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Peak Plasma Concentration of Azithromycin and Treatment Responses in *Mycobacterium avium* Complex Lung Disease

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Macrolides, such as azithromycin (AZM) and clarithromycin, are the cornerstones of treatment for *Mycobacterium avium* complex lung disease (MAC-LD). Current guidelines recommend daily therapy with AZM for cavitary MAC-LD and intermittent therapy for noncavitary MAC-LD, but the effectiveness of these regimens has not been thoroughly investigated. This study evaluated associations between microbiological response and estimated peak plasma concentrations (C_{max}) of AZM. The AZM C_{max} was measured in patients receiving daily therapy (250 mg of AZM daily, $n = 77$) or intermittent therapy (500 mg of AZM three times weekly, $n = 89$) for MAC-LD and daily therapy for *Mycobacterium abscessus* complex LD (MABC-LD) (250 mg of AZM daily, $n = 55$). The AZM C_{max} was lower with the daily regimen for MAC-LD (median, 0.24 μg/ml) than with the intermittent regimen for MAC-LD (median, 0.65 μg/ml; $P < 0.001$) or daily therapy for MABC-LD (median, 0.53 μg/ml; $P < 0.001$). After adjusting for confounding factors, AZM C_{max} was independently associated with favorable microbiological responses in MAC-LD patients receiving a daily regimen (adjusted odds ratio [aOR], 1.58; 95% confidence interval [CI], 1.01 to 2.48; $P = 0.044$) but not an intermittent regimen (aOR, 0.85; 95% CI, 0.58 to 1.23, $P = 0.379$). With the daily AZM-based multidrug regimen for MAC-LD, a low AZM C_{max} was common, whereas a higher AZM C_{max} was associated with favorable microbiologic responses. The results also suggested that the addition of rifampin may lower AZM C_{max}. When a daily AZM-based multidrug regimen is used for treating severe MAC-LD, such as cavitary disease, the currently recommended AZM dose might be suboptimal. (This study has been registered at ClinicalTrials.gov under identifier NCT00970801.)

Pulmonary disease caused by nontuberculous mycobacteria (NTM) is increasing worldwide (1, 2), and *Mycobacterium avium* complex (MAC) is the most common etiology of lung disease (LD) due to NTM (1, 2). The introduction of newer macrolides, such as clarithromycin (CLR) and azithromycin (AZM), was a major therapeutic advancement in the treatment of LD due to MAC (MAC-LD) (3–8). However, conversion to negative sputum culture is achieved in only 60% to 80% of patients receiving macrolide-based regimens (9–12). The often unsuccessful results of current treatment regimens are partly due to an incomplete understanding of the relationships between the dosages of the drugs used and the level of exposure achieved in target organs, as determined by the pharmacokinetics and pharmacodynamics of the drugs (13–15).

Therapeutic drug monitoring (TDM), that is, individualized drug dosing guided by drug plasma concentrations, could be of help in improving our understanding of drug interactions in the current treatment regimens for MAC-LD (13–15). Rifampin (RIF), one drug component of macrolide-based antibiotic regimens for the treatment of MAC-LD, is well known to induce cytochrome P450 isoenzymes and reduce peak plasma concentrations (C_{max}) of CLR and AZM (13–15). Although a lack of an association between the C_{max} of CLR and treatment outcomes was reported (14), little is known regarding the relationship between the C_{max} of AZM and treatment outcomes of MAC-LD.

The current American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) guidelines recommend a daily regimen of CLR or AZM, RIF, and ethambutol (EMB), with or without the initial use of parenteral aminoglycoside, for patients with fibrocavitary MAC-LD, cavitary nodular bronchiectatic MAC-LD, or previously treated MAC-LD (3). For patients with noncavitary nodular bronchiectatic MAC-LD, a three-times-weekly intermittent regimen of CLR or AZM, RIF, and EMB is recommended (3). Although the same CLR dose (1,000 mg) is used in both the daily and intermittent regimens, different AZM doses are recommended for the daily (250 to 300 mg) and intermittent regimens (500 to 600 mg) for MAC-LD in the current guidelines (3). This difference in dosing between CLR and AZM is likely why the AZM C_{max} differed significantly between patients receiving 500-mg and 250-mg doses of AZM (13), whereas the CLR C_{max} values were similar in patients receiving daily and intermittent therapy for MAC-LD (14). The present study (ClinicalTrials.gov identifier NCT00970801) was conducted to evaluate drug interactions between AZM and RIF and the association be-

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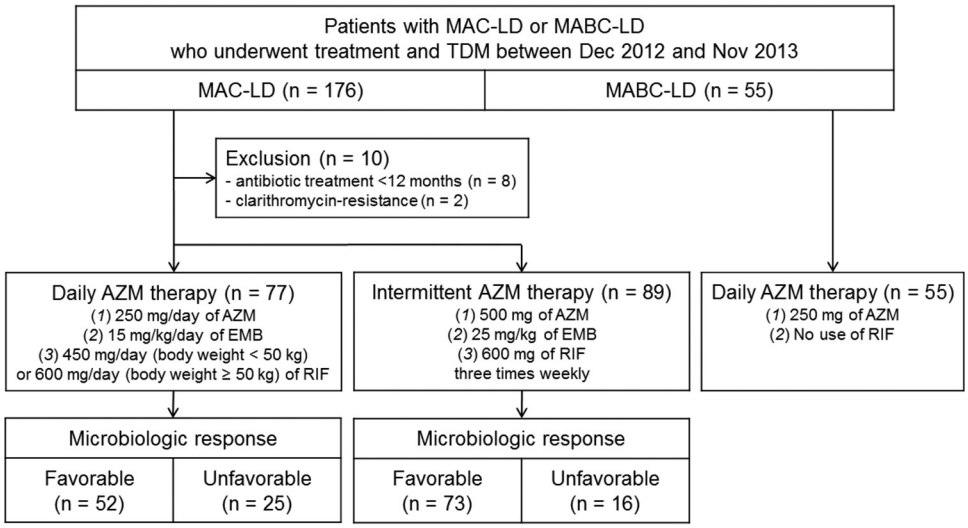


FIG 1 Treatment regimens and responses of study population. Flow chart shows the treatment regimens and microbiologic responses for the MAC-LD patients and MABC-LD control group. AZM, azithromycin; EMB, ethambutol; LD, lung disease; MABC, *M. abscessus* complex; MAC, *M. avium* complex; RIF, rifampin; TDM, therapeutic drug monitoring.

tween AZM C_{max} and treatment outcomes in patients with MAC-LD who received daily or intermittent AZM-based antibiotic treatment regimens.

MATERIALS AND METHODS

Study populations. This is a retrospective study investigating NTM lung disease, with some data prospectively collected for research purposes from an ongoing, institutional review board-approved, prospective, and observational cohort study that took place at Samsung Medical Center (a 1,961-bed university-affiliated tertiary referral hospital in Seoul, South Korea) between December 2012 and November 2013. Patients were identified using the NTM registry database of the Samsung Medical Center (10, 12, 16). The institutional review board (IRB) of the Samsung Medical Center approved this study and waived the requirement for additional informed consent (IRB no. 2015-05-111), as we used only deidentified data prospectively collected for research purposes.

Between December 2012 and November 2013, 176 patients were treated for MAC-LD with AZM-based antibiotic regimens and underwent TDM (Fig. 1). Of these patients, 10 patients who received antibiotic treatment for <12 months ($n = 8$) or who had MAC isolates that were resistant to CLR ($n = 2$) were excluded. As a control group in this study, 55 patients with LD due to *Mycobacterium abscessus* complex (MABC-LD) who underwent TDM for their plasma AZM levels during the same period were included, and their plasma AZM levels were compared with those of patients with MAC-LD. Because patients with MABC-LD received oral AZM without RIF during the entire treatment period (17–19), RIF-associated drug interactions could not influence their plasma AZM levels. A total of 166 patients with MAC-LD, including 77 patients who received daily antibiotic therapy and 89 patients who received intermittent antibiotic therapy, were used to evaluate the relationship between plasma AZM levels and microbiological treatment responses. All patients met the diagnostic criteria for NTM lung disease, according to the guidelines of the ATS and IDSA (3).

Antibiotic treatment. All patients with MAC-LD who began antibiotic therapy received the standardized combination of antibiotic therapy consisting of oral AZM, RIF, and EMB (3). For patients with cavitary MAC-LD, including the fibrocavitary and cavitary nodular bronchiectatic forms, and patients with previously treated MAC-LD, the following daily regimen was administered: (i) 250 mg/day AZM, (ii) 15 mg/kg of body weight/day EMB, and (iii) 450 mg/day (body weight, <50 kg) or 600 mg/day (body weight, ≥50 kg) RIF. Streptomycin was administered intramuscularly in some patients with severe fibrocavitary disease. For patients with noncavitary nodular bronchiectatic disease, the following intermittent regimen was administered: (i) 500 mg AZM, (ii) 25 mg/kg EMB, and (iii) 600 mg RIF three times weekly.

Drug susceptibility tests were performed at the Korean Institute of Tuberculosis (Cheongju, South Korea). The MICs of CLR were determined by the broth microdilution method. MAC isolates with MICs of 32 mg/ml or greater were considered to be resistant to CLR (20). Drug susceptibility tests for AZM were not performed during the study period.

Sputum examinations for acid-fast bacilli (AFB) were performed 1, 3, and 6 months after the initiation of antibiotic treatment and then at 2- to 3-month intervals until the end of treatment during the study period (12). Sputum conversion was defined as three consecutive negative cultures, and a favorable treatment outcome was defined as sputum culture conversion and maintenance of negative sputum cultures for more than 12 months (12).

Therapeutic drug monitoring. TDM for AZM was available and has been included in the research protocol at our institution since December 2012. Peripheral venous blood sampling was performed after 2 weeks of AZM treatment in the majority of patients (163/166 [98%]) with MAC-LD and 44/55 (80%) patients with MABC-LD. Samples were taken 2 h after drug intake to estimate the C_{max} (21).

Plasma concentrations of AZM, RIF, and EMB were determined with a Waters 2795 Alliance high-performance liquid chromatographic system and a Quattro Micro API tandem mass spectrometer (Waters, Manchester, United Kingdom). The linear ranges of the assay were 0.25 to 2.5 μg/ml for AZM, 0.5 to 50 μg/ml for RIF, and 0.5 to 10 μg/ml for EMB. The intra- and interday precisions, expressed as coefficient variations, were less than 10%. In accordance with previously published reference ranges, C_{max} values were dichotomized as either normal or low, with low concentrations defined as <0.2 μg/ml for AZM, <8 μg/ml for RIF, and <2 μg/ml for EMB (13).

Statistical analyses. All data are presented as medians and interquartile ranges (IQR) for the continuous variables and as numbers (percentages) for the categorical variables. The data were compared using the Mann-Whitney *U* test or Kruskal-Wallis test with *post hoc* paired comparisons using the Bonferroni method for continuous variables and Pearson χ^2 test or Fisher's exact test for categorical variables.

To assess whether the C_{max} values of AZM, RIF, and EMB were asso-

TABLE 1 Baseline characteristics of patients with *M. avium* complex lung disease or *M. abscessus* complex lung disease[a]

Characteristic[b]	*M. avium* complex				*M. abscessus* complex
	Total ($n = 166$)	Daily therapy ($n = 77$)	Intermittent therapy ($n = 89$)	*P* value	($n = 55$)
Sex, male	68 (41.0)	33 (42.9)	35 (39.3)	0.645	16 (29.1)
Age (yr)	61 (52–69)	59 (51–70.5)	61 (52.5–68.5)	0.707	56 (50–64)
Body mass index (kg/m^2)	20.1 (18.5–21.8)	19.8 (17.5–21.2)	20.7 (19.0–22.2)	0.003	20.5 (19.1–22.3)
Nonsmoker	163 (98.2)	75 (97.4)	88 (98.9)	0.597	53 (96.4)
Comorbid disease					
Bronchiectasis	137 (82.5)	52 (67.5)	85 (95.5)	<0.001	45 (81.8)
Previous tuberculosis	80 (48.2)	49 (63.6)	31 (34.8)	<0.001	37 (67.3)
Previous NTM lung disease	25 (15.1)	13 (16.9)	12 (13.5)	0.541	6 (10.9)
Cancer	33 (19.9)	16 (20.8)	17 (19.1)	0.787	4 (7.3)
Chronic lung disease[c]	11 (6.6)	9 (11.7)	2 (2.2)	0.015	3 (5.5)
Diabetes mellitus	10 (6.0)	4 (5.2)	6 (6.7)	0.753	0
Chronic liver disease	8 (4.8)	5 (6.5)	3 (3.4)	0.474	3 (5.5)
Chronic heart disease	5 (3.0)	2 (2.6)	3 (3.4)	1.000	4 (7.3)
Chronic kidney disease	2 (1.2)	1 (1.3)	1 (1.1)	1.000	0
Etiology					
Mycobacterium avium	87 (52.4)	36 (46.8)	51 (57.3)	0.175	
Mycobacterium intracellulare	79 (47.6)	41 (53.2)	38 (42.7)		
Mycobacterium abscessus					25 (45.5)
Mycobacterium massiliense					30 (54.5)
Type of disease					
Fibrocavitary form	26 (15.7)	26 (33.8)	0	<0.001	12 (21.8)
Nodular bronchiectatic form	132 (79.5)	47 (61.0)	85 (95.5)		42 (76.4)
Unclassifiable form	8 (4.8)	4 (5.2)	4 (4.5)		1 (1.8)
Cavity on chest HRCT[d]	66 (39.8)	66 (85.7)	0	<0.001	29 (52.7)
Positive sputum smear[d]	76 (45.8)	53 (68.8)	23 (25.8)	<0.001	39 (70.9)

[a] Data are presented as median (interquartile range) or as number (%).
[b] NTM, nontuberculous mycobacteria; HRCT, high-resolution computed tomography.
[c] Chronic lung disease included chronic obstructive pulmonary disease ($n = 5$), interstitial lung disease ($n = 2$), and chronic pulmonary aspergillosis ($n = 9$). Some patients had more than one chronic lung disease.
[d] At the initiation of antibiotic treatment.

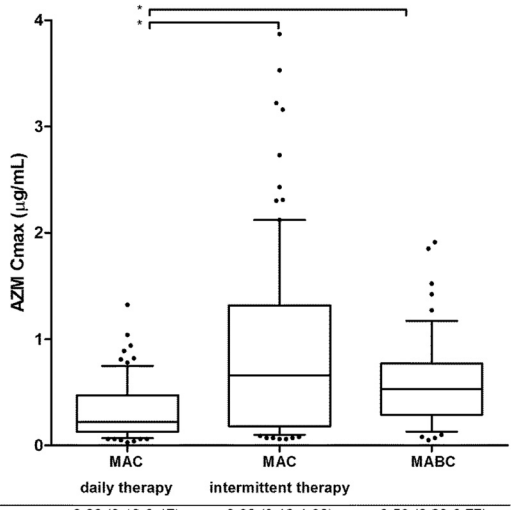
ciated with favorable microbiologic responses in patients with MAC-LD, we first log-transformed the C_{max} of AZM, RIF, and EMB to achieve a normal distribution and to mitigate the effects of outliers. Then, we performed multivariable logistic regressions in each daily and intermittent therapy group while adjusting for the variables with a *P* value of <0.25 in the univariable analysis. In addition, multivariable logistic regressions were performed with binomial data with referenced cut points (C_{max} of AZM, ≥0.2 μg/ml; C_{max} of RIF, ≥8 μg/ml; and C_{max} of EMB, ≥2 μg/ml) instead of continuous variables as AZM, RIF, and EMB C_{max}.

If correlations were shown between the C_{max} of each drug and favorable outcomes, we calculated the Youden index at each cut point to determine the best cut point of the C_{max} of each drug associated with favorable outcomes (22). Finally, to confirm associations between a new cut point and a favorable outcome, multivariable logistic regressions were performed with a new cut point. All statistical analyses were performed with PASW 18.0 (SPSS Inc., Chicago, IL), and a two-sided *P* value of <0.05 was considered significant.

RESULTS

Baseline characteristics. The baseline characteristics of patients with MAC-LD and MABC-LD are presented in Table 1. Of the 166 patients with MAC-LD, 68 (41.0%) were male. The median age was 61 years (IQR, 52 to 69 years), and the median body mass index was 20.1 kg/m^2 (IQR, 18.5 to 21.8 kg/m^2). None of the patients were positive for human immunodeficiency virus infection.

Seventy-seven patients (46.4%) received daily therapy, and 89 patients (53.6%) with the noncavitary nodular bronchiectatic form of MAC-LD received intermittent therapy. Of the 77 patients receiving daily therapy, 66 (85.7%) had cavitary MAC-LD, including either fibrocavitary disease ($n = 26$) or cavitary nodular bronchiectatic disease ($n = 40$), seven (9.1%) had a history of previous treatment for NTM lung disease with macrolide-based antibiotic regimens, and four (5.2%) had a nonclassifiable form of the disease, such as noncavitary consolidation on chest computed tomography (Table 1). Patients receiving daily therapy had lower body mass indexes, a more frequent history of previous tuberculosis, more frequent fibrocavitary disease, and sputum smears that were more frequently positive for acid-fast bacilli than did patients receiving intermittent



	MAC daily therapy	MAC intermittent therapy	MABC
AZM Cmax (μg/mL)[†]	0.22 (0.13-0.47)	0.66 (0.18-1.32)	0.53 (0.29-0.77)
AZM Cmax < 0.2 μg/mL	36/77 (46.8%)	23/89 (25.8%)	9/55 (16.4%)

Kruskal-Wallis test, $P < 0.001$;

*, Mann-Whitney *U* test with Bonferroni correction, $P < 0.001$.

†, Data are presented as medians (interquartile ranges).

FIG 2 A box-and-whisker plot of peak plasma azithromycin concentrations. The bottom and top of each box indicate the 25th and 75th percentiles, respectively, and the line within each box indicates the median. The whiskers indicate the 10th and 90th percentiles. AZM C_{max}, peak plasma azithromycin concentrations; MABC, *M. abscessus* complex; MAC, *M. avium* complex.

therapy. All patients had CLR-susceptible MAC isolates at treatment initiation.

***C*$_{max}$ of AZM according to treatment regimen.** As shown in Fig. 2, the C_{max} values of AZM were significantly lower in patients with MAC-LD receiving daily therapy that included both AZM and RIF (median C_{max}, 0.22 μg/ml; IQR, 0.13 to 0.47 μg/ml) than in patients with MABC-LD receiving AZM without RIF (median C_{max}, 0.53 μg/ml; IQR, 0.29 to 0.77 μg/ml; $P < 0.001$). In patients with MAC-LD who received intermittent therapy, which included both AZM and RIF, the AZM C_{max} (median, 0.66 μg/ml; IQR, 0.18 to 1.32 μg/ml) was higher than that in patients with MAC-LD receiving daily therapy ($P < 0.001$). In addition, 46.8% (36/77) of MAC-LD patients receiving daily therapy had an AZM C_{max} below the target of 0.2 μg/ml, which was a higher proportion than that found with patients receiving intermittent therapy for MAC-LD (25.8% [23/89], $P = 0.005$) or daily therapy without RIF for MABC-LD (16.4% [9/55], $P < 0.001$). The C_{max} of RIF did not differ between patients receiving daily therapy (median, 12.5 μg/ml; IQR, 7.6 to 17.6 μg/ml) or intermittent therapy (median, 11.3 μg/ml; IQR, 4.5 to 21.2 μg/ml; $P = 0.788$). However, the C_{max} of EMB was lower in patients receiving daily therapy (median, 2.8 μg/ml; IQR, 1.8 to 4.2 μg/ml) than in those receiving intermittent therapy (median, 3.8 μg/ml; IQR, 2.2 to 5.8 μg/ml; $P = 0.009$). The median AZM C_{max} values and the proportion of patients whose AZM C_{max} was below the target of 0.2 μg/ml did not differ significantly between patients with MAC-LD receiving intermittent therapy and patients with MABC-LD.

***C*$_{max}$ of AZM and microbiological responses.** Patients receiving the intermittent therapy for noncavitary treatment-naive MAC-LD had a higher favorable microbiological response (73/89 [82.0%]) than those receiving the daily therapy for cavitary or previously treated MAC-LD (52/77 [67.5%], $P = 0.031$), but given that cavitary disease is a more severe form of MAC-LD, it is unclear from these findings how the therapy regimen, versus disease status, factored into the differing microbiological responses between the two groups. Within each group, however, there were no significant differences in the demographic data, disease status, and treatment details between patients with favorable and unfavorable microbiological responses, except for a higher AFB-positive smear rate in patients with unfavorable microbiological responses than in those with favorable microbiological response (50.0% versus 20.5%, $P = 0.025$) in the intermittent therapy group (Table 2).

Table 3 shows the associations between the C_{max} of AZM, RIF, and EMB and microbiological response according to treatment regimen. In the daily therapy group, a higher C_{max} of AZM was associated with a favorable microbiological response (adjusted odds ratio [OR], 1.58; 95% confidence interval [CI], 1.01 to 2.48; $P = 0.044$). Although the association between microbiological response and a cutoff value of 0.2 μg/ml was not statistically significant, a C_{max} for AZM of ≥0.4 μg/ml, which was the best cut point with the highest Youden index, was significantly associated with favorable outcomes (adjusted OR, 3.98; 95% CI, 1.06 to 14.85; $P = 0.040$) (see Table S1 in the supplemental material). In the intermittent therapy group, the higher C_{max} of AZM was not associated with favorable microbiological response (adjusted OR, 0.85; 95% CI, 0.58 to 1.23; $P = 0.379$). In addition, a referenced cut point of 0.2 μg/ml was not associated with microbiological response (adjusted OR, 1.01; 95% CI, 0.27 to 3.72; $P = 0.991$) in the intermittent therapy group. The C_{max} levels of RIF and EMB were not associated with the microbiologic response in either the daily or intermittent therapy group (Table 3).

DISCUSSION

This study evaluated the associations between the C_{max} of AZM and the microbiologic response in patients with MAC-LD, as well as the effects of RIF on the C_{max} of AZM. A high C_{max} of AZM was associated with a favorable microbiological response in patients with MAC-LD treated with a daily AZM-based regimen, although this association was not found in patients treated with an intermittent regimen. In addition, we found that RIF significantly reduced the C_{max} of AZM when used in a daily regimen.

Although the use of TDM in the treatment of patients with tuberculosis has become more widely accepted (23–26), there are few reports of the clinical usefulness of TDM in patients with NTM lung disease (13–15). We previously reported that low plasma CLR concentrations were common in patients treated for MAC-LD, although we found no association between low plasma CLR concentrations and treatment outcomes (14). However, there have been no reports on the associations between the C_{max} of AZM and treatment outcomes in patients with MAC-LD.

Several reports have been published on the pharmacokinetics and pharmacodynamics of AZM in patients with MAC-LD (13, 15, 27). To the best of our knowledge, however, there has only been one report on the interaction between C_{max} of AZM and the use of RIF in patients with MAC-LD (13). That study demonstrated that the C_{max} of AZM decreased by 23% from 0.35 μg/ml to 0.27 μg/ml in conjunction with the administration of RIF (13). Our study showed that the C_{max} of AZM was 58% lower in patients with MAC-LD who received daily AZM with rifampin (median, 0.22 μg/ml) than that in patients with MABC-LD who received daily AZM without RIF (median, 0.53 μg/ml). Although a signif-

TABLE 2 Characteristics, treatment regimens, and microbiologic responses of 166 patients with *M. avium* complex lung disease[a]

Characteristic	Daily therapy ($n = 77$)			Intermittent therapy ($n = 89$)		
	Favorable microbiological responses ($n = 52$)	Unfavorable microbiological responses ($n = 25$)	*P* value	Favorable microbiological responses ($n = 73$)	Unfavorable microbiological responses ($n = 16$)	*P* value
Sex, male	21 (40.4)	12 (48.0)	0.527	27 (37.0)	8 (50.0)	0.334
Age (yr)	60 (51–69.5)	59 (52–71)	0.853	61 (53.5–69)	59.5 (50–67.5)	0.567
Body mass index (kg/m^2)	19.8 (17.5–20.7)	20.2 (16.9–22.0)	0.355	20.9 (19.1–22.3)	19.6 (17.9–22.0)	0.283
Nonsmoker	76 (98.1)	24 (96.0)	0.547	72 (98.6)	16 (100)	1.000
Comorbid disease						
Bronchiectasis	37 (71.2)	15 (60.0)	0.328	70 (95.9)	15 (93.8)	0.554
Previous tuberculosis	33 (63.5)	16 (64.0)	0.963	24 (32.9)	7 (43.8)	0.408
Previous NTM lung disease	10 (19.2)	3 (12.0)	0.529	10 (13.7)	2 (12.5)	1.000
Cancer	12 (23.1)	4 (16.0)	0.474	13 (17.8)	4 (25.0)	0.497
Chronic lung disease	5 (9.6)	4 (16.0)	0.461	2 (2.7)	0	1.000
Diabetes mellitus	3 (5.8)	1 (4.0)	1.000	6 (8.2)	0	0.586
Chronic liver disease	2 (3.8)	3 (12.0)	0.322	3 (4.1)	0	1.000
Chronic heart disease	1 (1.9)	1 (4.0)	0.547	2 (2.7)	1 (6.3)	0.452
Chronic kidney disease	1 (1.9)	0	1.000	1 (1.4)	0	1.000
Etiology			0.190			0.226
M. avium	27 (51.9)	9 (36.0)		44 (60.3)	7 (43.8)	
M. intracellulare	25 (48.1)	16 (64.0)		29 (39.7)	9 (56.3)	
Fibrocavitary form	15 (28.8)	11 (44.0)	0.188	0	0	
Positive sputum smear[b]	34 (65.4)	21 (84.0)	0.065	15 (20.5)	8 (50.0)	0.025
Additional treatment[c]						
Injectable drugs	11 (21.2)	10 (40.0)	0.082	0	0	
Surgical resection	3 (5.8)	3 (12.0)	0.383	0	0	

[a] Data are presented as median (interquartile range) or number (%).
[b] At the initiation of antibiotic treatment.
[c] Within 12 months of the start of antibiotic treatment.

icant lowering of the AZM C_{max} in conjunction with RIF was found in the present study, AZM seems to be less influenced by RIF than does CLR, as demonstrated by our previous study in which we observed a 92% reduction from a median C_{max} of CLR of 3.8 μg/ml in patients with MABC-LD to a median C_{max} of 0.3 μg/ml in patients with MAC-LD who received both CLR (1,000 mg/day) and RIF (14).

In addition, no reports on the interaction between AZM and RIF in an intermittent regimen for the treatment of noncavitary nodular bronchiectatic MAC-LD have been published. In our previous study, the C_{max} levels of CLR (median, 0.2 μg/ml) in MAC-LD patients, with an intermittent CLR-based multidrug regimen that included RIF, were significantly lower (−95%) than those (median, 3.8 μg/ml) in MABC-LD patients (14). In the present study, the C_{max} of AZM (median, 0.66 μg/ml) in the intermittent AZM-based multidrug regimen in MAC-LD patients was not lower than that in patients with MABC-LD (median, 0.53 μg/ml). This might be due to the use of a higher dosage of AZM in the intermittent regimen (500 mg) than in the daily regimen (250 mg) and also due to less interaction between AZM and RIF than between CLR and RIF.

Pharmacokinetic studies on AZM, like other macrolide antibiotics, have shown low plasma levels and high tissue concentrations (28, 29). Although the C_{max} of AZM after a single 500-mg oral dose is 5-fold lower than the C_{max} of CLR using the same dose (30), the ratio of tissue concentrations to plasma levels for AZM (10- to 100-fold) is higher than that for CLR (2- to 20-fold) (31–33). In combination with RIF, the induction of cytochrome P450 enzymes metabolizes CLR to its main metabolite, the 14-hydroxy form, which is 10 to 30 times less active against MAC *in vitro* (34, 35). However, AZM has no active metabolites, does not interact with cytochrome P450, and is eliminated in the feces as an unchanged drug (29, 36, 37). Therefore, AZM may be more advantageous than CLR in macrolide-based multidrug regimens with RIF (13).

This study is the first to document the relationship between the C_{max} of AZM and treatment responses in patients with MAC-LD under different treatment regimens. Our patients with MAC-LD were treated in accordance with the current guidelines (3), which recommend intermittent therapy for patients with treatment-naive noncavitary nodular bronchiectatic disease and daily therapy for patients with cavitary disease or previously treated disease. In patients who received an intermittent regimen that included 500 mg of AZM three times weekly, favorable microbiological responses and an AZM C_{max} of >0.2 μg/ml were achieved in 82.0% (73/89) and 74.2% (66/89) of patients, respectively. There was no association between the AZM C_{max} and microbiological response in patients who received intermittent therapy, and the basis for this is unclear. However, it is possible that given the high C_{max} achieved in this study group and the milder disease, the threshold level of AZM needed for effectiveness was present in the majority of patients, and that other factors were greater determi-

TABLE 3 Associations between estimated C_{max} of the antibiotics and microbiological response according to treatment regimen[a]

Therapy type	C_{max} (μg/ml) by drug[b]	Favorable responses	Unfavorable responses	Univariate analysis		Multiple logistic regression[c]	
				OR (95% CI)	*P*	aOR (95% CI)	*P*
Daily	AZM	0.24 (0.14–0.51)	0.18 (0.08–0.35)	1.53 (1.01–2.31)	0.045	1.58 (1.01–2.48)	0.044
	AZM ≥0.2	29/52 (55.8)	12/25 (48.0)	1.37 (0.53–3.56)	0.523	1.45 (0.52–4.04)	0.476
	AZM ≥0.4	21/52 (40.4)	4/25 (16.0)	3.56 (1.07–11.86)	0.039	3.98 (1.06–14.85)	0.040
	RIF[d]	11.2 (7.4–15.1)	13.7 (8.3–20.4)	0.92 (0.64–1.34)	0.678	1.00 (0.66–1.52)	0.989
	RIF ≥8.0	36/52 (69.2)	20/25 (80.0)	0.56 (0.18–1.76)	0.324	0.68 (0.19–2.38)	0.545
	EMB[d]	3.4 (1.8–4.6)	2.4 (1.7–3.5)	1.34 (0.79–2.25)	0.279	1.30 (0.73–2.31)	0.367
	EMB ≥2.0	37/52 (71.2)	17/25 (68.0)	1.16 (0.41–3.26)	0.777	1.14 (0.38–3.46)	0.810
Intermittent	AZM[d]	0.65 (0.18–1.31)	1.00 (0.18–1.44)	0.84 (0.59–1.19)	0.322	0.85 (0.58–1.23)	0.379
	AZM ≥0.2	54/73 (74.0)	12/16 (75.0)	0.95 (0.27–3.30)	0.932	1.01 (0.27–3.72)	0.991
	RIF[d]	11.2 (3.0–21.6)	12.0 (6.5–19.5)	0.87 (0.64–1.18)	0.382	0.93 (0.67–1.27)	0.636
	RIF ≥8.0	47/73 (64.4)	11/16 (68.8)	0.82 (0.26–2.62)	0.740	1.12 (0.33–3.84)	0.861
	EMB[d,e]	3.5 (2.0–5.6)	4.8 (3.0–6.6)	0.65 (0.35–1.21)	0.177	0.63 (0.33–1.23)	0.176
	EMB ≥2.0	55/72 (76.4)	14/16 (87.5)	0.46 (0.10–2.24)	0.338	0.41 (0.08–2.13)	0.291

[a] Data are presented as median (interquartile range) or number/total number (%). CI, confidence interval; OR and aOR, odds ratio and adjusted odds ratio, respectively.
[b] AZM, azithromycin; C_{max}, peak plasma concentration; EMB, ethambutol; RIF, rifampin.
[c] In univariate and multiple logistic regression analyses, the C_{max} of each drug was analyzed after $\log_2$ transformation.
[d] Adjusted for etiologic pathogen, fibrocavitary disease, positive sputum smear, and use of injectable drugs in patients with daily therapy and adjusted for etiologic pathogen and positive sputum smear in patients with intermittent therapy.
[e] One patient with favorable response had a missing C_{max} value for EMB.

nants of outcome. In patients who received a daily regimen that included 250 mg of daily AZM, favorable microbiological responses and an AZM C_{max} of >0.2 μg/ml were achieved in only 67.5% (52/77) and 53.2% (41/77) of patients, respectively. In contrast to the intermittent-therapy group, a higher C_{max} of AZM was associated with a favorable microbiological response in patients receiving daily therapy. However, the overall poorer responses of patients on daily therapy may be largely due to the greater severity of cavitary disease than noncavitary disease.

The currently recommended treatment regimens for MAC-LD resulted in significant drug interactions and low C_{max} levels of AZM, which is the most important drug within the regimen, especially in patients who receive daily therapy. Several modifications, such as increased AZM doses or replacement of RIF with another drug, may increase the C_{max} of AZM and improve treatment outcomes in severe MAC-LD. A daily AZM-based regimen with a higher dose of AZM has not been fully evaluated for its efficacy and safety in the treatment of MAC-LD. A previous study reported that a daily 600-mg AZM-based regimen resulted in higher AZM C_{max} than did a daily 300-mg AZM-based regimen (27). However, a higher dose of AZM was associated with more frequent complications, such as gastrointestinal symptoms and hearing impairment (27). Interestingly, a recent study using the hollow-fiber system model of MAC suggested that 500 mg of AZM might be also suboptimal and that higher doses of AZM may be necessary to treat MAC-LD (38). Another possible means to increase the AZM C_{max} is to use a two-drug regimen (AZM and EMB) without RIF or with the substitution of other drugs, such as clofazimine, for RIF. A preliminary randomized study showed that the clinical efficacy of a daily two-drug regimen (CLR and EMB) was similar to that of a daily three-drug regimen (CLR, EMB, and RIF) for MAC-LD (39). In addition, the replacement of RIF with clofazimine and daily treatment with CLR or AZM, EMB, and clofazimine achieved similar treatment outcomes in patients with MAC-LD in two retrospective studies (40, 41). Further clinical studies are warranted to evaluate these treatment options for MAC-LD.

This study has several limitations. First, this retrospective study was performed at a single referral center. Second, only one sample was collected after 2 h of drug administration in the outpatient clinical setting. Third, drug susceptibility tests for AZM were not performed during the study period. Therefore, we could not evaluate the associations between C_{max} and MIC (C_{max}/MIC) or the area under the curve (AUC)/MIC of AZM. Fourth, we did not measure AZM concentrations in the epithelial lining fluid or in alveolar macrophages at the site of MAC infection. Finally, there was no validation group for confirming the associations between the new cut point of C_{max} AZM (≥0.4 μg/ml) and favorable treatment responses in patients with MAC-LD who received daily therapy. Therefore, the generalizability of our findings may be limited, and further large-scale studies are needed to evaluate the associations between the pharmacokinetics and pharmacodynamics of the investigated drugs and microbiological response.

In summary, a low AZM C_{max} was common in patients receiving a daily AZM-based multidrug regimen for MAC-LD, and a higher AZM C_{max} was associated with favorable microbiologic outcomes. When a daily AZM-based multidrug regimen is used for treating severe MAC-LD, such as cavitary disease, the currently recommended AZM dose may be suboptimal. Further analyses, including investigating the effects of increased AZM doses or substituting RIF with another drug, are needed to confirm the associations between AZM C_{max} and microbiologic outcomes.

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