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의학석사 학위논문

Progression and Treatment Outcomes of Lung Disease Caused by *Mycobacterium abscessus* and *Mycobacterium massiliense*

Mycobacterium abscessus 폐 질환 및 *Mycobacterium massiliense* 폐 질환의 진행 및 치료 성적에 대한 비교 및 고찰

2017 년 2 월

서울대학교 대학원
임상의과학과
박 지 명

A thesis of the Degree of Master of Science

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Progression and Treatment Outcomes of Lung Disease Caused by *Mycobacterium abscessus* and *Mycobacterium massiliense*

February 2017

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ABSTRACT

Introduction: *Mycobacterium abscessus* and *Mycobacterium massiliense* are grouped as the *Mycobacterium abscessus* complex. Although *M. massiliense* lung diseases are reported to have better treatment response than *M. abscessus* lung diseases, clinical comparisons between these two species remain limited. The aim of this study was to elucidate the differences between *M. abscessus* lung diseases and *M. massiliense* lung diseases in terms of progression rate, treatment outcomes, and the predictors thereof.

Methods: Between January 1, 2006 and June 30, 2015, 56 patients and 54 patients were diagnosed with *M. abscessus* and *M. massiliense* lung diseases, respectively. The time to progression requiring treatment and treatment outcomes were compared between the two groups of patients, and predictors of progression and sustained culture conversion with treatment were analyzed. In addition, mediation analysis was performed to evaluate the effect of susceptibility to clarithromycin on treatment outcomes.

Results: During follow-up, 21 of 56 patients with *M. abscessus* lung diseases and 21 of 54 patients with *M. massiliense* lung diseases progressed requiring treatment. No difference was detected in the time to progression between the two patient groups. Lower body mass index, bilateral lung involvement, and fibrocavitary type disease were identified as predictors of disease progression. Among the patients who began treatment, infection with *M. massiliense* rather than *M. abscessus* and the use of azithromycin rather than clarithromycin were associated with sustained culture conversion, while lower body mass index was a negative predictive factor. The difference in treatment outcomes between these two species was partly mediated by the organism's susceptibility to clarithromycin.

Conclusions: Progression rates were similar but treatment outcomes differed significantly between patients with lung disease caused by *M. abscessus* and *M. massiliense*. This difference in treatment outcomes was partly explained by the susceptibility of these organisms to clarithromycin.

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Keywords: Nontuberculous mycobacteria, *Mycobacterium abscessus*, *Mycobacterium massiliense*, Disease progression, Treatment outcome

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INTRODUCTION

Nontuberculous mycobacteria (NTM) comprise mycobacterial species other than those of *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. Although determining the incidence and prevalence of NTM lung diseases remains challenging, recent epidemiologic reports indicate that the prevalence of NTM lung diseases is increasing, especially in many developed countries [1-4].

The *Mycobacterium abscessus* complex, one of the rapid growing NTM, is an important cause of chronic pulmonary infection [2, 3, 5]. Although there is a significant variability in geographic distribution, *M. abscessus* complex is the most common cause of pulmonary infection worldwide among the rapid growing NTM [6]. In South Korea, the *M. abscessus* complex is the second-most common pathogen responsible for NTM lung diseases after the *Mycobacterium avium* complex (MAC) [7]. Currently, the *M. abscessus* complex comprises three different species (*M. abscessus*, *M. massiliense*, and *M. bolletii*) based on gene sequence analysis.

Treatment outcomes for *M. abscessus* complex lung diseases vary significantly with culture conversion rates ranging from 40%

to 80% according to previous reports [8–10]. This variance in treatment outcomes may be due to the heterogeneity in treatment strategies employed and the different proportions of *M. abscessus* and *M. massiliense* in each study population [11]. *M. abscessus* is known to be more resistant to antibiotics [12] and to carry a worse prognosis compared with *M. massiliense* [13–16]. However, few studies have reported a detailed comparison of the clinical characteristics of lung disease caused by *M. abscessus* and *M. massiliense*.

The aim of this study was to elucidate the differences in progression rate, treatment outcomes, and the predictors thereof between *M. abscessus* lung diseases and *M. massiliense* lung diseases. In addition, we evaluated whether susceptibility to clarithromycin correlated with treatment outcomes.

MATERIALS AND METHODS

1. Study Population

We retrospectively reviewed the medical records of patients diagnosed as having a lung disease caused by *M. abscessus* complex at the Seoul National University Hospital between January 1, 2006 and June 30, 2015. We excluded patients with a follow-up period of less than 6 months. Patients who did not undergo chest computed tomography (CT) or who had malignant diseases at stage IV were also excluded. The protocol of this study was approved by the institutional review board of Seoul National University Hospital.

2. Microbiologic evaluation

Acid-fast bacilli (AFB) smear and mycobacterial cultures were performed as recommended in the standard guidelines [17, 18]. All cultures were grown in both solid Ogawa media and the BACTEC MGIT 960 system. NTM species were identified by gene sequence analysis of the 16S rRNA gene using the algorithm described in the Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. Sequencing of the rpoB and tuf

genes was performed for further identification. In particular, differentiation between *M. abscessus* and *M. massiliense* was performed based on analysis of the rpoB gene sequence [20, 21].

Antimycobacterial drug susceptibility tests were referred to the Korean Institute of Tuberculosis and performed using broth microdilution. Minimum inhibitory concentrations (MICs) of antibiotics (i.e. amikacin, cefoxitin, ciprofloxacin, clarithromycin, imipenem, moxifloxacin) were determined according to the CLSI guideline as well [22]. Determining the MIC of azithromycin is difficult due to its poor solubility at high concentrations, so instead the MIC of clarithromycin was determined as being representative of this class of macrolides [23]. Isolates were regarded as susceptible if the MIC of clarithromycin was 2 μg/mL or less, and as resistant if the MIC of clarithromycin was 8 μg/mL or greater. Isolates with an MIC between 2 and 8 μg/mL were regarded as having an intermediate level of resistance. Susceptibility to clarithromycin was evaluated after 3 and 14 days of incubation to detect resistance. If isolates were susceptible after 3 days of incubation and resistant after 14 days of incubation, they were considered to have inducible resistance to clarithromycin.

3. Radiographic evaluation

The CT scan images obtained at the time of diagnosis of *M. abscessus* complex lung disease were reviewed as the baseline CT in terms of radiographic extents (laterality and number of involved lobes) and disease patterns (i.e. either nodular bronchiectatic or fibrocavitary type) [14, 18]. Follow-up CT examinations were typically performed every 6 months to 1 year and as needed according to the decision of duty physician.

4. Diagnosis of NTM lung disease

Patients were diagnosed with *M. abscessus* complex lung diseases based on the diagnostic criteria of American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) guideline [18]. Because strains of both *M. abscessus* and *M. massiliense* were isolated in some patients, patients were classified into three groups: *M. abscessus* lung diseases, *M. massiliense* lung diseases, and *M. abscessus*/*M. massiliense* mixed lung diseases. Patients with *M. abscessus*/*M. massiliense* mixed lung diseases were defined as those who had more than two positive cultures for both *M. abscessus* and *M. massiliense*. Additionally, patients were considered to have co-infection with

NTM species other than *M. abscessus* complex if those NTM species were isolated at least twice.

5. Treatment protocol

Patients underwent follow-ups every 3 to 6 months and treatment was offered in cases of significant radiographic progression (i.e. new cavity formation), or worsening respiratory symptoms (i.e. development of hemoptysis). For this study, patients were defined as having disease progression if treatment was offered to them. Once patients had agreed a course of treatment, they were hospitalized and central catheters were peripherally inserted for long-term parenteral therapy. Patients received combination antibiotic therapy including one macrolide agent (clarithromycin or azithromycin) and at least two parenteral agents such as amikacin, cefoxitin, or imipenem.

Patients were hospitalized for at least three weeks. The decision whether to discharge was made by the duty physician, taking account of symptomatic and radiographic improvements. After discharge, macrolide treatment was continued with concurrent outpatient parenteral antibiotic therapy comprising amikacin three or five times a week via peripherally-inserted

central catheters. The duration of amikacin administration was not predefined and patients continued to receive amikacin until either clinical improvement or the development of significant adverse events was observed.

6. Treatment response assessment

Culture conversion was defined as the production of three consecutive negative cultures from sputum specimens. If patients could not expectorate sputum after one or two consecutive negative cultures, they were also regarded as having culture conversion. Final treatment responses were classified as either sustained culture conversion or as treatment failure. Treatment failure included failure of achieving culture conversion, or recurrence after initial culture conversion. If patients could not be followed-up before culture conversion, they were regarded as having treatment failure.

7. Statistical analysis

Data were summarized as means with standard deviations for continuous variables with normal distribution, or medians with interquartile range (IQR) for those with non-normal distribution.

For categorical variables, values were reported as frequencies and proportions. Differences between groups were analyzed using Student's t-test, Mann-Whitney test, Pearson's chi-square test, or Fisher's exact test, as appropriate. *P* values less than 0.05 were considered statistically significant in a two-tailed test.

Time to progression was compared between patients with *M. abscessus* and *M. massiliense* lung diseases using the Kaplan-Meier method. To elucidate risk factors for progression, multivariable logistic regression was performed. Among patients who started treatment, a comparison of the time to initial culture conversion between patients with *M. abscessus* and *M. massiliense* lung diseases was performed using the Kaplan-Meier method. Multivariable logistic regression was also used to determine predictors of sustained culture conversion. In both logistic regression models, variables with *P* values less than 0.1 in the univariable analysis were included in the multivariable analysis and adjusted odds ratios (aORs) with 95% confidence intervals (CI) were calculated.

In addition, we performed mediation analysis to evaluate whether susceptibility to clarithromycin could explain the

different treatment outcomes observed for *M. abscessus* and *M. massiliense* lung disease (Figure 1) [24]. Binary mediation with bootstrapping (500 replications) was used to estimate the direct effect (effect of the predictor on the outcome by itself) and the indirect effect (effect of the predictor on the outcome via the mediator) with CIs, as described in previous studies of similar design [25]. Where the CI of the indirect effect does not cross zero, such analysis can provide evidence of significant mediation. We also adjusted the model by including other variables significantly associated with sustained culture conversion as covariates. All the statistical analyses were carried out using STATA software version 14.0 (StataCorp LP. College Station, TX, USA).

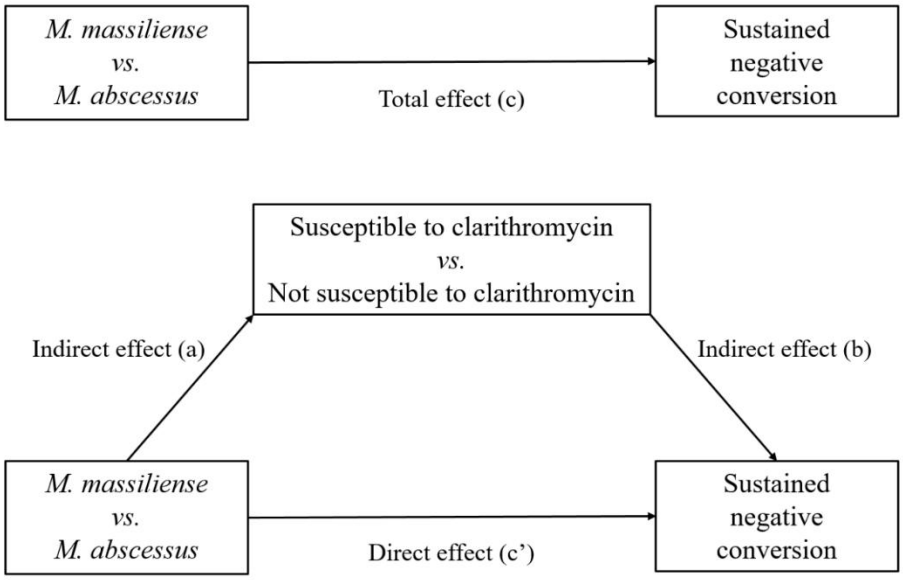

Figure 1. Mediation analysis for determining whether susceptibility to clarithromycin mediates the different treatment outcomes between *M. abscessus* and *M. massiliense* lung diseases.

This process was composed of a series of separate regression models to examine three effects: the effect of the NTM species (*M. abscessus* or *M. massiliense*) on sustained culture conversion (pathway c), the effect of the NTM species on susceptibility to clarithromycin (pathway a), and the effect of the NTM species on sustained culture conversion after accounting for susceptibility to clarithromycin as a mediator (pathways a–b and c').

RESULTS

1. Characteristics of patients

During the study period, a total of 143 patients were identified from whom *M. abscessus* complex had been isolated at least once. Thirty patients were excluded because they did not meet the ATS/IDSA criteria for NTM lung disease. The remaining 113 patients with *M. abscessus* complex lung diseases were classified into three predefined groups: 56 patients had *M. abscessus* lung diseases, 54 patients had *M. massiliense* lung diseases, and the remaining three patients had *M. abscessus*/*M. massiliense* mixed lung diseases. These three patients were not included in the main analyses of the current study. Subsequently, a total of 110 patients were analyzed (Figure 2).

The median age of patients with *M. abscessus* lung diseases and those with *M. massiliense* lung diseases was similar at 64 and 63 years, respectively (P = 0.990). The proportion of female patients was also similar (66.1% vs. 63.0%, respectively, P = 0.733). Less than one-fifth of patients (17.9% of *M. abscessus* and 18.5% of *M. massiliense*) showed a fibrocavitary pattern of disease. MAC was the most common species of co-infected

NTM (21 of 23 in the *M. abscessus* group and 15 of 17 in the *M. massiliense* group) (Table 1).

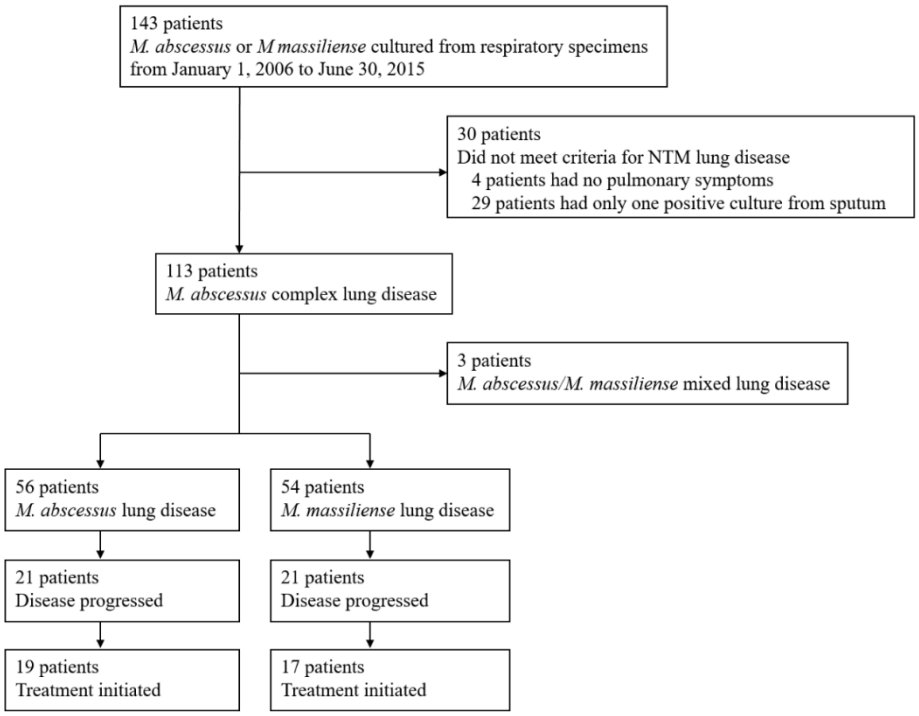

Figure 2. Selection of study patients.

Among 143 patients from whom *M. abscessus* complex had been isolated at least once during the study period, 113 patients met the ATS/IDSA criteria for NTM lung disease. They were classified into three predefined groups as follows: 56 patients with *M. abscessus* lung diseases, 54 patients with *M. massiliense* lung diseases, and three patients with *M. abscessus*/*M. massiliense* mixed lung diseases.

Table 1. Baseline characteristics of patients with *M. abscessus* and *M. massiliense* lung diseases

Characteristics	*M. abscessus* (N = 56)	*M. massiliense* (N = 54)	*P* value
Age, years	64 (57–70)	63 (52–71)	0.990
Female	37 (66.1%)	34 (63.0%)	0.733
BMI, kg/m^2	20.6 ± 2.6	20.8 ± 2.8	0.678
Ever−smoker	15 (26.8%)	13 (24.1%)	0.744
Medical history			
Tuberculosis	22 (39.3%)	26 (48.2%)	0.349
Diabetes	10 (17.9%)	3 (5.6%)	0.046
COPD	9 (16.1%)	9 (16.7%)	0.933
Asthma	3 (5.4%)	4 (7.4%)	0.714
CKD	6 (10.7%)	5 (9.3%)	0.799
Symptoms			
Sputum	56 (100.0%)	54 (100.0%)	
Cough	38 (67.9%)	35 (64.8%)	0.736
Hemoptysis	31 (55.4%)	16 (29.6%)	0.006
Radiographic feature			
Extent			
Bilateral involvement	46 (82.1%)	39 (72.2%)	0.215
No. of involved lobes	4 (3–5)	4 (2–4)	0.353
Disease pattern			0.928
Nodular bronchiectatic	46 (82.1%)	44 (81.5%)	
Fibrocavitary	10 (17.9%)	10 (18.5%)	
Initial AFB smear positivity	14 (25.0%)	12 (22.2%)	0.732
Co−infection with other NTM	23 (41.1%)	17 (31.5%)	0.296

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; AFB, acid−fast bacilli; NTM, nontuberculous mycobacteria

Data are shown as mean ± SD for normally distributed data, median (IQR) for non−normally distributed data, and as a number (%) for categorical variables.

2. *In vitro* drug susceptibility

The results of an in vitro antimicrobial susceptibility test were available for 95 patients (Table 2). A significant difference in the susceptibility to clarithromycin between *M. abscessus* and *M. massiliense* was identified ($P < 0.001$). Although 38 out of 46 (82.6%) strains of *M. massiliense* isolated were susceptible to clarithromycin, only 16 out of 49 (32.6%) strains of *M. abscessus* were susceptible. Inducible resistance was observed in 27 (55.1%) strains of *M. abscessus* isolated and in three (6.5%) strains of *M. massiliense*.

Table 2. *In vitro* drug susceptibilities of *M. abscessus* and *M. massiliense*

Drug	*M. abscessus* (N = 49)	*M. massiliense* (N = 46)	*P* value
Clarithromycin			
Susceptible, %	16 (32.6%)	38 (82.6%)	<0.001
Intermediate, %	2 (4.1%)	0 (0.0%)	
Resistant, %	4 (8.2%)	5 (10.9%)	
Inducible resistance, %	27 (55.1%)	3 (6.5%)	
MIC at day 3, μg/mL	0.5 (0.5–2.0)	0.5 (0.5–0.5)	0.003
MIC at day 14, μg/mL	64.0 (2.0–64.0)	0.5 (0.5–0.5)	<0.001
Amikacin			
Susceptible, %	40 (81.6%)	34 (73.9%)	0.053
Intermediate, %	4 (8.2%)	11 (23.9%)	
Resistant, %	5 (10.2%)	1 (2.2%)	
MIC, μg/mL	16 (16–16)	16 (16–32)	0.318
Cefoxitin			
Susceptible, %	7 (14.3%)	13 (28.3%)	0.248
Intermediate, %	36 (73.5%)	29 (63.0%)	
Resistant, %	6 (12.2%)	4 (8.7%)	
MIC, μg/mL	32 (32–64)	32 (16–64)	0.196
Imipenem			
Susceptible, %	6 (12.2%)	6 (13.0%)	1.000
Intermediate, %	34 (69.4%)	32 (69.6%)	
Resistant, %	9 (18.4%)	8 (17.4%)	
MIC, μg/mL	16 (8–16)	16 (8–16)	0.827
Moxifloxacin			
Susceptible, %	0 (0.0%)	1 (2.2%)	0.309
Intermediate, %	2 (4.1%)	4 (8.7%)	
Resistant, %	47 (95.9%)	41 (89.1%)	
MIC, μg/mL	8 (4–16)	8 (4–16)	0.524
Ciprofloxacin			
Susceptible, %	0 (0.0%)	0 (0.0%)	0.484
Intermediate, %	0 (0.0%)	1 (2.2%)	
Resistant, %	49 (100.0%)	45 (97.8%)	
MIC, μg/mL	16 (8–16)	16 (8–16)	0.782

Abbreviations: MIC, minimum inhibitory concentration

Data are shown as median (IQR) and number (%) as indicated.

Drug susceptibility tests were available for 49 of 56 (87.5%) patients from the *M. abscessus* group and for 46 of 54 (85.2%) patients from the *M. massiliense* group.

3. Disease progression rate

During the follow-up period (median 1,265 days; IQR 664-1,938), disease progression necessitated treatment of 21 of the 56 (37.5%) patients with *M. abscessus* lung diseases and 21 of the 54 (38.9%) patients with *M. massiliense* lung diseases (P = 0.881). There was no difference in time to progression between *M. abscessus* and *M. massiliense* lung diseases (Figure 3, P = 0.941). Lower body mass index (BMI) (aOR 4.8; 95% CI 1.4-16.5), bilateral lung involvement (aOR 3.8; 95% CI 1.1-13.8), and fibrocavitary type disease (aOR 3.6; 95% CI 1.1-12.8) were identified as risk factors for disease progression. The probability of disease progression did not differ between *M. abscessus* and *M. massiliense* lung diseases after adjusting for these factors (aOR 1.5; 95% CI 0.6-3.7; Table 3).

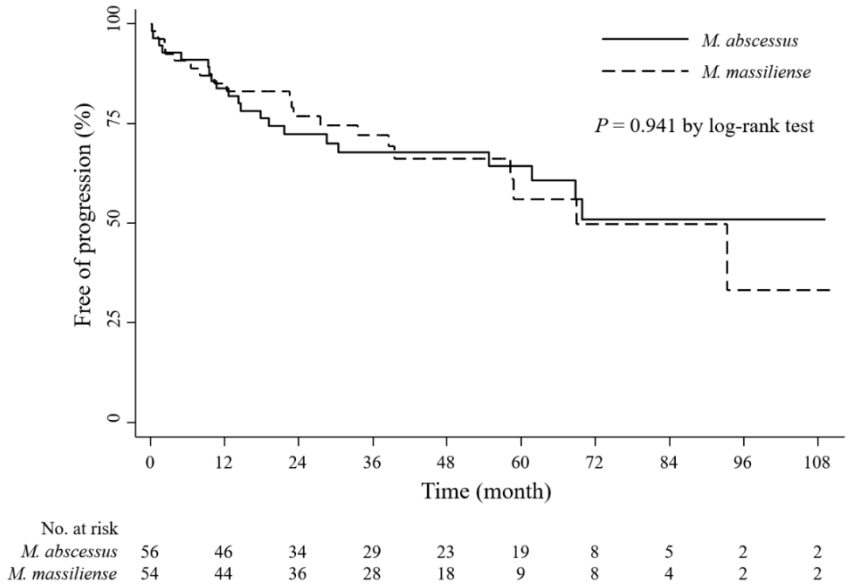

Figure 3. Kaplan–Meier analysis of the comparison of time to disease progression between *M. abscessus* and *M. massiliense* lung diseases.

There was no difference in time to progression between *M. abscessus* and *M. massiliense* lung diseases (P = 0.941 by log-rank test).

Table 3. Clinical characteristics associated with disease progression requiring treatment among 110 patients with *M. abscessus* or *M. massiliense* lung disease

Variables	Unadjusted		Adjusted	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Age				
≥65 yr	1.0		1.0	
<65 yr	2.8 (1.3–6.4)	0.012	2.7 (0.9–7.3)	0.055
Sex				
Male	1.0			
Female	2.0 (0.9–4.6)	0.113		
BMI				
≥18.5 kg/m^2	1.0		1.0	
<18.5 kg/m^2	8.6 (2.9–25.7)	<0.001	4.8 (1.4–16.5)	0.013
Smoking				
Never−smoker	1.0			
Ever−smoker	0.5 (0.2–1.2)	0.101		
Diabetes				
No	1.0			
Yes	0.7 (0.2–2.4)	0.560		
COPD				
No	1.0		1.0	
Yes	0.3 (0.1–1.1)	0.051	0.5 (0.1–2.2)	0.333
Disease extent				
Unilateral	1.0		1.0	
Bilateral	3.1 (1.1–9.0)	0.039	3.8 (1.1–13.8)	0.040
Radiographic pattern				
Nodular bronchiectatic	1.0		1.0	
Fibrocavitary	7.0 (2.3–21.2)	0.001	3.6 (1.1–12.8)	0.046
Initial AFB smear				
Negative	1.0		1.0	
Positive	2.3 (0.9–5.7)	0.063	2.3 (0.8–6.6)	0.128
NTM species				
M. abscessus	1.0		1.0	
M. massiliense	1.1 (0.5–2.3)	0.881	1.5 (0.6–3.7)	0.435

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; AFB, acid−fast bacilli; NTM, nontuberculous mycobacteria

4. Treatment outcomes

Among the 42 patients who displayed disease progression, 36 patients (19 with *M. abscessus* lung diseases and 17 with *M. massiliense* lung diseases) agreed to commence treatment. Macrolide treatment with parenteral agents including amikacin, cefoxitin, or imipenem was initiated in 16 patients (84.2%) with *M. abscessus* lung diseases and in 13 patients (76.5%) with *M. massiliense* lung diseases. Surgical resection was included for three patients (15.8%) with *M. abscessus* lung diseases and two patients (11.8%) with *M. massiliense* lung diseases (Table 4).

Patients with *M. massiliense* lung diseases were significantly more likely to achieve initial culture conversion (Figure 4, P = 0.045). However, recurrence occurred in 6 out of 11 patients with *M. abscessus* lung disease who achieved initial culture conversion, while no recurrence was observed in 14 patients with *M. massiliense* lung disease. Consequently, sustained culture conversion was more common in patients with *M. massiliense* lung diseases than those with *M. abscessus* lung diseases (82.4% vs. 26.3%, P = 0.001). Being infected with *M. massiliense* rather than *M. abscessus* (aOR 17.2; 95% CI 2.2–136.9) and use of azithromycin rather than clarithromycin (aOR

9.0; 95% CI 1.1-74.7) were identified as predictors of sustained culture conversion, while BMI less than 18.5 kg/m2 (aOR 0.1; 95% CI 0.0-0.7) was identified as a negative predictor. However, the use of parenteral agents was not associated with sustained culture conversion (Table 5).

Table 4. Treatment regimen and response for patients treated for *M. abscessus* and *M. massiliense* lung diseases

	M. abscessus (n = 19)[a]	*M. massiliense* (n = 17)[a]	*P* value
AFB smear prior to initiating treatment			0.196
Positive, %	14 (73.7%)	9 (52.9%)	
Negative, %	5 (26.3%)	8 (47.1%)	
Initial treatment regimen			0.684
Macrolide with parenteral agents[b]	16 (84.2%)	13 (76.5%)	
Macrolide with or without other oral agents[b]	3 (15.8%)	4 (23.5%)	
Initial macrolide			0.463
Clarithromycin	9 (47.4%)	6 (35.3%)	
Azithromycin	10 (52.6%)	11 (64.7%)	
Surgical treatment	3 (15.8%)	2 (11.8%)	1.000
Treatment duration, weeks[c]			
Total duration of treatment	61 (28–116)	87 (64–120)	0.318
Use of amikacin	16 (2–50)	7 (2–30)	0.455
Use of cefoxitin	2 (0–6)	3 (0–6)	0.676
Use of imipenem	3 (0–5)	0 (0–4)	0.493
Initial culture conversion			
No. of patients achieving culture conversion	11 (57.9%)	14 (82.4%)	0.112
Time to initial culture conversion, weeks[c]	14 (2–29)	6 (4–15)	0.511
Final treatment response			0.001
Sustained culture conversion	5 (26.3%)	14 (82.3%)	
Treatment failure	14 (73.7%)	3 (17.7%)	

Abbreviations: AFB, acid–fast bacilli

[a] Among patients with disease progression, 19 of 21 patients with *M. abscessus* lung disease and 17 of 21 patients with *M. massiliense* lung disease received treatment.

[b] Parenteral agents included amikacin, cefoxitin, or imipenem, while oral agents included fluoroquinolone, amoxicillin/clavulanate, rifampin, or ethambutol.

[c] Treatment duration and time to initial negative conversion were described as median (IQR).

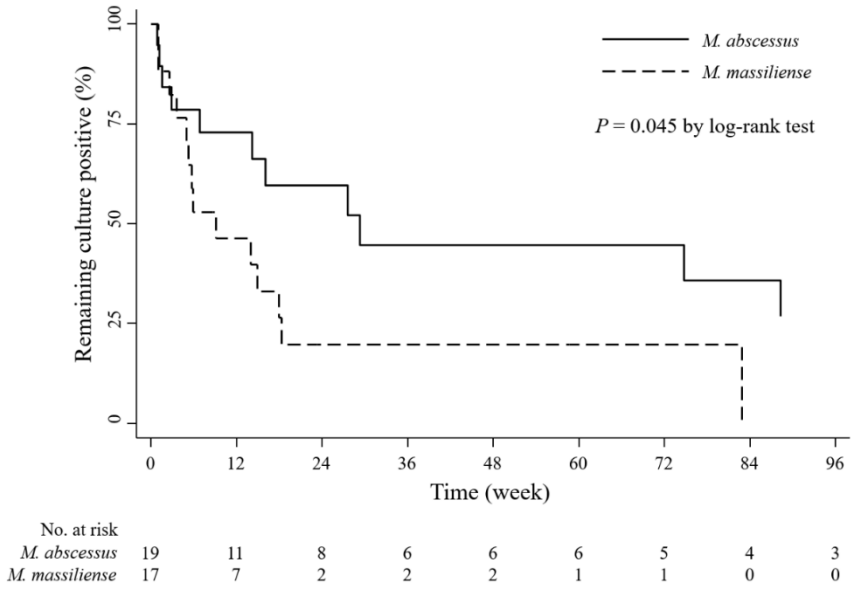

Figure 4. Kaplan–Meier analysis of the comparison of time to culture conversion between *M. abscessus* and *M. massiliense* lung diseases.

Patients with *M. massiliense* lung diseases were significantly more likely to achieve initial culture conversion compared to those with *M. abscessus* lung diseases (P = 0.045 by log–rank test).

Table 5. Predictors of sustained culture conversion among the 36 patients treated for *M. abscessus* or *M. massiliense* lung disease

Variables	Unadjusted		Adjusted	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Age				
≥65 yr	1.0			
<65 yr	1.2 (0.3–4.6)	0.790		
Sex				
Male	1.0			
Female	2.2 (0.4–11.2)	0.333		
BMI				
≥18.5 kg/m^2	1.0		1.0	
<18.5 kg/m^2	0.2 (0.1–0.6)	0.011	0.1 (0.0–0.7)	0.021
Radiographic pattern				
Nodular bronchiectatic	1.0			
Fibrocavitary	0.4 (0.1–1.6)	0.201		
NTM species				
M. abscessus	1.0		1.0	
M. massiliense	13.1 (2.6–65.5)	0.002	17.2 (2.2–136.9)	0.007
Treatment regimen				
Without parenteral agents	1.0			
With parenteral agents	0.8 (0.2–4.3)	0.797		
Initial macrolide				
Clarithromycin	1.0		1.0	
Azithromycin	4.0 (0.9–16.3)	0.053	9.0 (1.1–74.7)	0.041
Susceptibility to clarithromycin				
Not susceptible	1.0			
Susceptible	27.5 (3.9–193.5)	0.001		

Abbreviations: BMI, body mass index; AFB, acid-fast bacilli; NTM, nontuberculous mycobacteria

5. Mediation analysis

The causative organism's susceptibility to clarithromycin was shown to be a significant predictive factor of sustained culture conversion (aOR 27.5; 95% CI 3.9-193.5). Since strains of *M. abscessus* and strains of *M. massiliense* showed a significant difference in the susceptibility to clarithromycin, we performed mediation analysis and tested the susceptibility to clarithromycin as a mediator of treatment outcomes. We adjusted the mediation analysis model with the BMI and the use of azithromycin rather than clarithromycin, which were identified as predictive factors of sustained culture conversion. As a result, the effect of infection with *M. abscessus* or *M. massiliense* on the treatment outcomes was shown to be significantly mediated by the susceptibility of the isolated strain to clarithromycin (indirect effect 0.28; CI 0.08-0.95). The proportion of the indirect effect to the total effect was 38.1%.

DISCUSSION

The comparison of *M. abscessus* and *M. massiliense* lung diseases in our study revealed several interesting findings. First, the disease progression rate did not differ between the two groups during the follow-up period. Instead, lower BMI, bilateral lung involvement, and fibrocavitary type disease were predictive of disease progression. Second, better treatment response was observed for *M. massiliense* lung diseases, which is consistent with previous reports [13, 15, 16]. It is worth noting that this difference in treatment outcomes between *M. abscessus* and *M. massiliense* lung diseases was only partly mediated by their different susceptibilities to clarithromycin. Third, azithromycin showed superior efficacy in achieving sustained culture conversion compared with clarithromycin.

The natural history of NTM lung diseases remains elusive, which makes it difficult to decide when to initiate treatment [26]. Mycobacterial virulence and host factors influence the progression of NTM lung diseases [18]. For MAC lung diseases, patients with *M. intracellulare* lung diseases were reported to be more likely to receive antibiotic therapy and have unfavorable microbiologic response after treatment compared with those with

M. avium lung diseases [27]. However, our study showed no difference in disease progression rate between *M. abscessus* and *M. massiliense* lung diseases, which suggests that these two species may not differ in virulence in spite of their difference in treatment response. Instead, clinical characteristics such as lower BMI and the radiographic pattern of involvement appear to be associated with disease progression. These findings are similar to those reported for MAC lung diseases [28–30].

For patients who received treatment for *M. abscessus* complex lung diseases, patients infected with *M. massiliense* rather than *M. abscessus* were more likely to have sustained culture conversion, which was consistent with previous studies [13, 15, 16]. It has been suggested that the better treatment response observed for *M. massiliense* lung diseases is attributable to *M. massiliense* isolates rarely having inducible resistance to clarithromycin, and their more frequent susceptibility to macrolides [12, 15, 31]. However, according to the mediation analysis carried out in our study, this difference in susceptibility to clarithromycin accounted for only about 40% of the difference in treatment outcomes. This means that microbiologic factors other than susceptibility to clarithromycin as well as host factors

influence treatment outcomes. Whole genome sequencing and analysis of the causative mycobacteria could therefore play a role in identifying additional markers that may help to explain the different treatment outcomes observed between the two species.

We determined several factors, including BMI and choice of macrolide, that influenced treatment outcomes. Lower BMI is a well-known factor associated with the development and/or progression of various chronic lung diseases and several infectious diseases [32-34]. However, our study is the first to report the superiority of azithromycin to clarithromycin in terms of achieving sustained culture conversion in patients with *M. abscessus* complex lung diseases. In MAC lung diseases, no difference was reported in treatment efficacy between these two drugs [35]. The reasons why azithromycin showed better response in our study is unclear, but the superiority of azithromycin in preventing inducible resistance may provide an explanation [31]. However, another study found no difference in terms of inducible resistance between these two agents, leaving some controversy around this issue [12]. Another possible explanation may lie in the different anti-inflammatory or immunomodulatory effects of these macrolides, but these

pharmacologic differences have not been consistent in previous reports [36, 37]. The longer half-life and improved tissue penetration of azithromycin could also explain the higher treatment efficacy of this drug [38].

Although ATS/IDSA guideline recommended the use of parenteral agents as well as macrolide treatment [18], in our study, the use of parenteral agents did not increase the chance of achieving sustained culture conversion. Longer treatment periods with parenteral agents than those used in our study may be needed to improve outcomes. However, such longer treatment periods would be impractical because of adverse drug reactions, inconvenience to patients, and increased costs. The observation that four out of seven patients (all four patients with *M. massiliense* lung diseases) for whom azithromycin or clarithromycin was prescribed without parenteral agents achieved sustained culture conversion, also raises a question on the role of using parenteral agents, especially for *M. massiliense* lung diseases. Recent report showing no difference in treatment outcomes between shorter and longer use of parenteral agents for the treatment of *M. massiliense* lung diseases further empathizes this issue [39].

The current study has several limitations. First of all, the initiation of treatment was determined by the duty physician. Although this decision was made taking account of radiographic and symptomatic changes, the subjective judgment of the physicians may have introduced bias. Second, several patients refused admission and did not receive parenteral therapy. Although this introduced variations to the predefined treatment protocol, it also enabled us to compare treatment response between patients who received parenteral therapy with those who did not. Third, only microbiologic response was assessed as a treatment outcomes in this study. Considering the chronic course of disease, improvement in patient quality of life as well as their length of survival may be more appropriate treatment response targets for *M. abscessus* complex lung diseases, as was illustrated by a recent study [40]. Finally, the small number of patients included in this study limited the statistical power of the analysis. A nationwide multi-center study should be performed to provide more reliable results.

CONCLUSIONS

In conclusion, the progression rates of *M. abscessus* lung diseases and *M. massiliense* lung diseases were similar but treatment outcomes were better for patients with *M. massiliense* lung diseases. Lower BMI, bilateral lung involvement, and fibrocavitary type disease were identified as predictors of disease progression. Among those patients who underwent treatment, infection with *M. massiliense* rather than *M. abscessus* and the use of azithromycin rather than clarithromycin were associated with sustained culture conversion. The difference in treatment outcomes observed for these two species was partly mediated by their difference in susceptibility to clarithromycin.

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국문 초록

서론: 비결핵 항산균 폐 질환 중 *Mycobacterium abscessus* complex 에 의한 폐 질환은 치료가 어렵고 예후가 좋지 않은 것으로 알려져 있다. *M. abscessus* complex 는 미생물학의 발전으로 최근 *M. abscessus, M. massiliense,* 그리고 *M. bolletii* 라는 3 가지 다른 균으로 이루어져 있다는 것이 밝혀졌으며, 국내에서는 이 중 *M. abscessus* 와 *M. massiliense* 가 대부분을 차지하고 있다. 본 연구는 *M. abscessus* 폐 질환과 *M. massiliense* 폐 질환의 질병의 진행 양상 및 치료 성적을 비교 고찰하고자 하였다.

방법: 서울대학교 병원에서 2006 년 1 월부터 2015 년 6 월 사이의 기간 동안 진단된 56 명의 *M. abscessus* 폐 질환 환자와 54 명의 *M. massiliense* 폐 질환 환자의 의무 기록을 검토 및 분석하였다. 치료를 필요로 할 수준으로 질병이 진행하는 데 걸리는 시간, 그리고 질병이 진행하여 치료를 시행하였을 때 치료 성적을 양 군에서 비교하였다. 또한 그 외에 질병의 진행 여부와 치료를 하였을 때 배양 음전 여부를 예측할 수 있는 관련 인자를 확인하고자 하였다. 마지막으로 *M. abscessus* 와 *M. massiliense* 사이 치료 성적의 차이가 양 균 사이의 Clarithromycin 감수성 차이에 의해 매개되는지를 분석하였다.

결과: *M. abscessus* 폐 질환 환자 중에서는 56 명 중 21 명에서, 그리고 *M. massiliense* 폐 질환 환자 중에서는 54 명 중 21 명에서 임상적으로 치료를 필요할 정도로 폐 질환이 진행 및 악화되었다. 양 군에서 질병이 진행하는 데까지 걸리는 시간은 유의한 차이가 없었다. 대신 진단 당시 낮은 체질량 지수, 양측 폐 침범, 그리고 섬유공동형 질환이 질병의 진행을 예측할 수 있는 인자로 확인되었다. 폐 질환의 진행 속도에는 차이가 없었지만, 질환이 진행하여 치료를 시행하였을 때에는 *M. massiliense* 폐 질환에서 *M. abscessus* 폐 질환에 비하여 배양 음전이 지속적으로 유지되는 치료 성공률이 유의하게 높았다. 이러한 치료 성적의 차이는 부분적으로 양 균의 Clarithromycin 감수성 차이에 의해 부분적으로 매개된다는 것을 확인할 수 있었으며, 그 외에 초치료 약제로 Clarithromycin 보다는 Azithromycin 을 사용하였을 때 배양 음전을 유의하게 더 많이 이룰 수 있었다.

결론: *M. abscessus* 폐 질환과 *M. massiliense* 폐 질환의 진행 속도는 비슷하였다. 그러나 질병이 진행하여 치료를 시행하게 되었을 때에는 *M. massiliense* 폐 질환이 *M. abscessus* 폐 질환에 비해 치료 성적이 우수하였다. 이러한 치료 성적의 차이는 양 균 사이의 Clarithromycin 감수성 차이에 의해 부분적으로 매개됨을 확인할 수 있었다.

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주요어 : 비결핵 항산균, 폐 질환, *Mycobacterium abscessus*, *Mycobacterium massiliense*

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