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The Significance of *Mycobacterium abscessus* Subspecies *abscessus* Isolation During *Mycobacterium avium* Complex Lung Disease Therapy

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BACKGROUND: Isolation of *Mycobacterium abscessus* subspecies *abscessus* (MAA) is common during *Mycobacterium avium* complex (MAC) lung disease therapy, but there is limited information about the clinical significance of the MAA isolates.

METHODS: We identified 53 of 180 patients (29%) treated for MAC lung disease who had isolation of MAA during MAC lung disease therapy. Patients were divided into those without (group 1) and those with (group 2) MAA lung disease.

RESULTS: There were no significant demographic differences between patients with and without MAA isolation or between groups 1 and 2. Group 1 and 2 patients had similar total sputum cultures obtained ($P = .7$; 95% CI, −13.4 to 8.6) and length of follow-up ($P = .8$; 95% CI, −21.5 to 16.1). Group 2 patients had significantly more total positive cultures for MAA (mean ± SD, 15.0 ± 11.1 vs 1.2 ± 0.4; $P < .0001$; 95% CI, −17.7 to −9.9), were significantly more likely to develop new or enlarging cavitary lesions while on MAC therapy ($P > .0001$), and were significantly more likely to meet all three American Thoracic Society diagnostic criteria for nontuberculous mycobacterial disease (21 of 21 [100%] vs 0 of 32 [0%]; $P < .0001$) compared with group 1 patients. Group 1 patients were significantly more likely to have single, positive MAA cultures than group 2 patients (25 of 31 vs 0 of 21; $P < .0001$).

CONCLUSIONS: Microbiologic and clinical follow-up after completion of MAC lung disease therapy is required to determine the significance of MAA isolated during MAC lung disease therapy. Single MAA isolates are not likely to be clinically significant.

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ABBREVIATIONS: AFB = acid-fast bacilli; ATS = American Thoracic Society; IDSA = Infectious Diseases Society of America; MAA = *Mycobacterium abscessus* subspecies *abscessus;* MAC = *Mycobacterium avium* complex; MAM = *Mycobacterium abscessus* subspecies *massiliense;* NB = nodular/bronchiectatic; NTM = nontuberculous mycobacteria; VNTR = variable number tandem repeat

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For > 20 years, it has been recognized that *Mycobacterium abscessus* subspecies *abscessus* (MAA) can sometimes be cultured from the sputum of patients who also have sputum acid-fast bacilli (AFB) cultures positive for *Mycobacterium avium* complex (MAC).[1] For many patients, dual isolation of MAA and MAC has uncertain clinical significance that is complicated by the complexities of treating MAA lung infection. First, there is no convenient or effective dual therapy for MAC and MAA lung infections.[2] Simply continuing MAC therapy will not provide adequate treatment of MAA. Second, empirical therapy for presumed MAA infection is daunting because of the need for parenteral antibiotics and the generally slow and unpredictable clinical response to therapy.[1,3,4] Ultimately, the gravity of committing a patient to prolonged potentially toxic medication for MAA therapy gives pause to clinicians even when the diagnosis is not in doubt.

The significance of co-isolation of MAA and MAC, or more generally the co-isolation of more than one mycobacterial species from the same patient, has not been rigorously studied, although there is some potentially pertinent information from patients treated for TB.[5-10] To date, however, there has not been sufficient data amassed to make confident conclusions about optimal management of patients with nontuberculous mycobacterial (NTM) isolation during a course of TB therapy. The major shortcomings of the available studies have been either insufficient duration of patient follow-up or inadequate, individual patient clinical assessment.[5-10]

We recently reported microbiologic treatment outcomes for 180 patients with MAC lung disease.[11] A substantial number of those patients had concomitant isolation of other NTM with MAC, primarily MAA. We report the clinical significance of the concomitant MAC and MAA isolation after extensive patient follow-up.

Materials and Methods

Between 2000 and the 2012 we identified patients treated for nodular/bronchiectatic (NB), macrolide susceptible MAC lung disease at our institution who were also found to have MAA respiratory isolates during the course of MAC therapy.[11] Patients diagnosed with MAA lung disease met diagnostic criteria for NTM lung disease including multiple isolations of MAA from sputum with clinical and radiographic deterioration after prior improvement on MAC therapy while those not diagnosed with MAA disease met no diagnostic criterion or only the microbiologic criterion.[2,12] The clinical treatment outcome studies, retrospective chart reviews, and maintenance of a database were approved by the institutional review board of University of Texas Health Science Center, Tyler (no. 760, 11-009).

Sputum was collected for AFB analysis as previously described.[13,14] Briefly, three routine, expectorated sputum AFB cultures were collected at initiation of MAC therapy either spontaneously or by induction with nebulized hypertonic saline. Sputum samples were collected at 1- to 2-month intervals while on therapy and then every 2 to 3 months for the length of follow-up.

Sputum samples were processed in the University of Texas Health Science Center, Tyler, 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 Co; and/or VersaTREK, Thermo Fisher Scientific Inc), as previously described.[13,14] MAC isolates were identified using AccuProbe (Hologic Inc). Semiquantitative AFB smear and culture results for each submitted clinical specimen during and after therapy were recorded as previously described.[13,14]

Isolates of rapidly growing mycobacteria were identified to species level using polymerase chain reaction-restriction fragment length analysis of an approximately 441-base pair heat shock protein (*hsp*) gene using two restriction endonucleases, *BstE*II and *Hae*III, as previously described.[15] A third restriction enzyme, *Sml*I, was added to differentiate *M abscessus* subspecies *massiliense* from *M abscessus* subspecies *bolletii*.

Culture media including sheep blood agar, chocolate, and MacConkey and/or eosin methylene blue agar were inoculated and examined for the presence of potential pathogens (eg, *Streptococcus pneumoniae*, *Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus,* and gram-negative rods, including *Pseudomonas* and other). For most patients, additional selective media including *Pseudomonas cepacia* agar were used.

Sputum conversion for both MAC and MAA disease was defined as three or more consecutive, negative AFB cultures over a minimum of 3 months. The primary treatment end point for MAC and MAA therapy was 12 months of negative cultures while on therapy. Failure to convert sputum to culture negative with 12 months of therapy was considered treatment failure.

Group data are expressed as means and SD. Comparison of outcomes between patient treatment groups was done with the Fisher exact test or Pearson χ^2 test. Analysis of other clinical variables between groups was done with the *t* test for equality of means after evaluation of the data with the 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 $< .05$. SPSS Statistics, version 21 (IBM Corp) was used to calculate these values.

Results

Fifty-three of 180 patients (29%) with NB MAC treated from 2000 until 2012 at our institution also had MAA isolated from respiratory specimen(s) at some point during their MAC therapy. The patients in the analysis were 100% non-Hispanic white; 92% women; 75% lifetime nonsmokers; and 25% former smokers (24.0 ± 26.0 pack-years), with a mean age at time of the first positive culture for MAC of 73.2 ± 7.6 years. Patients had a mean weight of 57.4 ± 9.5 kg and BMI of 20.4 ± 4.1. None of these parameters was significantly different among patients with both MAC and MAA