CASE REPORT

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

Yusuke Yamaba¹, Osamu Takakuwa¹,², Manami Saito¹, Daisuke Kawae¹, Misuzu Yoshihara¹, Yuta Mori¹, Eiji Kunii¹, Yutaka Ito³, Shiomi Yoshida⁴ and Kenji Akita¹

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.
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 from the right middle lobe, lingula segment, and bilateral lower lobes. Acid-fast bacilli were 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).

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°C 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                   | 1 μg/mL (S) | 2 μg/mL (S) |
| BrothMIC NTM®         | 0.5 μg/mL (S) | 8 μg/mL (R) |

CLSI criteria of CAM susceptibility:
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°C. 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 therefore...
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).**

**References**

1. Adjemian J, Olivier KN, Setz AE, Holland SM, Prevots DR. Prevalence of nontuberculous mycobacterial lung disease in U.S. Medicare beneficiaries. Am J Respir Crit Care Med **185**: 881-888, 2016.

2. Lai CC, Tan CK, Chou CH, et al. Increasing incidence of nontuberculous mycobacteria, Taiwan, 2000-2008. Emerg Infect Dis **16**: 294-296, 2010.

3. Moore JE, Kruipjaah ME, Ormerod LP, Drobniewski F, Abubakar I. Increasing reports of non-tuberculous mycobacteria in England, Wales and Northern Ireland, 1995-2006. BMC Public Health **10**: 612, 2012.

4. Morimoto K, Hasegawa N, Izumi K, et al. A Laboratory-based analysis of nontuberculous mycobacterial lung disease in Japan from 2012 to 2013. Ann Am Thorac Soc **14**: 49-56, 2017.

5. Thomson RM. Changing epidemiology of pulmonary nontuberculous mycobacteria infections. Emerg Infect Dis **16**: 1576-1583, 2010.

6. Koh WJ, Kwon OJ, Jeon K, et al. Clinical significance of nontuberculous mycobacteria isolated from respiratory specimens in Korea. Chest **129**: 341-348, 2006.

7. Tortoli E, Kohl TA, Brown-Elliott BA, et al. Emended description of *Mycobacterium abscessus*, *M. abscessus* subsp. *abscessus*, *Mycobacterium bolletii* and designation of *Mycobacterium abscessus* subsp. *bolletii* comb. nov. Int J Syst Evol Microbiol **66**: 4471-4479, 2016.

8. A’dekambi T, Burger P, Raoult D, Drancourt M. *rpoB* gene sequence-based characterization of emerging non-tuberculous mycobacteria with descriptions of *Mycobacterium bolletii* sp. nov., *Mycobacterium phocaciun* sp. nov. and *Mycobacterium auhagen* sp. nov. Int J Syst Evolution Microbiol **56**: 133-143, 2006.

9. Wallace RJ Jr, Meier A, Brown BA, et al. Genetic basis for clarithromycin resistance among isolates of *Mycobacterium chelonae* and *Mycobacterium abscessus*. Antimicrob Agents Chemother **40**: 1676-1681, 1996.

10. Rubio M, March F, Garrigó M, Moreno C, Español M, Coll P. Inducible and acquired clarithromycin resistance in the *Mycobacterium abscessus* complex. PLoS One **10**: e0140166, 2015.

11. Maurer FP, Castelberg C, Quiblier C, Böttger EC, Somoskői A. *Erm*(41)-dependent inducible resistance to azithromycin and clarithromycin in clinical isolates of *Mycobacterium abscessus*. J Antimicrob Chemother **69**: 1559-1563, 2014.

12. Nash KA, Brown-Elliott BA, Wallace RJ Jr. A novel gene, *erm* (41), confers inducible macrolide resistance to clinical isolates of *Mycoparacterium abscessus* but is absent from *Mycobacterium chelonae*. Antimicrob Agents Chemother **53**: 1367-1376, 2009.

13. Bastian S, Veziris N, Roux AL, et al. Assessment of clarithromycin susceptibility in strains belonging to the *Mycobacterium abscessus* group by *erm*(41) and *rrl* sequencing. Antimicrob Agents Chemother **55**: 775-781, 2011.

14. Harris K A, Kenna D T, Blauwendraat C, et al. Molecular fingerprinting of *Mycobacterium abscessus* strains in a cohort of pediatric cystic fibrosis patients. J Clin Microbiol **50**: 1758-1761, 2012.

15. Yoshida S, Tsuyuguchi K, Kobayashi T, et al. Discrepancies between the genotypes and phenotypes of clarithromycin-resistant *Mycobacterium abscessus* complex. Int J Tuberc Lung Dis **22**: 413-418, 2018.

16. Shallom SJ, Gardina PJ, Myers TG, et al. New rapid scheme for distinguishing the subspecies of the *Mycobacterium abscessus* group and identification of *Mycobacterium massiliense* with inducible clarithromycin resistance. J Clin Microbiol **51**: 2943-2949, 2013.

17. In: Susceptibility Testing of Mycobacterium, Nocardiae, and Other Aerobic Actinomycetes. Second Edition: approved standard M24-A2. Clinical and Laboratory Standards Institute. CLSI, Wayne, PA, USA, 2011.

18. Brown-Elliott BA, Nash KA, Wallace RJ Jr. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. Clin Microb Rev **25**: 545-582, 2012.

19. Choi GE, Shin SJ, Won CJ, et al. Macrolide treatment for *Mycobacterium abscessus* and *Mycobacterium massiliense* infection and inducible resistance. Am J Respir Crit Care Med **186**: 917-925, 2012.

20. Kobashi Y, Mouri K, Obuse Y, Kato S, Oka M. Clinical analysis of patients with pulmonary nontuberculous mycobacterial disease complicated by pneumothorax. Intern Med **52**: 2511-2515, 2013.

21. Ueyama M, Asakura T, Morimoto K, et al. Pneumothorax associated with nontuberculous mycobacteria: a retrospective study of 69 patients. Medicine **95**: e2426, 2016.

22. Pang YK, Ngewow YF, Wong YL, Liam CK. Mycobacterium abscessus - to treat or not to treat. Respiroil Case Reports I: 31-33, 2013.

23. Tomas JE, Taoka CR, Gibbs BT, Fraser SL. Fatal pulmonary *Mycobacterium abscessus* infection in a patient using etanercept. Hawaii Med J **65**: 12-15, 2006.

The Internal Medicine is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License. To view the details of this license, please visit (https://creativecommons.org/licenses/by-nc-nd/4.0/).