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Mycobacterial Characteristics and Treatment Outcomes in *Mycobacterium abscessus* Lung Disease

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Background. Treatment outcomes of patients with *Mycobacterium abscessus* subspecies *abscessus* lung disease are poor, and the microbial characteristics associated with treatment outcomes have not been studied systematically. The purpose of this study was to identify associations between microbial characteristics and treatment outcomes in patients with *M. abscessus* lung disease.

Methods. Sixty-seven consecutive patients with *M. abscessus* lung disease undergoing antibiotic treatment for ≥12 months between January 2002 and December 2012 were included. Morphotypic and genetic analyses were performed on isolates from 44 patients.

Results. Final sputum conversion to culture negative occurred in 34 (51%) patients. Compared to isolates from 24 patients with persistently positive cultures, pretreatment isolates from 20 patients with final negative conversion were more likely to exhibit smooth colonies (9/20, 45% vs 2/24, 8%; $P = .020$), susceptibility to clarithromycin (7/20, 35% vs 1/24, 4%; $P = .015$), and be of the C28 sequevar with regard to the *erm*(41) gene (6/20, 30% vs 1/24, 4%; $P = .035$). *Mycobacterium abscessus* lung disease recurred in 5 (15%) patients after successful completion of antibiotic therapy. Genotypic analysis revealed that most episodes (22/24, 92%) of persistently positive cultures during antibiotic treatment and all cases of microbiologic recurrence after treatment completion were caused by different *M. abscessus* genotypes within a patient.

Conclusions. Precise identification to the subspecies level and analysis of mycobacterial characteristics could help predict treatment outcomes in patients with *M. abscessus* lung disease. Treatment failures and recurrences are frequently associated with multiple genotypes, suggesting reinfection.

Clinical Trials Registration. NCT00970801.

Keywords. nontuberculous mycobacteria; *Mycobacterium abscessus*; macrolides; lung disease.

The incidence and prevalence of lung disease due to nontuberculous mycobacteria (NTM) is increasing worldwide [1, 2]. *Mycobacterium abscessus* complex is the most important source of pulmonary infections caused by rapidly growing mycobacteria in patients with chronic lung diseases, such as bronchiectasis and cystic fibrosis [3, 4]. Although their taxonomic status remains controversial, the *M. abscessus* complex is currently divided into 3 subspecies: *M. abscessus* subspecies *abscessus*, hereafter referred to as *M. abscessus* (Mab); *M. abscessus* subspecies *massiliense* (*M. massiliense*); and *M. abscessus* subspecies *bolletii* (*M. bolletii*). Mab is the most common pathogen (45%–65%) of the complex, followed by *M. massiliense* (20%–55%) and *M. bolletii* (1%–18%), with treatment outcomes differing according to the etiologic organism [5].

Mab is a highly drug-resistant mycobacterial pathogen. For treatment of *M. abscessus* lung disease, current guidelines recommend macrolide-based antibiotic therapy combined with intravenous amikacin with cefoxitin or imipenem, based on results of drug susceptibility testing (DST) [3]. However, sputum culture conversion rates are only 25%–42% [6, 7], and recurrence rates are high, even after successful treatment completion [7]. These poor treatment outcomes are attributable to inducible macrolide resistance (susceptible at day 3 but resistant at day 14 of DST) conferred by the *M. abscessus* ribosomal methyl transferase gene *erm*(41) [6, 8, 9]. However, a polymorphism (T or C) occurs at position 28 of *erm*(41), and isolates of the C28 sequevar are usually susceptible to macrolides because of loss of function of *erm*(41) [9]. In addition to inducible macrolide resistance, acquired macrolide resistance can develop during antibiotic treatment due to mutations in the 23S rRNA gene (*rrl*) [9].

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Although a relationship between treatment outcome and various Mab characteristics, such as morphotype, susceptibility to macrolides, and the T28C substitution in the *erm*(41) gene, has been suggested [10], such an association has not yet been studied systematically in Mab lung disease. The purpose of this study was to evaluate the relationship between mycobacterial characteristics and treatment outcomes, including recurrence after treatment completion, in patients with Mab lung disease.

METHODS

Study Population

Through the NTM registry, a dataset of an ongoing, prospective, observational cohort study of NTM lung disease, we identified 167 consecutive patients with Mab lung disease diagnosed between January 2002 and December 2012 at Samsung Medical Center (a 1979-bed referral hospital in Seoul, South Korea). The Samsung Medical Center Institutional Review Board approved the study protocol, and all patients met the diagnostic criteria for NTM lung disease [3]. After excluding patients who did not undergo antibiotic treatment (n = 74), who were transferred to our institution after ≥1 month of antibiotic treatment (n = 21), and those with a total duration of antibiotic therapy <12 months (n = 5), 67 patients with Mab lung disease who received antibiotic therapy for ≥12 months were included in the study (Figure 1).

Antibiotic Treatment

As reported previously [6, 11], patients were hospitalized for 4 weeks and received an oral macrolide (clarithromycin or azithromycin), fluoroquinolone (ciprofloxacin or moxifloxacin), and doxycycline, together with an initial 4-week course of amikacin and cefoxitin. If an adverse reaction associated with cefoxitin occurred, such as leukopenia, imipenem was substituted. Oral doxycycline and fluoroquinolone were excluded from the treatment protocol beginning in February 2008 and March 2011, respectively, because of the high resistance rates of Mab to these drugs in vitro [6, 11]. After discharge, patients took an oral regimen (macrolide and/or fluoroquinolone and/or doxycycline) for at least 12 months after sputum culture conversion.

Sputum examinations were performed at 1, 3, and 6 months after initiation of antibiotic treatment and then at 2- to 3-month intervals during treatment. Sputum conversion was defined as 3 consecutive negative cultures, and the time of conversion was defined as the date of the first negative culture. A favorable response was defined as sputum

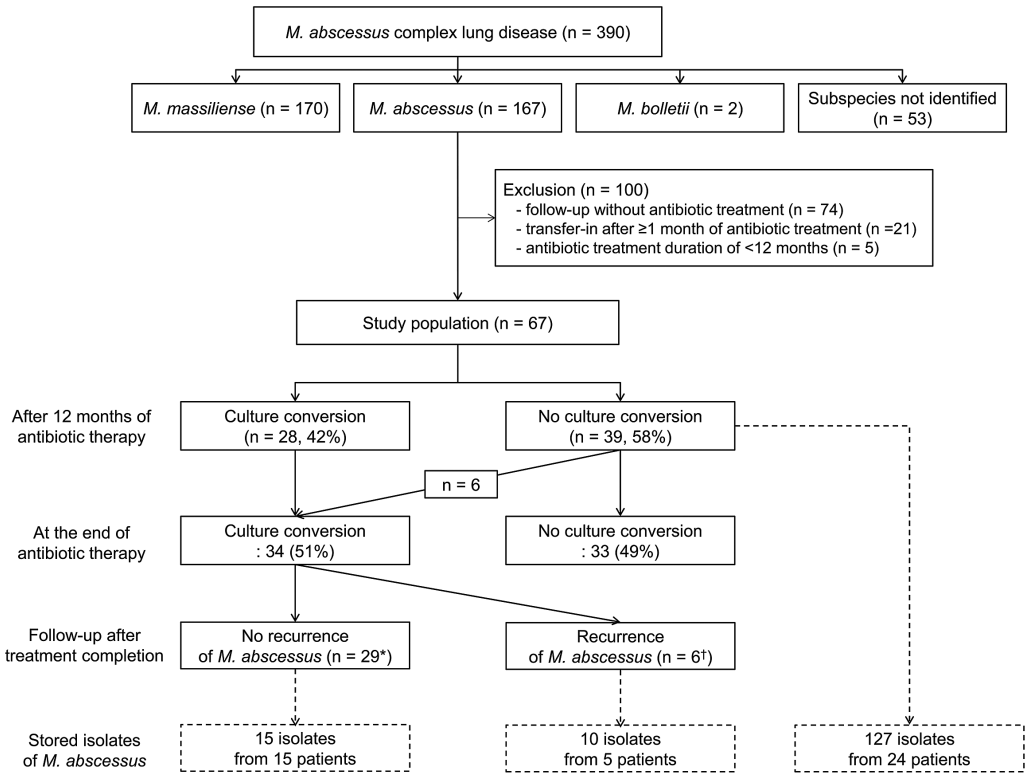

Figure 1. Study population. *, Mycobacterium avium complex lung disease developed in 9 patients (M. intracellulare in 6 and M. avium in 3) after the successful completion of treatment for M. abscessus lung disease. †, 1 patient had only 1 positive M. abscessus culture post-treatment.

culture conversion within 12 months after initiation of treatment and maintenance of a negative culture for 12 months or longer on treatment. An unfavorable response was defined as no sputum culture conversion. Microbiologic recurrence was defined as 2 or more Mab-positive cultures after treatment completion [12]. Some clinical data were included in previous studies [6, 11]. Final treatment outcomes, follow-up information including microbiologic recurrence, and laboratory examination of stored Mab isolates were included in the current study.

Radiographic and Microbiological Examinations

The fibrocavitary form (previously referred to as the upper lobe cavitary form) was defined by the presence of cavitary opacities and pleural thickening, mainly in the upper lobes. The nodular bronchiectatic form was defined by the presence of multifocal bronchiectasis and clusters of small nodules on chest high-resolution computed tomography (HRCT), regardless of the presence of small cavities in the lungs. Disease deemed "unclassifiable" did not meet the definition for either the fibrocavitary or nodular bronchiectatic form [13].

Sputum acid-fast bacilli (AFB) smears and mycobacterial cultures were performed according to standard methods [6, 11]. Clinical isolates were identified using polymerase chain reaction (PCR)–restriction fragment length polymorphism analysis of the *rpoB* gene or reverse-blot hybridization assay of *rpoB* [14, 15], followed by multilocus sequencing analysis of *rrs*, *hsp65*, and *rpoB* [16]. DST was performed using the broth microdilution method [17]. The minimal inhibitory concentration (MIC) of clarithromycin was determined on day 3 and day 14 after incubation; Mab isolates were considered susceptible (MIC ≤2 μg/mL at day 3 and day 14), resistant (MIC ≥8 μg/mL at day 3), or inducibly resistant (susceptible at day 3 but resistant at day 14) to clarithromycin [17].

For morphotype and genetic analyses, a single colony was obtained from stored Mab isolates, following propagation on Middlebrook 7H10 agar plates supplemented with 10% oleic acid-albumin-dextrose-catalase. Single colonies were classified macroscopically as smooth or rough; if isolates included colonies of both morphotypes, a colony of each morphotype was genetically analyzed separately. All single colonies were re-identified using multilocus sequence analysis [16]. Genetic analyses, such as the presence of *erm*(41) and mutations in the 23S rRNA gene (*rrl*), were performed by PCR sequencing as described previously [9]. Mycobacterial genotyping was performed using repetitive sequence-based PCR (rep-PCR), which was standardized according to the DiversiLab *Mycobacterium* kit protocol [18]. Rep-PCR reports were generated based on the Kullback-Leibler method, and isolates with identical profiles or >97% similarity were considered indistinguishable [19].

Statistical Analyses

The data are presented as numbers (percentages) for categorical variables and medians (interquartile range [IQR]) for continuous variables. The data were compared using Pearson χ^2 tests or Fisher exact tests for categorical variables and Mann-Whitney *U* test for continuous variables. The Kaplan-Meier method was used to estimate the cumulative rates of negative conversion and of microbiologic recurrence. All statistical analyses were performed using PASW (version 18.0, SPSS Inc., Chicago, Illinois), and a 2-sided $P < .05$ was considered significant.

RESULTS

Baseline Characteristics

The baseline characteristics of the patients are summarized in Table 1. The median age was 57 years (IQR, 48–64 years), and 52 (78%) were female. No patient tested positive for human immunodeficiency virus. Sixty (90%) patients had a positive AFB smear at the time of antibiotic therapy initiation. Chest HRCT scans were available for all patients. Cavities (either single or multiple) were visible on chest HRCT in 29 (43%) patients. Fifty-three (79%) patients had nodular bronchiectatic disease, 11 (16%) had fibrocavitary disease, and 3 (4%) had an unclassifiable form of Mab lung disease.

Treatment Outcomes

Among the initial oral antibiotics used, clarithromycin was the most commonly prescribed, administered to 40 (60%) patients, with azithromycin the second most common, administered to 15 (22%) patients. Fluoroquinolones and doxycycline were used for 42 (63%) and 17 (25%) patients, respectively. Surgical resection was performed in 9 (13%) patients (Table 2). A favorable response was achieved in 28 (42%) patients, and the median time of sputum conversion was 3.4 weeks (IQR, 1.9–9.4 weeks). Among the baseline patient characteristics, the incidence of an AFB-positive sputum smear at the time of antibiotic treatment initiation was lower in patients with a favorable response (21/28, 75%) compared with an unfavorable response (39/39, 100%; $P = .001$). Although surgical resection was performed in more patients with a favorable response (6/28, 21%) than an unfavorable response (3/39, 8%), the difference was not statistically significant ($P = .149$) (Table 2).

Of the 39 patients with an unfavorable response after 12 months of initial treatment, final negative sputum culture conversion was achieved in 6 (15%) patients by the end of treatment (Figure 1). Surgical resection had been performed more frequently in patients with later negative culture conversion (4/6, 67%) than in those who failed to achieve culture conversion by the end of treatment (5/33, 15%; $P = .018$).

During the initial 12 months of treatment, the cumulative rate of negative conversion with antibiotics alone was 34% (Figure 2). With a combination of surgical resection

Table 1. Baseline Characteristics of Patients According to Treatment Outcomes After 12 Months of Treatment

Characteristic	Total (n = 67)	Favorable Response[a] (n = 28)	Unfavorable Response[b] (n = 39)	*P* Value
Age, y	57 (48–64)	59 (47–65)	57 (48–63)	.859
Female	52 (78)	22 (79)	30 (77)	.873
Body mass index, kg/m^2	20.3 (18.5–22.0)	20.3 (18.7–22.0)	20.0 (18.2–22.5)	.698
Never smoker	58 (87)	23 (82)	35 (90)	.474
Underlying disease				
Previous tuberculosis treatment	45 (67)	17 (61)	28 (72)	.341
Previous nontuberculous mycobacteria treatment[c]	12 (18)	4 (14)	8 (21)	.512
Malignancy	7 (10)	4 (14)	3 (8)	.440
Diabetes mellitus	6 (9)	3 (11)	3 (8)	.688
Chronic liver disease	4 (6)	0	4 (10)	.134
Radiographic type				
Nodular bronchiectatic form	53 (79)	21 (75)	32 (82)	.707
Fibrocavitary form	11 (16)	5 (18)	6 (15)	
Unclassifiable form	3 (4)	2 (7)	1 (3)	
Cavity on chest high-resolution computed tomography	29 (43)	12 (43)	17 (44)	.952
Time interval between diagnosis and treatment initiation, mo	5.8 (1.5–13.4)	4.9 (1.4–9.5)	6.2 (1.5–15.6)	.412
Laboratory findings				
Positive sputum acid-fast bacilli smear	60 (90)	21 (75)	39 (100)	.001
Erythrocyte sedimentation rate, mm/h	44 (31–60)	41 (29–53)	48 (31–67)	.215
C-reactive protein, mg/dL	0.42 (0.13–1.10)	0.43 (0.17–0.73)	0.41 (0.13–1.43)	.633

Data are presented as medians (interquartile ranges) or numbers (%).

[a] Sputum culture conversion within 12 months after initiation of treatment and maintenance of a negative culture for 12 months or longer on treatment.

[b] No sputum culture conversion.

[c] The etiologic organisms were *Mycobacterium abscessus* (n = 7), *M. avium* complex (n = 2), and others (n =3).

and antibiotics during the initial 12 months of treatment, the cumulative rate of negative conversion increased to 42%. After the initial 12 months of treatment, the continuation of antibiotic treatment resulted in additional negative conversion in only a small proportion of patients (0% at 24 months and 8% at 36 months), whereas surgical resection led to negative conversions in more of these patients (11% at 24 months and 12% at 36 months). Finally, the cumulative rate of negative conversion at 36 months of treatment was 42% of patients who received antibiotic treatment alone and 54% of those who received combined surgical resection and antibiotics.

Table 2. Treatment of Patients and Treatment Outcomes After 12 Months of Initial Treatment

Treatment of Patients	Total (n = 67)	Favorable Response[a] (n = 28)	Unfavorable Response[b] (n = 39)	*P* Value
Duration of initial intravenous antibiotic treatment, mo	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	.429
Macrolide				
Clarithromycin	40 (60)	16 (57)	24 (62)	.105
Azithromycin	15 (22)	4 (14)	11 (28)	
Clarithromycin followed by azithromycin	12 (18)	8 (29)	4 (10)	
Fluoroquinolone	42 (63)	17 (61)	25 (64)	.777
Doxycycline	17 (25)	6 (21)	11 (28)	.530
Surgical resection	9 (13)	6 (21)	3 (8)	.149
Segmentectomy	1	0	1	
Lobectomy	6	5	1	
Lobectomy + segmentectomy	2	1	1	

Data are presented as medians (interquartile ranges) or as numbers (%).

[a] Sputum culture conversion within 12 months after initiation of treatment and maintenance of a negative culture for 12 months or longer on treatment.

[b] No sputum culture conversion.

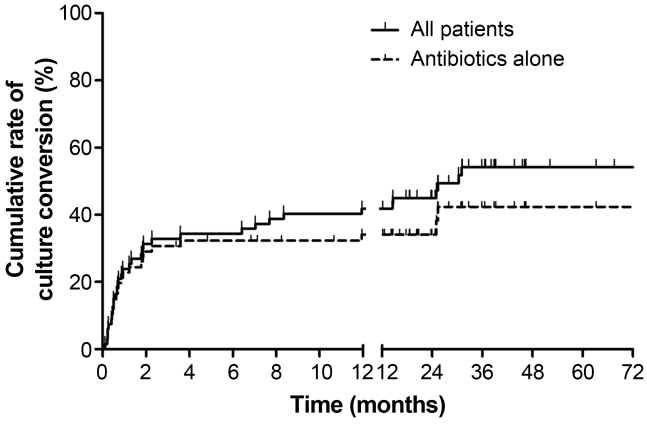

Figure 2. Cumulative sputum conversion rates in 67 patients with Mycobacterium abscessus lung disease. The solid line shows the cumulative conversion rate in patients until the end of antibiotic treatment. The dotted line shows the effects of antibiotic treatment alone on the cumulative conversion rate in the 67 patients. Data from the 18 surgically treated patients were censored at the time of surgery.

Table 3. Comparison of the Mycobacterial Characteristics of Pretreatment *Mycobacterium abscessus* Isolates According to Treatment Outcomes

Mycobacterial Characteristics	Patients With Final Negative Conversion (n = 20)	Patients With Persistently Positive Cultures (n = 24)	*P* Value
Initial morphotype			
Smooth	9 (45)	2 (8)	.020
Mixed (smooth + rough)	4 (20)	8 (33)	
Rough	7 (35)	14 (58)	
Initial susceptibility to clarithromycin			
Susceptible	7 (35)	1 (4)	.015
Inducible resistance	13 (65)	23 (96)	
Resistant	0	0	
Initial 28th sequevar of *erm*(41)			
C28	6 (30)	1 (4)	.035
T28	14 (70)[a]	23 (96)	
Initial *rrl* mutation			

Data are presented as medians (interquartile ranges) or numbers (%).

[a] An isolate from 1 patient had a C19→T point mutation in the *erm*(41) gene, and this isolate was susceptible to clarithromycin [16].

Morphotype and Genetic Analysis of Mab Isolates

Mab isolates from 44 patients (66% of the study population) had been stored and were available for further morphotypic and genetic analyses (Figure 1). Among the 39 patients who failed to achieve negative conversion after 12 months of treatment, 24 isolates obtained before treatment and 103 isolates obtained during treatment were available for 24 (62%) patients. The median number of isolates per patient was 5 (IQR, 3–7) during the median treatment period of 21 months (IQR, 13–40 months). Thirty-five single, "pretreatment" colonies and 129 single, "during-treatment" colonies were sampled from these isolates. Among the 34 patients with final negative conversion, 20 pretreatment isolates were available for 20 (59%) patients, and 24 single colonies were sampled from these isolates.

The mycobacterial characteristics of pretreatment Mab isolates, including colony morphotypes, susceptibility to clarithromycin, and sequence analysis of the *erm*(41) and *rrl* genes, were compared between patients with final negative conversion and those with persistently positive cultures; results are shown in Table 3 and in the Supplementary Material (Tables S2 and S3 and Figure S1). Compared to those from the 24 patients with persistently positive cultures, pretreatment Mab isolates from the 20 patients with final negative conversion more frequently exhibited the smooth colony morphotype (9/20, 45% vs 2/24, 8%; $P = .020$) and susceptibility to clarithromycin (7/20, 35% vs 1/24, 4%; $P = .015$). Seven isolates of the C28 sequevar for *erm*(41) were susceptible to clarithromycin and were more frequently obtained from patients with final negative conversion (6/20, 30%) than from patients with persistently positive cultures (1/24, 4%; $P = .035$).

Of the 24 patients with persistently positive cultures, clarithromycin resistance developed in only 3 (13%) after 4, 12, or 46 months of treatment, and this resistance correlated with mutation of the *rrl* gene in their Mab strains (Table S2). Mutation of *rrl* and clarithromycin resistance did not develop in patients with final negative conversion. Genotype analysis identified a median of 4.0 (IQR, 2.0–5.0) distinguishable rep-PCR profiles per patient, and 22/24 (92%) patients had polyclonal Mab isolates during treatment. There were no statistically significant differences in the number of polyclonal isolates according to disease type (Table S4).

Mab Lung Disease Recurrence After Successful Treatment Completion

Of the 34 patients who achieved final treatment success, 14 (41%) experienced a recurrence of NTM lung disease during the median follow-up period of 11.8 months (IQR, 3.6–27.0 months) after successful treatment for Mab lung disease, with the etiologic agents of recurrent disease including *M. avium* complex (MAC) in 9 patients (*M. intracellulare* in 6 and *M. avium* in 3 patients) and Mab in 5 patients. All patients met the diagnostic criteria for NTM lung disease, and the cumulative rates of NTM lung disease recurrence at 6, 12, and 24 months after completion of treatment for Mab lung disease were 22%, 33%, and 47%, respectively. The cumulative rates of Mab lung disease microbiologic recurrence, defined as 2 or more Mab-positive cultures after treatment completion, were 8%, 16%, and 22%, respectively, at 6, 12, and 24 months after treatment completion (Figure 3). In addition to 5 patients with microbiologic recurrence of Mab lung disease, 1 had only 1 positive Mab culture and consecutive negative sputum cultures during 18 months of follow-up after successful completion of therapy (Figure 1). Five isolates obtained before treatment and 5 isolates obtained after treatment completion were available for 5 (83%) of these 6 patients, and from each of these isolates, 6 single colonies were selected for genetic analysis. All recurrent

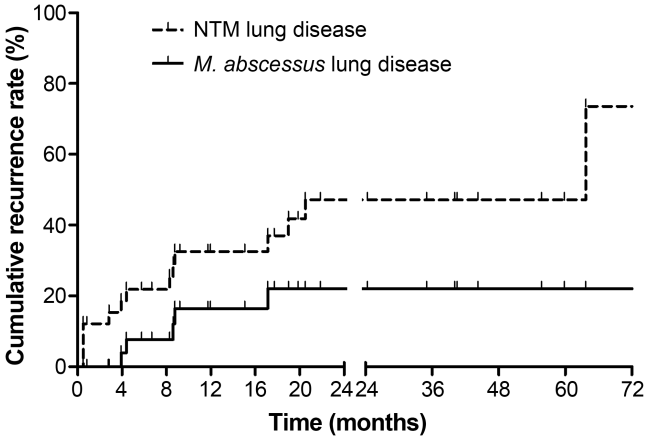

Figure 3. Cumulative rate of nontuberculous mycobacteria lung disease recurrence after successful treatment completion in 34 patients with Mycobacterium abscessus lung disease. Abbreviation: NTM, nontuberculous mycobacteria.

isolates contained the T28 sequevar of *erm*(41) and wild type *rrl* (Table S5). Rep-PCR profiles indicated that all recurrent Mab isolates had genotypes different from those of the original isolates (Figure S2).

DISCUSSION

This is the first study to comprehensively evaluate treatment outcomes in patients with Mab lung disease after subspecies differentiation. Previous studies did not differentiate subspecies within the *M. abscessus* complex [11, 20, 21] and did not include detailed information regarding mycobacterial characteristics [6, 7]. In our study, approximately 50% (34/67) of patients achieved final sputum culture conversion after multidrug antibiotic treatment and combined surgical resection. However, it was very difficult to predict treatment outcome at the time of initiation of antibiotic therapy because no clinical characteristics were associated with treatment outcomes except sputum AFB smear positivity.

Phenotypically, Mab manifests 2 distinct colony morphotypes: smooth and rough [22, 23]. The smooth morphotype initially colonizes airway surfaces and forms biofilms, with subsequent conversion to the rough morphotype believed to result in a more invasive and persistent infection [22, 23]. This is supported by case reports showing more chronic and invasive Mab lung disease with the rough morphotype [24, 25]. We found that the rough Mab morphotype was more refractory to antibiotic therapy than the smooth morphotype. Our findings suggest that morphotype distinction could help predict antibiotic response.

Mab has inducible macrolide resistance. This resistance is evidenced as susceptibility at day 3 of clarithromycin exposure but resistance at day 14 and is related to a functional *erm*(41) gene [8, 26]. However, 7%–18% of Mab clinical isolates have a T→C polymorphism at nucleotide 28 of *erm*(41). These isolates are susceptible to macrolides [27–31], suggesting that macrolides can be useful for treating patients with the C28 sequevar [29]. The present study clearly demonstrated that most patients with clarithromycin-susceptible Mab lung disease achieved treatment success (7/8, 88%), whereas the treatment success rate was 36% (13/36) in patients with isolates with inducible macrolide resistance. These results suggest that precise subspecies identification, macrolide susceptibility testing, and sequencing of *erm*(41) can predict treatment response in Mab lung disease.

Mab can also develop acquired macrolide resistance (resistant at day 3 of DST) through point mutations at positions 2058 and 2059 in the *rrl* gene, selected during macrolide-based antibiotic treatment [9, 32]. In our study, acquired macrolide resistance from *rrl* mutation was found in only 13% (3/24) of patients with persistently positive cultures during antibiotic therapy. Additionally, genotyping revealed that recurrent infections involved genotypes that differed from the initial Mab strain in most patients (22/24, 92%). Polyclonal infections during or after antibiotic therapy are well established in MAC lung disease [33, 34]. Multiple and repeated polyclonal MAC infections usually occur in patients with nodular bronchiectatic MAC lung disease, whereas individuals with the fibrocavitary form are usually infected with a single strain during or after antibiotic treatment [33, 34]. We found that repeated infections with different genotypes are common for both nodular bronchiectatic and fibrocavitary Mab lung disease. However, for cystic fibrosis patients with Mab lung disease, monoclonal infections are reportedly common [35].

Another important finding of our study was that all microbiologic recurrences of Mab lung disease were caused by different Mab genotypes. After treatment completion, 30%–50% of patients with MAC lung disease experienced microbiologic recurrence [36–39], mainly because of reinfection with MAC and particularly with nodular bronchiectatic disease [38]. Additionally, Mab lung disease can develop during or after treatment for MAC lung disease [40]. Our study revealed that NTM lung disease caused by different species, including MAC, can develop in patients during or after the completion of treatment for Mab lung disease.

There are several limitations to our study. First, this study was conducted at a single referral center. Because the proportion of clarithromycin-susceptible isolates (C28 sequevar) and treatment practices for Mab lung disease may vary by geographic region, some of our findings may not be generalizable. Second, Mab isolates were only available for two-thirds of the patients. Third, we selected only 1 or 2 colonies for genetic analysis, based on colony morphotype, from each stored isolate. Therefore, some relapses could be misclassified as reinfection if the patients had several genotypes before antibiotic treatment. Fourth, surgical resection was performed in 9 patients before 12 months of antibiotic therapy were completed. Finally, the microbiologic recurrence rate could have been underestimated because the follow-up duration after successful treatment completion was relatively short.

In conclusion, approximately 50% of patients with Mab lung disease achieved sputum culture conversion after combined antibiotic therapy and surgical resection. Smooth colony morphotype, macrolide susceptibility, and the T28C substitution in *erm*(41) were associated with favorable patient responses. Precise subspecies identification and analysis of mycobacterial characteristics may help predict treatment outcomes for Mab lung disease. In addition, genotypic analysis suggested that most episodes of persistently positive cultures during antibiotic treatment and all microbiologic recurrences after treatment completion were caused by Mab genotypes differing from the original genotype. Further research is needed to determine the source of repeated infections and to identify effective measures for reducing exposure to these difficult-to-treat pathogens in patients with Mab lung disease.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Author contributions. Study conception and design: W-J. K., B-H. J., S-Y. K.; data acquisition and analysis: W-J. K., B-H. J., S-Y. K.; experimental work: S-Y. K., K. U. P., H. J. H., C-S. K., N. Y. L., S-H. L., C. K. K., S. J. S.; data interpretation and manuscript writing: W-J. K., B-H. J., S-Y. K.; critical revision and final approval of the manuscript: W-J. K., B-H. J., S-Y. K., J. K., K. U. P., B. W. J., H. L., H. Y. P., D. H. K., H. J. H., C-S. K., N. Y. L., H. K. K., Y. S. C., J. K., S-H. L, S. J. S., C. K. K., C. L. D., H. K., O. J. K.

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