Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.2391-18
Intern Med 58: 2675-2678, 2019
http://internmed.jp

【 CASE REPORT 】

Pulmonary *Mycobacterium abscessus* Subspecies *abscessus* Disease That Showed a Discrepancy Between the Genotype and Phenotype of Clarithromycin Resistance

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Abstract:
Mycobacterium abscessus subspecies *abscessus* is major subspecies in the *M. abscessus* complex and is usually refractory to standard antibiotherapy. Genetic tracing of *erm*(41) T28 is a mechanism for monitoring macrolide resistance. We treated a patient with a pulmonary infection caused by *M. abscessus* subsp. *abscessus* with the *erm*(41) T28 polymorphism, which was susceptible to clarithromycin, and his clinical treatment course was good. The identification of the *M. abscessus* complex genotype is important, but clinical confirmation of clarithromycin susceptibility is also needed to plan individual treatment strategies.

Key words: *Mycobacterium abscessus*, inducible resistance, *erm*(41)

(Intern Med 58: 2675-2678, 2019)
(DOI: 10.2169/internalmedicine.2391-18)

Introduction

The prevalence of non-tuberculous mycobacteria (NTM) infection has been increasing worldwide (1-3). *Mycobacterium abscessus* complex belongs is a member of the rapidly growing mycobacteria (RGM) group among NTM, and the frequency of RGM differs among regions; for example, it is 3% in Japan (4) and 5% in Australia (5). However, in Korea, the frequency is 33%, which is the second highest frequency after that of *Mycobacterium avium* complex (MAC) (6).

From a clinical aspect, the importance of this species is that it is often refractory to antibacterial treatment. In recent years, *M. abscessus* complex has been classified into *M. abscessus* subsp. *abscessus* (*M. abscessus*), *M. abscessus* subsp. *massiliense* (*M. massiliense*), and *M. abscessus* subsp. *bolletii* (*M. bolletii*) (7, 8). The *Mycobacterium abscessus* complex has acquired resistance by point mutations in the *rrl* gene at positions 2,057-2,059 (9, 10). In addition, *M. abscessus* and *M. bolletii* have inducible resistance to macrolide, which is induced by *erm*(41), whereas *M. massiliense* has a dysfunctional *erm*(41) due to two characteristic deletions and is susceptible to macrolides (11, 12). The response rate to antibiotic therapy including clarithromycin (CAM) was much higher in patients with pulmonary *M. massiliense* disease than in those with pulmonary *M. abscessus* disease due to the function of *erm*(41). Furthermore, *M. abscessus* strains harbor a T/C polymorphism at the 28th nucleotide in *erm*(41). T28 sequevar strains (Trp10 codon) demonstrate inducible CAM resistance, while C28 strains (Arg10 codon) are susceptible to CAM (13). Therefore, identification of *M. abscessus* complex subspecies and genetic typing of *erm*(41) have clinical value for predicting the efficacy of antibiotic therapy and developing appropriate treatment strategies.

We herein report the case of a 55-year-old man with pulmonary *M. abscessus* infection whose clinical course differed from that predicted based on subspecies identification and *erm*(41) typing.

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Received: November 15, 2018; Accepted: March 19, 2019; Advance Publication by J-STAGE: June 7, 2019
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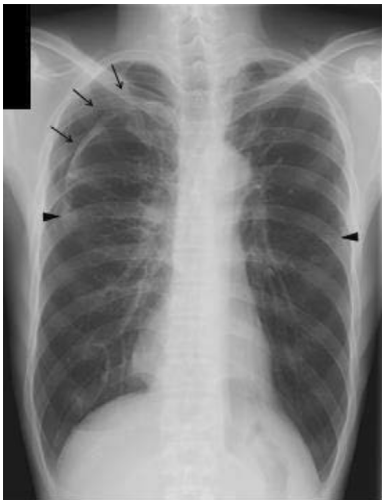

Figure 1. Chest X-ray on the first visit shows right pneumothorax (arrows) and granular shadows of both middle lung fields (arrowheads).

Case Report

A 55-year-old man visited our hospital because of right chest pain. The patient was an ex-smoker (40 pack-year history) and had no history of immunosuppressive treatment or malignant disease. He had no fever and oxygenation was within the normal range. Right pneumothorax and granular shadows of both middle lung fields were observed on chest X-ray (Fig. 1A). On chest computed tomography (CT), granular shadows and consolidation were observed in the middle lobe, lingula segment, and bilateral lower lobes. Additionally, peripheral consolidation with cavities was found in the lower left lobe (Fig. 2A and B). Right pneumothorax spontaneously improved without specific treatment, but pulmonary mycobacterial infection was suspected as a background disease due to his chest CT findings. Blood and serologic examinations revealed a slightly increased white blood cell count (7,950 cells/μL) but C-reactive protein was not increased (0.2 mg/mL). T-SPOT® TB (an interferon-gamma release assay) was negative and levels of IgA antibodies against the glycopeptidolipid core (anti-MAC antibody) were under the detection limit. Acid-fast bacilli were cultured from sputum and bronchial lavage fluid collected by bronchoscopy and both strains were identified as *M. abscessus* complex by the DNA-DNA hybridization method. We clinically diagnosed the patient with pulmonary infection of *M. abscessus* complex and administered antibiotherapy comprising imipenem/cilastatin (IPM/CS, 1,000 mg/day), amikacin (AMK, 400 mg/day), and CAM (800 mg/day). IPM/CS was switched to faropenem (FAPM, 600 mg/day) due to nausea as an adverse effect. AMK was substituted with levofloxacin [LVFX, 500 mg/day, orally (p.o.)] 1 month later. After three months of treatment, image findings (Fig. 2C and D) had improved, and sputum culture was negative.

We then performed subspecies identification and drug susceptibility testing (DST). The presence of acquired and inducible resistance was also examined. The isolates were identified using *rpoB* and *hsp65* gene sequencing, as described previously (14). *rrl* sequencing was performed as described by Rubio et al. (10). DST and *erm*(41) sequencing was performed as reported previously (13, 15). All tests were performed at Kinki-Chuo Chest Medical Center. Partial sequencing of the *rrl* gene of the isolates revealed no mutations at these positions. In contrast, the isolate was identified as *M. abscessus* by multigene sequencing, and a sequence analysis of the *erm*(41) gene revealed T28 sequevar with a full-length gene product. The sequencing of *erm*(41) sequevar types revealed mixed sequevar type 6 and 7, which those associated with an A238G substitution were primarily found to be functional, and such types usually demonstrate inducible resistance to CAM. However, DST indicated the strain was sensitive to CAM, and inducible CAM resistance was also not found (Table).

At the one-year follow-up, the patient has continued treatment comprising CAM, LVFX, and FAPM; sputum cultures remain negative,; image findings remain improved;, and recurrent infection has not been detected (Fig. 2E and F).

Discussion

We herein present a case of pulmonary *M. abscessus* disease caused by *M. abscessus* carrying *erm*(41) T28 sequevar. Although this genotype tends to suggest resistance to CAM (13), the strain was susceptible to CAM without inducible resistance and the patient showed a good clinical response to CAM-containing antimicrobial treatment. Therefore, the genotype of *erm*(41) T28 sequevar in pulmonary *M. abscessus* disease did not predict a poor clinical response in this case.

Recently, Yoshida et al. reported that a subset of *M. abscessus* isolates (9.5%) presented with genetically functional *erm*(41) but no phenotypic inducible resistance (15), as observed in the strain isolated from our patient. Previous reports that investigated the *erm*(41) sequevar classification may have failed to predict inducible resistance correctly (15, 16). These findings suggest the importance of carrying out DST for CAM during the development of treatment strategies for *M. abscessus* infection without relying solely on identification using a genetic approach.

The Clinical and Laboratory Standards Institute recommends that DST be performed with culturing at 30℃ and determined 3 days later using cation-adjusted Mueller-Hinton broth (pH 7.4) for RGM (17). If the strain is resistant on day 3, then resistance is caused by the *rrl* gene mutation. If the strain is judged to be sensitive to CAM on day 3, assessing the inducible resistance, which is associated with the *erm*(41) gene (18, 19), should be carried out using an additional extended culture with an assessment on day 14. In Japanese clinical practice, DST of NTM is usually performed using a BrothMIC NTM® kit with Middlebrook 7

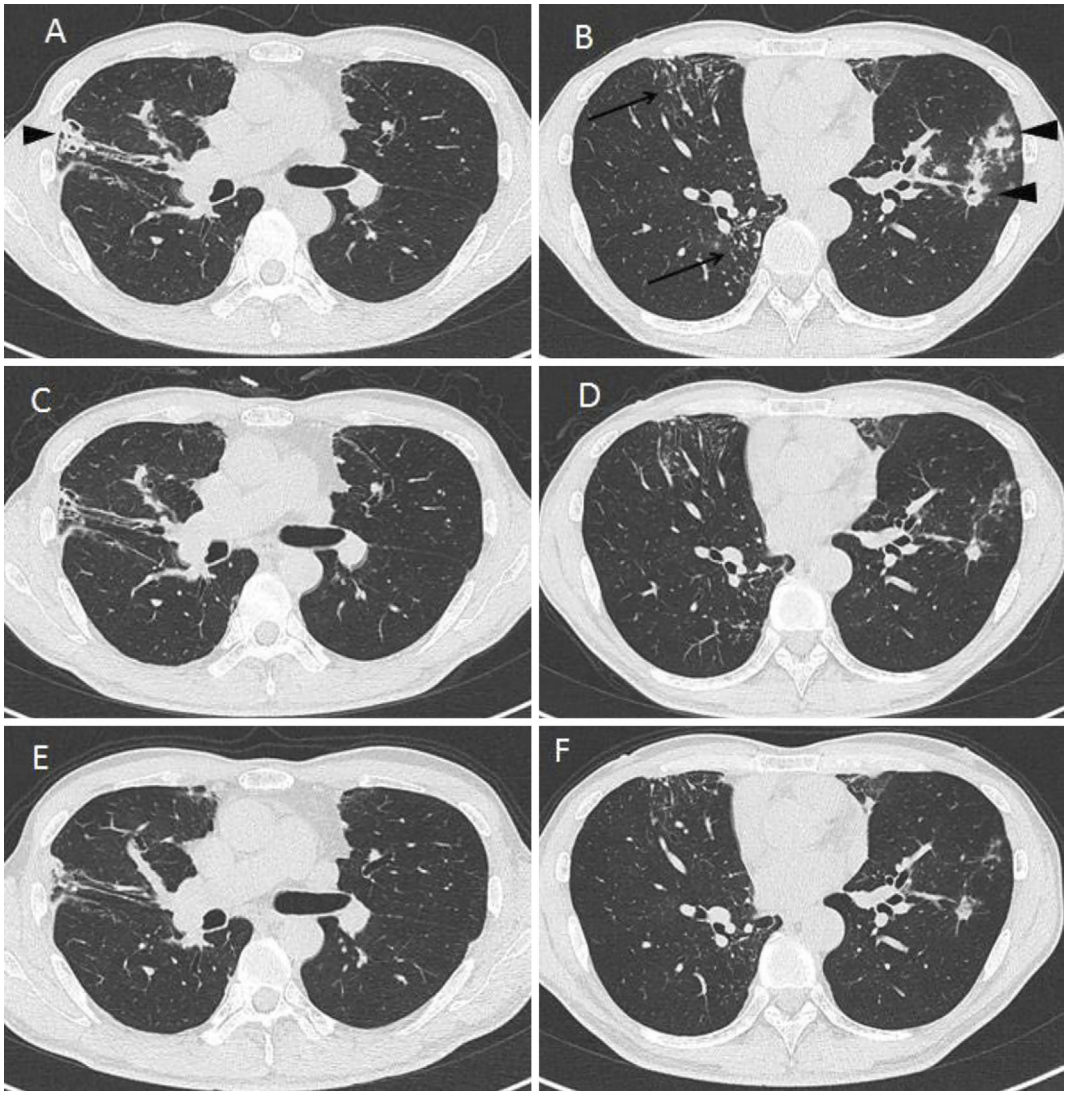

Figure 2. **Chest CT before treatment for *Mycobacterium abscessus* complex (A, B), 3 months after treatment (C, D), and 1 year after treatment (E, F). Before treatment, chest CT shows granular shadows in both lung fields (arrows) and consolidation with cavity lesions (arrowheads) are also found in both lung fields (A, B). Continual improvement was observed within 3 months (C, D) and maintained 1 year after treatment (E, F).**

Table. **CAM MIC Values on Days 3 and 14.**

Test method	Day 3	Day 14
CLSI[16]	1 μg/mL (S)	2 μg/mL (S)
BrothMIC NTM®	0.5 μg/mL (S)	8 μg/mL (R)

CLSI criteria of CAM susceptibility[16]
S: susceptible (MIC≤2 μg/mL), (2 μg/mL<MIC<8 μg/mL), R: resistant (MIC≥8 μg/mL)
CAM: clarithromycin, MIC: minimum inhibitory concentration, CLSI: Clinical and Laboratory Standards Institute, NTM: non-tuberculous mycobacteria

H9 (Kyokuto Pharmaceutical Industrial, Tokyo, Japan) as the liquid growth medium and culturing at 37℃. However, a BrothMIC NTM® kit is inadequate for RGM. We attempted DST using BrothMIC NTM® and obtained a different result at the late phase on day 14 compared to our findings using the recommended method (Table). In addition, sequencing of the *erm*(41) gene is a particularly important diagnostic tool for assessing the clarithromycin susceptibility in isolates of *M. abscessus* complex, although, genetic identification for *M. abscessus* complex is not performed in clinical *Mycobacterium* laboratories in Japan. The development of kits that can be used for RGM is therefore needed.

In the present case, *M. abscessus* infection was detected by the onset of pneumothorax. The frequency of pneumothorax complications is reported to be 4.1% in pulmonary NTM disease (20). Regarding the *M. abscessus* complex, some cases with complication of pneumothorax have been reported (21-23). In the present case, lung image findings revealed consolidation with cavities near the pleura, which is a possible cause of pneumothorax. If *M. abscessus* complex infection is misdiagnosed as MAC, inadequate treatment can result in a poor treatment course. Physicians should threfore

consider *M. abscessus* complex as a causative disease of pneumothorax.

In conclusion, we encountered a case of pulmonary *M. abscessus* infection in which the isolated strain showed discrepancies between the genotype and phenotype concerning CAM resistance. Identifying the *M. abscessus* complex subspecies and the *erm*(41) genotype is crucial; however, carrying out DST for CAM also has importance in properly treating this infection.

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

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