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Mycobacteriological characteristics and treatment outcomes in extrapulmonary *Mycobacterium abscessus* complex infections

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SUMMARY

Objectives: The differentiation between *Mycobacterium abscessus* subspecies *abscessus* (*M. abscessus*) and *Mycobacterium abscessus* subspecies *massiliense* (*M. massiliense*) and determination of the presence of inducible resistance to macrolide antibiotics are important factors in the management of patients with *Mycobacterium abscessus* complex (MABC) infections. Unlike pulmonary MABC infections, little information on extrapulmonary MABC infections is available.
Methods: The molecular identification of clinical isolates was performed, and the clinical characteristics and treatment outcomes of 20 consecutive patients with extrapulmonary MABC infections were assessed.
Results: *M. abscessus* and *M. massiliense* each caused 10 (50%) of the cases. Eight (80%) *M. abscessus* isolates that had inducible resistance to clarithromycin harbored an intact *erm*(41) gene of the T28 variant, whereas two (20%) *M. abscessus* isolates had the C28 *erm*(41) variant and were susceptible to clarithromycin. All *M. massiliense* isolates had a truncated *erm*(41) gene and were susceptible to clarithromycin. The drug susceptibility profiles other than clarithromycin were similar for the *M. abscessus* and *M. massiliense* isolates. Of the 20 patients, 17 (85%) showed a favorable outcome, including all patients with *M. massiliense* infection and 70% (7/10) of patients with *M. abscessus* infection. Favorable outcomes were associated with *M. massiliense* and *M. abscessus* isolates with a non-functional *erm*(41) gene ($p = 0.049$).
Conclusions: Precise species and subspecies identification and the determination of macrolide susceptibility are recommended for the optimal treatment of extrapulmonary MABC infections.

Introduction

Pulmonary and extrapulmonary infections caused by non-tuberculous mycobacteria (NTM) are increasingly reported worldwide (Prevots and Marras, 2015; Stout et al., 2016). The *Mycobacterium abscessus* complex (MABC) is the most important cause of NTM infections by rapidly growing mycobacteria (Griffith et al., 2007; Floto et al., 2016). Moreover, MABC is a highly drug-resistant pathogen and very difficult to treat (Kasperbauer and De Groote, 2015; Lee et al., 2015; Ryu et al., 2016).

The guidelines of the American Thoracic Society and Infectious Diseases Society of America recommend macrolide-based antibiotic therapy combined with intravenous amikacin and cefoxitin or imipenem, based on the results of drug susceptibility testing (DST), for the treatment of MABC infections (Griffith et al., 2007). However, the guidelines recommend different approaches for the treatment of extrapulmonary and pulmonary MABC infections (Griffith et al., 2007). For extrapulmonary MABC infections, such as skin, soft tissue, and bone infections, a total of 4–6 months of antibiotic therapy with at least 2 weeks of an initial combination of parenteral antibiotics is recommended, with a high likelihood of

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cure (Griffith et al., 2007). However, pulmonary MABC disease is regarded as a chronic, incurable infection for most patients, even with the recommended 2–4 months of parenteral antibiotics followed by long-term macrolide-based antibiotic therapy (Griffith et al., 2007). The exact reasons for these different treatment outcomes are largely unknown.

Currently, MABC can be divided into three subspecies: *M. abscessus* subspecies *abscessus* (hereafter *M. abscessus*), *M. abscessus* subspecies *massiliense* (hereafter *M. massiliense*), and *M. abscessus* subspecies *bolletii* (hereafter *M. bolletii*) (Tortoli et al., 2016). *M. abscessus* is the most common pathogen, causing 45–65% of MABC cases, followed by *M. massiliense* (20–55%) and *M. bolletii* (1–18%); the treatment outcomes of patients with pulmonary MABC infections differ according to the etiologic organism (Koh et al., 2014).

The treatment response rates to macrolide-based antibiotic therapy are much higher in patients with pulmonary *M. massiliense* infections than in those with pulmonary *M. abscessus* infections (Koh et al., 2011; Lyu et al., 2014; Koh et al., 2016; Koh et al., 2017). This difference is likely due to the presence of a functional ribosomal methyltransferase gene, *erm*(41), in *M. abscessus* that results in inducible macrolide resistance (susceptible on day 3 but resistant on day 14 of DST). In contrast, the *erm*(41) gene in *M. massiliense* is non-functional due to truncation, so inducible resistance does not occur (Nash et al., 2009; Choi et al., 2012; Brown-Elliott et al., 2015). In addition, previous studies have demonstrated that 7–18% of *M. abscessus* clinical isolates have a T → C polymorphism at nucleotide 28 of the *erm*(41) gene, which inactivates the gene, and as these isolates are susceptible to macrolides (Brown-Elliott et al., 2015; Bastian et al., 2011; Yoshida et al., 2013; Lee et al., 2014; Shallom et al., 2015), macrolides can be useful for treating *M. abscessus* infections caused by the C28 sequevar (Koh et al., 2017). However, information on the responsiveness of extrapulmonary MABC infections to macrolide treatment and the relevance of the different sequevars is very limited.

Because of the high cure rate compared to pulmonary infections, it was hypothesized that a substantial proportion of extrapulmonary MABC infections are caused by *M. massiliense* or the C28 sequevar of *M. abscessus*. The aim of this study was to determine the proportions of M. massiliense and the M. abscessus C28 sequevar among extrapulmonary MABC infections and to examine treatment outcomes of these infections based on causative organisms.

Materials and methods

Study population

The medical records of 22 consecutive patients with extrapulmonary MABC infections treated at Samsung Medical Center (a 1979-bed referral hospital in Seoul, South Korea) from October 2010 to December 2014 were identified using an electronic database. These medical records were reviewed. After the exclusion of two patients who were transferred to another hospital or lost to follow-up during treatment, 20 patients were included in the study. This retrospective study was approved by the Institutional Review Board of Samsung Medical Center (IRB No. 2016-03-050). Informed consent was waived for the use of medical data because patient information was anonymized and de-identified prior to analysis.

Microbiological examination

Acid-fast bacillus (AFB) smears and cultures were performed using standard methods, as described previously (Koh et al., 2016). During the study period, NTM species were identified using a reverse blot hybridization assay of the *rpoB* gene (REBA Myco-ID test; YD Diagnostics, Yongin, South Korea) (Wang et al., 2014). For precise species identification of the isolates, multilocus sequencing analysis of the nearly complete 16S rRNA gene, the 16S–23S rRNA internal transcribed spacer (ITS) sequences, partial *rpoB* sequences, and partial *hsp65* sequences was performed (Ben Salah et al., 2008; Frothingham and Wilson, 1993; Turenne et al., 2001; Adekambi et al., 2003). Detection of *erm*(41) and mutations in the 23S rRNA gene (*rrl*) was performed by PCR sequencing, as described previously (Bastian et al., 2011; Jamal et al., 2000). DST was performed by broth microdilution method, and *Mycobacterium peregrinum* ATCC 700686 was used for quality control, in accordance with the guidelines of the Korean Institute of Tuberculosis (Clinical Laboratory Standards Institute, 2011). The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration capable of inhibiting visible growth of the mycobacteria. The MIC_{50} and MIC_{90} were defined as the concentrations required to inhibit the growth of 50% and 90% of the strains, respectively. The MIC of clarithromycin was determined on days 3 and 14 after incubation; MABC isolates were considered susceptible (MIC $\leq$2 μg/ml on days 3 and 14), resistant (MIC $\geq$8 μg/ml on day 3), or inducibly resistant (susceptible on day 3, but resistant on day 14) to clarithromycin (Clinical Laboratory Standards Institute, 2011).

Antibiotic therapy and treatment outcomes

Treatment regimens and durations for extrapulmonary MABC infections were not standardized but were determined by the attending physician in the institution during the study period. Patients with mild disease received oral or topical antibiotics, such as macrolides (clarithromycin 1000 mg/day or azithromycin 250 mg/day) and fluoroquinolones (moxifloxacin 400 mg/day or ciprofloxacin 500 mg/day), in the outpatient clinic. Patients with severe disease were hospitalized and received intravenous amikacin (10–15 mg/kg/day) combined with cefoxitin (6–12 g/day) or imipenem (2000 mg/day) for several weeks, together with oral antibiotics. Surgical treatment was performed during antibiotic treatment based on discussions among the attending physicians and surgeons. Favorable outcomes were defined as the resolution of clinical symptoms and site lesions after antibiotic treatment and/or initial surgical treatment. Unfavorable treatment outcomes were defined as hospital readmission for the second surgical treatment due to clinical and radiographic deterioration during antibiotic therapy that included at least 2 weeks of combination parenteral antibiotic therapy.

Statistical analysis

All data are presented as the median value and range for continuous variables and as the number and percentage for categorical variables. Data were compared using the Mann–Whitney *U*-test and Kruskal–Wallis test for continuous variables and using the Pearson Chi-square test or Fisher's exact test for categorical variables. A two-sided *p*-value of $<$0.05 was considered statistically significant for all analyses. All analyses were performed using IBM SPSS version 23 statistical software (IBM Corp., Armonk, NY, USA).

Results

Clinical characteristics of patients

A total of 20 patients with extrapulmonary MABC infections were included in the study. Baseline characteristics of the patients