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Macrolide/Azalide Therapy for Nodular/Bronchiectatic *Mycobacterium avium* Complex Lung Disease

Richard J. Wallace Jr, MD, FCCP; Barbara A. Brown-Elliott, MS; Steven McNulty, BS; Julie V. Philley, MD; Jessica Killingley, BS; Rebecca W. Wilson, BS; Deanna S. York, RN; Sara Shepherd, MS; and David E. Griffith, MD, FCCP

BACKGROUND: There is no large study validating the appropriateness of current treatment guidelines for *Mycobacterium avium* complex (MAC) lung disease. This is a retrospective single-center review evaluating the efficacy of macrolide/azalide-containing regimens for nodular/bronchiectatic (NB) MAC lung disease.

METHODS: Patients were treated according to contemporary guidelines with evaluation of microbiologic responses. Macrolide susceptibility of MAC isolates was done at initiation of therapy, 6 to 12 months during therapy, and on the first microbiologic recurrence isolate. Microbiologic recurrence isolates also underwent genotyping for comparison with the original isolates.

RESULTS: One hundred eighty patients completed > 12 months of macrolide/azalide multidrug therapy. Sputum conversion to culture negative occurred in 154 of 180 patients (86%). There were no differences in response between clarithromycin or azithromycin regimens. Treatment regimen modification occurred more frequently with daily (24 of 30 [80%]) vs intermittent (2 of 180 [1%]) therapy ($P = .0001$). No patient developed macrolide resistance during treatment. Microbiologic recurrences during therapy occurred in 14% of patients: 73% with reinfection MAC isolates, 27% with true relapse isolates ($P = .03$). Overall, treatment success (ie, sputum conversion without true microbiologic relapse) was achieved in 84% of patients. Microbiologic recurrences occurred in 74 of 155 patients (48%) after completion of therapy: 75% reinfection isolates, 25% true relapse isolates.

CONCLUSIONS: Current guidelines for macrolide/azalide-based therapies for NB MAC lung disease result in favorable microbiologic outcomes for most patients without promotion of macrolide resistance. Intermittent therapy is effective and significantly better tolerated than daily therapy. Microbiologic recurrences during or after therapy are common and most often due to reinfection MAC genotypes. CHEST 2014; 146(2):276-282

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ABBREVIATIONS: AFB = acid-fast bacilli; MAC = *Mycobacterium avium* complex; MR = microbiologic recurrence; NB = nodular/bronchiectatic; NTM = nontuberculous mycobacteria; tiw = three times weekly; UTHSCT = University of Texas Health Science Center, Tyler

AFFILIATIONS: From the Department of Microbiology (Dr Wallace; Mss Brown-Elliott, Killingley, and York; and Mr McNulty), the Department of Medicine (Drs Wallace, Philley, and Griffith and Ms Brown-Elliott), and the Department of Pathology (Dr Wallace and Mss Wilson and Shepherd), University of Texas Health Science Center at Tyler, Tyler, TX.

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CORRESPONDENCE TO: David E. Griffith, MD, FCCP, The University of Texas Health Science Center at Tyler, 11937 US Hwy 271, Tyler, TX 75708; e-mail: david.griffith@uthct.edu

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Introduction of the macrolide clarithromycin and the closely related azalide azithromycin in the early 1990s was associated with significant improvement in the therapy of *Mycobacterium avium* complex (MAC) infections.[1-4] Several single-center, uncontrolled trials and one prospective randomized trial of MAC lung disease therapy demonstrated favorable treatment responses to macrolide/azalide-based regimens.[3-13] These studies are difficult to compare, as doses of the macrolide/azalide, choices of companion drugs, and definitions of sputum conversion and disease relapse varied.[3-13] Additionally, patients with MAC disease characterized by nodules and bronchiectasis (nodular/bronchiectatic [NB] MAC disease) and those with disease characterized by upper lobe fibronodular and fibrocavitary disease were analyzed together, which is questionable from a pathophysiologic perspective.[14-16]

A further complicating factor emerged with the recognition that patients with NB disease can be infected with multiple MAC genotypes and that microbiologic recurrences (MRs) during or after therapy may be the result of new MAC genotypes and presumed reinfection rather than true disease relapse.[17,18] We report results of a consistent protocol for treatment of MAC lung disease at a single center using a microbiologic end point and contemporary diagnostic and MAC lung disease treatment standards.[19,20]

FOR EDITORIAL COMMENT SEE PAGE 244

Materials and Methods

Patients treated at The University of Texas Health Science Center, Tyler, (UTHSCT), Texas, for NB MAC lung disease not previously reported are included in this report. The clinical treatment outcome studies, retrospective chart reviews, and maintenance of a database were approved by the Institutional Review Board of UTHSCT (Institutional Review Board #760, #11-009).

Daily therapy consisted of rifampin 600 mg or rifabutin 150 mg, ethambutol 15 mg/kg, clarithromycin 1,000 mg in divided doses or 15 mg/kg for patients weighing $<$ 50 kg, or azithromycin 250 mg. Three times weekly (tiw) therapy consisted of rifampin 600 mg or rifabutin 150 to 300 mg, ethambutol 25 mg/kg, and clarithromycin 1,000 mg in divided doses, or azithromycin 500 mg. Medication choices and frequency of dosing were at the discretion of the investigator.

Three routine expectorated sputum cultures for acid-fast bacilli (AFB) were collected at initiation of therapy. For patients unable to produce sputum by spontaneous expectoration, sputum induction was performed with nebulized hypertonic saline with directions for home use. Sputum samples were collected monthly from patients receiving therapy and then every 1 to 2 months for the length of follow-up. Patients initially diagnosed bronchoscopically did not routinely undergo repeat bronchoscopies, as most patients were successful in submitting samples after induction.

Sputum samples were processed in the UTHSCT clinical laboratory using standard decontamination procedures, fluorochrome microscopy, solid media culture on a biplate of Middlebrook 7H10 agar with and without antibiotics, and a broth culture (BACTEC 960; Becton, Dickinson and Company; ESP; Thermo Fisher Scientific) as previously described.[4,5] MAC isolates were identified using AccuProbe (Hologic Gen-Probe Inc). Semiquantitative AFB smear and culture results for each submitted clinical specimen during and after therapy were recorded as previously described.[4,5]

Macrolide/azalide susceptibilities were performed at initiation of therapy, at 6 to 12 months while receiving therapy, or on the first MR isolate. Susceptibilities used broth microdilution according to contemporary guidelines.[21,22] Clarithromycin was used as the class drug for both macrolide and azalide susceptibility.

Sputum conversion was defined as three or more consecutive negative AFB cultures over a minimum of 3 months. In patients unable to expectorate, a single culture-negative bronchoscopically obtained specimen was also considered conversion. The primary treatment end point was 12 months of negative cultures for the initial MAC genotype(s). A single positive culture during therapy after sputum conversion did not change the duration of therapy if other monthly cultures were negative. Failure to convert sputum to culture negative with 12 months of macrolide/azalide therapy was considered treatment failure.

Two or more positive AFB cultures for MAC after sputum conversion constituted MR. Patients with positive cultures receiving therapy after sputum conversion or after successful completion of therapy had genotyping performed on three pretreatment MAC isolates and all MR isolates. Two or more positive MR cultures for the pretreatment genotype were considered a true relapse. Two or more positive cultures for a new genotype(s) were considered reinfection. If genotyping was not performed, two or more positive cultures after sputum conversion were considered a probable true relapse. Treatment success was defined as 12 months with negative sputum cultures while receiving therapy without isolation of a true relapse MAC isolate.

Genotyping was performed using pulsed-field gel electrophoresis as previously described.[17,18] Definitions of isolates as indistinguishable (no band differences), probably related (four to six band differences), or unrelated (more than seven band differences) were the same as in previous studies.[17,18] Restriction enzymes used were *Xba*I and AseI or *Dra*I.

Group data are expressed as means and SD. Comparison of outcomes between patient treatment groups was done with Fisher exact test or Pearson χ^2 test. The binomial test was used to compare the frequencies of new infections vs true relapse in episodes with subsequent MR. Analysis of other clinical variables between groups was done with the *t* test for equality of means after evaluation of the data with Levene test for equality of variances. Two-tailed *P* values were used for all *t* tests. Significance of all comparisons was determined with a *P* value $<$ .05. IBM SPSS Statistics, version 21 was used to calculate these values.

Results

Two hundred seven consecutively treated patients were started on MAC therapy for NB MAC lung disease during the study period. Twenty-seven patients were excluded from the main analysis because they did not receive at least 12 months of macrolide/azalide-based therapy, leaving 180 patients who met the inclusion criteria for analysis. Fifty-five of 180 patients (31%) received $>$ 6 months macrolide-based therapy prior to treatment at our facility. Twenty-one patients (15%) received

either streptomycin (78%) or amikacin (22%) for 2 to 3 months at the outset of therapy, too few patients to allow statistical analysis. Six patients (3%) had surgical resection, which also did not permit statistical analysis.

The patients with NB MAC in the analysis were 95% white, 90% female, 68% lifetime nonsmokers, and 32% former smokers (23.4 ± 27.0 pack-years), with a mean age at the time of the first positive culture for MAC of 67.0 ± 12.1 years. Patients had a mean weight of 57.4 ± 9.5 kg and BMI of 20.9 ± 4.3. There were no significant differences in these demographic parameters between patients receiving clarithromycin or azithromycin regimens.

All 180 patients underwent one or more chest CT scans; 94% were diagnosed with bronchiectasis, and 95% had multiple pulmonary nodules. All patients had bronchiectasis and/or multiple pulmonary nodules. There were no differences in radiographic abnormalities between patients who received clarithromycin or azithromycin regimens. Cavitating lesions in mid and lower lung zones were present in four patients (2%); three of four lesions (75%) were < 2.0 cm in diameter. Sputum AFB smears and culture results are summarized in Table 1. There were no significant differences in any parameters between patients receiving clarithromycin or azithromycin regimens. All patients had documented macrolide-susceptible (minimum inhibitory concentration ≤ 8.0 μg/mL) MAC isolates prior to treatment. No patient developed macrolide/azalide resistance during study-related treatment.

Patients taking daily medication commonly changed treatment regimens because of medication intolerance, primarily GI symptoms (Table 2). Sixty-seven percent of daily clarithromycin treatment episodes and 77% of daily azithromycin treatment episodes were changed to tiw therapy compared with only 4% tiw clarithromycin ($P = .001$) and 3% azithromycin ($P = .001$) episodes changed to daily therapy. Overall, 71% daily macrolide/azalide treatment episodes and 3% tiw macrolide/azalide treatment regimens were not completed with the initially prescribed regimen ($P = .001$). Despite frequent treatment modification, only 18 of 207 treatment episodes (9%) were stopped before completion of 12 months of therapy because of medication intolerance, and only two treatment episodes (1%) were not completed because of macrolide/azalide intolerance.

Seventy-eight of 91 clarithromycin treatment episodes (86%) and 76 of 89 azithromycin treatment episodes (85%) resulted in sputum conversion ($P = .4$), with a combined rate of sputum conversion for all macrolide/azalide episodes of 86% (154 of 180) (Table 3). Treatment episodes associated with sputum conversion were significantly shorter than those not associated with sputum conversion: 17.1 ± 7.2 months vs 25.1 ± 16.8 months ($P \leq .001$). In contrast to prior reports, there was no significant difference in sputum conversion rates between subjects who received > 6 months of macrolide/azalide therapy (83%) and those with < 6 months prior macrolide/azalide therapy (87%) ($P = .4$).

Twenty-seven patients were treated < 12 months: two with macrolide/azalide intolerance, two with ethambutol optic neuritis, one with ethambutol peripheral neuropathy, 12 with rifamycin intolerance, nine lost to follow-up, and one patient who died of unrelated causes. Six of 27 patients (22%) had sputum conversion, compared with 86% of patients who completed at least 12 months of therapy ($P \leq .001$).

Twenty-five of 180 patients (14%) with sputum conversion subsequently had two or more positive sputum cultures for MAC while still receiving therapy (Table 4). Of the 21 of 25 episodes (84%) wherein MAC isolates had genotyping, 16 of 21 of the isolates were new genotypes (76%) (reinfections), whereas five of 31 were genotypes identical to the original MAC genotype (34%) (true relapses) ($P = .04$). For patients with a single MAC isolate after sputum conversion, 18 of 23 were new genotypes (78%), whereas five of 23 were identical to the original MAC genotype (22%) ($P = .001$) (Table 4).

Overall treatment success, defined as sputum conversion without MR with the original infecting MAC genotype (ie, true relapse), was achieved in 84% of patients.

TABLE 1] AFB Smear and Culture Results per Patient

AFB Smears/Cultures[a]	Total Patients (N = 180)
Total specimens for AFB smear and culture obtained/patient	28.7 ± 17.3
Total specimens for AFB smear and culture obtained on therapy/patient	13.3 ± 7.7
AFB smear-positive specimens on ≥ 1 specimen	52 of 180 (29)
Total positive cultures for MAC/patient	7.6 ± 7.8
Positive single MAC cultures from bronchoscopy	23 of 180 (13)

Data are presented as mean ± SD or No. patients (%). AFB = acid-fast bacilli; MAC = *Mycobacterium avium* complex.
[a]Includes pretreatment, during treatment, and posttreatment. specimens processed at the University of Texas Health Science Center at Tyler microbiology laboratory.

TABLE 2] Treatment Episodes With Initial Intermittent or Daily Macrolide/Azalide-Based Therapy For NB MAC Lung Disease

Episode	tiw, No. (%)	Daily, No. (%)	Combined tiw/Daily, No. (%)	*P* Value
Regimen modification[a]				
Clarithromycin	3 of 74 (4)	14 of 21 (67)	17 of 95 (18)	.0001[b]
Azithromycin	2 of 72 (3)	10 of 13 (77)	13 of 85 (15)	.0001[b]
Clarithromycin + azithromycin	5 of 180 (3)	24 of 34 (71)	40 of 339 (12)	.001[b]
Sputum conversion[c]				
Clarithromycin	75 of 87 (86)	3 of 4 (75)	78 of 91 (86)	.1[d]
Azithromycin	72 of 85 (85)	4 of 4 (100)	76 of 89 (85)	…
Clarithromycin + azithromycin	147 of 172 (85)	7 of 8 (88)	154 of 180 (86)	…

NB = nodular/bronchiectatic; tiw = three times weekly or Monday-Wednesday-Friday. See Table 1 legend for expansion of other abbreviation.
[a]Macrolide/azalide given at the initiation of treatment.
[b]tiw vs daily therapy.
[c]Macrolide/azalide given at the completion of therapy.
[d]Clarithromycin-containing regimen vs azithromycin-containing regimen.

Seventy-four of 155 successful treatment episodes (48%) subsequently had two or more positive sputum cultures for MAC (Table 4). Of the 53 of 74 episodes (72%) that underwent genotyping, 75% represented reinfection, whereas 25% were true relapse ($P = .0001$). True relapse isolates occurred significantly earlier after successful completion of therapy than reinfection isolates: 6.2 ± 12.5 months vs 17.5 ± 21.0 months (95% CI, 4.1-18.4; $P = .003$). For patients with only a single MAC isolate after successful completion of therapy, 18 of 23 represented new genotypes (78%), whereas five of 23 were identical to the original genotype (22%) ($P \leq .001$).

Discussion

Patients with macrolide-susceptible NB MAC lung disease had a high rate of sputum conversion to AFB culture negative (86%) with macrolide/azalide-based regimens. There were no significant differences in microbiologic responses between clarithromycin and azithromycin treatment regimens. Overall treatment success was achieved in 84% of patients. Consistent with prior reports, our findings support currently recommended macrolide/azalide treatment regimens for NB MAC lung disease.[4,5,11-13,20,23]

Also consistent with prior experience, there was a high degree of intolerance to MAC treatment medications, especially when given daily.[24-31] It is clear that tiw regimens are significantly better tolerated than daily regimens and associated with comparably favorable microbiologic outcomes, although the number of patients completing daily therapy was too small to allow

TABLE 3] Microbiologic Results for All Patients With Clarithromycin and Azithromycin-Containing Regimens

Results	Clarithromycin	Azithromycin	Total	*P* Value
No. patients	91	89	180	…
Sputum conversion, No. (%)	78 of 91 (86)	76 of 89 (85)	154 of 180 (86)	.4[a]
Days to convert	130.6 ± 149.7	123.2 ± 109.8	…	.7[a]
Months negative cultures on therapy	12.1 ± 2.5	12.6 ± 3.5	…	.3[a]
Treatment duration, mo	18.6 ± 8.8	18.8 ± 6.3	…	.9[a]
MR on therapy, No. (%)[b]	14 of 91 (15)	11 of 89 (12)	25 of 180 (14)	.4[a]
MR off therapy, No. (%)[c]	41 of 77 (53)	33 of 78 (42)	74 of 180 (48)	.6[a]
Months microbiologic follow-up off therapy	44.1 ± 31.4	40 ± 25.5	…	.1
Months clinical follow-up off therapy	44.4 ± 31.3	40.7 ± 27.1	…	.2

Data are presented as mean ± SD or No. (%). Macrolide/azalide given at the completion of treatment. MR = microbiologic recurrence. See Table 1 legend for expansion of other abbreviations.
[a]Clarithromycin-containing regimen vs azithromycin-containing regimen.
[b]MR: two or more positive sputum AFB cultures for MAC after sputum conversion on therapy.
[c]MR: two or more positive sputum AFB cultures for MAC after successful completion of therapy.

TABLE 4] Patients With Sputum Conversion to AFB Culture Negative With Subsequent MR While Still on Therapy or After Completion of Therapy

Measure	Still on Therapy[a]	After Completion of Therapy[b]
MR after sputum conversion	25 of 180 (14)	74 of 155 (48)
Genotyping on ≥ 2 MR isolates	21 of 25 (84)	53 of 74 (72)
New infection	10 of 21 (48)	40 of 53 (75)
True relapse	11 of 21 (52)	13 of 53 (25)
Genotyping of single MAC isolates	22 of 23 (93)	37 of 45 (82)
New infection	18 of 23 (78)	28 of 37 (76)
True relapse	5 of 23 (22)	9 of 37 (24)
Mean length of follow-up off therapy, mo	…	41.8 ± 37.4
Mean no. cultures off therapy	…	13.2 ± 7.0
Mean no. negative cultures off therapy before MR	…	9.5 ± 28.7
Mean no. positive AFB cultures off therapy	…	5.5 ± 6.0
Time to first microbiologic relapse isolate, mo	…	12.8 ± 15.3

Data are presented as mean ± SD or No. (%). See Table 1 and 3 legends for expansion of abbreviations.
[a]MR: two or more positive sputum AFB cultures for MAC after sputum conversion on therapy.
[b]MR: two or more positive sputum AFB cultures for MAC after successful completion of therapy.

statistical comparison with patients receiving tiw therapy. In contrast to previous studies, microbiologic response was not different between patients with or without ≥ 6 months prior macrolide/azalide therapy.

For patients with NB MAC lung disease who had sputum conversion, the subsequent isolation of MAC from sputum or MR was common, either while still receiving therapy (14%) or after completion of successful therapy (48%). In either circumstance, MR most frequently represented reinfection with the isolation of new MAC genotype(s), rather than true relapse with recurrence of the pretreatment MAC genotype. MR isolates obtained < 6 months after discontinuation of therapy were most often true relapse isolates, whereas MR isolates obtained > 6 months after discontinuation of therapy were most often reinfection isolates.

Patients with a single MAC isolate either during or after therapy most often had reinfection isolates and would not meet the nontuberculous mycobacteria (NTM) disease microbiologic diagnostic criterion.[19] In this circumstance, the clinician can be reassured that the patient probably would not require reinstitution of therapy, although continued clinical and microbiologic follow-up is necessary to confirm that other MAC isolates are not obtained.

Patients with two positive MAC reinfection isolates are more challenging, since these patients would meet the NTM microbiologic diagnostic criterion.[19] Determining the clinical significance of these isolates requires an approach similar to that for patients with an initial MAC isolation including evaluation of symptoms and radiographic findings. Whether two or more reinfection isolates are associated with progressive MAC disease can only be established with clinical assessment and follow-up. Alternatively, patients with multiple true relapse MAC isolates are considered treatment failures and usually require treatment intensification. Without MAC genotyping, patients with MR during therapy would be uncritically labeled as treatment failures, triggering more aggressive treatment efforts. The recently introduced mycobacterial interspersed repetitive-unit-variable-number tandem repeat is simpler and more rapid than pulsed-field gel electrophoresis and may facilitate more widespread use of this type of analysis.[32]

The explanation for the reinfection phenomenon is likely multifactorial, including ongoing host susceptibility to NTM infection due to bronchiectasis with unavoidable environmental MAC exposure. Mounting evidence supports exposure to water from household plumbing as the major risk factor for *M avium* NB lung disease.[32-34] Alternatively, it is possible that MAC infections are polyclonal and that new predominant genotypes emerge during therapy, a possibility that would not change the diagnostic approach previously outlined.

In this series, no treatment episode resulted in a macrolide-resistant MAC isolate. The development of macrolide resistance is associated with increased rates of treatment failure and mortality compared with macrolide-susceptible MAC isolates.[35] Consistent with current laboratory

guidelines for the management of MAC lung disease, we did not routinely obtain in vitro susceptibility information for therapeutic agents other than macrolide/azalide, as evidence supports a correlation between in vitro susceptibility and in vivo clinical response for macrolides only.[5,9,11-13,20-22,36,37]

This study has several limitations. These results are not based on a randomized controlled NB MAC treatment trial comparing clarithromycin vs azithromycin or daily vs intermittent treatment regimens. This report also focuses on microbiologic response as a primary MAC treatment outcome rather than symptomatic or radiographic treatment responses. It is also difficult to determine if treatment nonadherence contributed to treatment failure, although compliance with frequent clinic visits, toxicity monitoring, and submission of specimens for AFB analysis suggest an overall patient motivation.

In summary, this is the first study in the 20 years of macrolide/azalide therapy for MAC lung disease to our knowledge that includes a large number of patients with MAC lung disease with a defined and consistent disease presentation (NB disease), uniform treatment according to published guidelines, presumptive patient adherence with the treatment regimen, extended follow-up after completion of therapy, and state-of-the-art laboratory support including genotyping analysis for MR MAC isolates. This study shows that adherence to current treatment guidelines for MAC lung disease is associated with a high rate of favorable microbiologic response that does not place patients at risk for the development of macrolide-resistant MAC isolates. MRs are frequent, requiring genotyping of MR MAC isolates to interpret their significance. With increasing MAC lung disease prevalence, reliable treatment guidance is increasingly important.[38,39] Pending improved treatment regimens or strategies, use of the currently recommended macrolide/azalide-based MAC treatment regimens is effective for the majority of patients with NB MAC lung disease.

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