous infection with Mycobacterium parascrofulaceum. Ther Clin Risk Manag 8: 353-358, 2012.
11. Kim KB, Park SG, Park JS, et al. First Case of Pulmonary Mycobacterium parascrofulaceum Infection in a Patient With Bronchiectasis in Korea. Ann Lab Med 35 (3): 379-381, 2015.
12. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 175 (4): 367-416, 2007.
13. Izumikawa K, Tashiro T, Tashiro M, et al. Pathogenesis and clinical features of chronic pulmonary aspergillosis - is it possible to distinguish CNPA and CCPA clinically? J Infect Chemother 20 (3): 208-212, 2014.
14. Kobashi Y, Fukuda M, Yoshida K, Miyashita N, Niki Y, Oka M. Chronic necrotizing pulmonary aspergillosis as a complication of pulmonary Mycobacterium avium complex disease. Respirology 11 (6): 809-813, 2006.
15. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 35 (6): 1547-1549, 2018.

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