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

David E. Griffith, MD, FCCP; Julie V. Philley, MD; Barbara A. Brown-Elliott, MS; Jeana L. Benwill, MD; Sara Shepherd, MS; Deanna York, RN; and Richard J. Wallace Jr, MD, FCCP

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 $\pm$ 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 cavitory 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.

CHEST 2015; 147(5):1369-1375

Manuscript received May 30, 2014; revision accepted October 1, 2014; originally published Online First October 30, 2014.

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

AFFILIATIONS: From the Departments of Medicine (Drs Griffith, Philley, Benwill, and Wallace) and Microbiology (Mss Brown-Elliott, Shepherd, and York and Dr Wallace), the University of Texas Health Science Center, Tyler, TX.

Data included in this manuscript were presented in part at the European Respiratory Society Annual Meeting, September 7-11, 2013, Barcelona, Spain.

FUNDING/SUPPORT: This manuscript was supported in part by institutional funds from the University of Texas Health Science Center, Tyler; the Amon G. Carter Foundation (Dr Wallace), Ft. Worth, TX; and the Moncrief Foundation (Dr Griffith), Ft. Worth, TX.

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

© 2015 AMERICAN COLLEGE OF CHEST PHYSICIANS. Reproduction of this article is prohibited without written permission from the American College of Chest Physicians. See online for more details.

DOI: 10.1378/chest.14-1297

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.

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}

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;

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.

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, *Bst*EII 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.

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

isolated from sputum compared with the entire 180-patient cohort.

Patients with MAA isolation were divided into two groups: Group 1 comprised patients not diagnosed with clinically significant MAA lung disease, and group 2 comprised those diagnosed with clinically significant MAA lung disease. There were no significant demographic differences between groups 1 and 2 (Table 1). There were no differences between groups 1 and 2 in the composition of MAC treatment regimens, the frequency of drug administration (daily vs intermittent), the duration of MAC therapy, number of patients with prior macrolide-based MAC therapy, and duration between start of MAC therapy and recovery of first MAA isolate (Table 1). All patients in groups 1 and 2 met MAC treatment-success criteria.

Other parameters that were compared between groups 1 and 2 are summarized in Table 2. Patients in groups 1 and 2 had a similar total number of respiratory specimens obtained and similar length of follow-up. Group 2 patients had significantly more respiratory AFB cultures positive for MAA than group 1 patients ($P < .0001$; 95% CI, -17.7 to -9.9), were significantly more likely to develop new or expanding cavitary lesions while on MAC therapy, and were significantly more likely to meet joint American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) diagnostic criteria for NTM lung disease ($P < .0001$). No patient in group 1 met either radiographic or symptomatic diagnostic criteria, even with more than one MAA-positive sputum culture. There was no significant difference between groups 1 and 2 in the isolation of other

NTM known to contaminate municipal (tap) water or bronchiectasis-related bacteria. Even after the diagnosis of MAA lung disease, not all patients were felt to require treatment of MAA. For those patients treated for MAA lung disease, treatment-response rates were similar to previous reports.^{1,3,4}

MAC/MAA Case

A 78-year-old woman presented with cough, sputum production, fatigue, weight loss, and multiple sputum specimens culture positive for MAC. Her initial chest CT scan showed bilateral nodular and reticulonodular densities, which improved while on standard (azithromycin/rifampin/ethambutol) macrolide-based MAC therapy (Figs 1A, 1B). She initially improved symptomatically, but near completion of MAC therapy, she had symptomatic deterioration associated with the advent of multiple positive sputum cultures for MAA and a chest CT scan showing a new, left-lung, cavitary mass-like lesion (Fig 1C). A biopsy specimen of the lesion was AFB culture positive for MAA. Sputum AFB cultures were negative for MAC for > 12 months while on MAC therapy; therefore, the MAC medications were stopped. She was subsequently started on MAA therapy, including parenteral amikacin and tigecycline with oral linezolid. She improved symptomatically, microbiologically, and radiographically with MAA therapy, which was stopped after 6 months per patient request.

Discussion

Recovery of MAA during the course of therapy for MAC lung disease was common in our cohort of patients with NB MAC lung disease. The dual recovery of *M tuberculosis* and NTM has also been noted.⁵⁻¹⁰

TABLE 1 Comparison of Group 1 Patients Who Were Not Diagnosed With MAA Lung Disease vs Group 2 Patients Who Were Diagnosed With MAA Lung Disease

Characteristics	Group 1	Group 2	P Value
Patients, No.	32	21	...
Age, y	72.3 $\pm$ 6.7	75.5 $\pm$ 8.5	.2
Female sex	30 (94)	19 (90)	.9
BMI	20.8 $\pm$ 4.0	19.6 $\pm$ 3.7	.3
Never smoker	23 (72)	16 (76)	.7
TIW MAC therapy (at completion of MAC therapy)	29 (91)	19 (90)	.9
Total duration of MAC therapy, mo	17.9 $\pm$ 6.1	18.7 $\pm$ 8.6	.7
Patients with more than one 6-mo macrolide-based treatment course	11 (34)	8 (38)	.9
Time from initiation of MAC therapy and first MAA isolate, mo	9.9 $\pm$ 5.9	11.3 $\pm$ 7.2	.4

Data given as mean $\pm$ SD or No. (%) unless otherwise indicated. MAA = *Mycobacterium abscessus* subspecies *abscessus*; MAC = *Mycobacterium avium* complex; TIW = three times per week.

TABLE 2] Comparison of Group 1 Patients Who Were Not Diagnosed With MAA Lung Disease vs Group 2 Patients Who Were Diagnosed With MAA Lung Disease

Characteristics	Group 1	Group 2	P Value (95% CI)
Patients, No.	32	21	...
Total AFB cultures obtained	39.3 ± 19.4	41.7 ± 19.7	0.7 (95% CI, −13.4 to 8.6)
Total AFB cultures positive for MAA	1.2 ± 0.4 (range, 1-3)	15.0 ± 11.1	<.0001 (95% CI, −17.7 to −9.9)
Single, positive MAA AFB culture	25/32 (78)	0/21	<.0001
ATS/IDSA microbiologic diagnostic criteria met	7/32 (22)	21/21 (100)	<.0001
All three ATS/IDSA diagnostic criteria met	0/32 (0)	21/21 (100)	<.0001
New or enlarging cavitary lesion associated with MAA isolation ^a	0/32 (0)	8/21 (38)	<.001
Isolation of other waterborne NTM ^b	13/32 (41)	5/21 (24)	.2
Isolation of bronchiectasis-related bacteria ^c	28/32 (86)	17/21 (81)	.7
Mean duration of follow-up after successful MAC therapy, mean ± SD, mo	45.6 ± 36.1	48.3 ± 28.7	.8 (95% CI, −21.5 to 16.1)
Patients treated for MAA	0/32	11/21 (52)	<.0001
Treated concomitantly for MAC and MAA	...	5/11 (45)	...
Successful treatment of MAC and MAA	...	3/5 (60)	...
Successful treatment of MAA after completion of MAC therapy	...	3/6 (50)	...

Data given as No./total (%) unless otherwise indicated. AFB = acid-fast bacilli; ATS = American Thoracic Society; IDSA = Infectious Diseases Society of America; NTM = nontuberculous mycobacteria. See Table 1 legend for expansion of other abbreviations.

^a≥ 2.0-cm diameter.

^b*Mycobacterium gordonae*, *Mycobacterium terrae*, and so forth.

^c*Pseudomonas* species, *Stenotrophomonas* species, and so forth.

The underlying bronchial conditions or milieu promoting mycobacterial acquisition and persistence in the lung are likely very similar, if not identical, regardless of the primary mycobacterial pathogen. The challenge is to determine the significance of an NTM isolated in this context, as identification of clinically relevant NTM isolates requires satisfying a combination of clinical,

radiologic, and microbiologic criteria, which is neither facile nor inconsequential, as it could obligate the patient to further prolonged antimycobacterial therapy.²

In this study, we were able to follow over an extended period a large cohort of patients treated for MAC lung disease and who had co-isolation of MAA during the

Figure 1 – A, Chest CT scan cut from 78-y-old woman with symptomatic *Mycobacterium avium* complex lung disease prior to initiation of therapy. Scan shows bilateral nodular and reticulonodular densities on the left greater than the right. **B,** Chest CT scan cut at a comparable level to A after 12 mo of *M avium* complex therapy, including 6 mo of negative sputum cultures for *M avium* complex, showing improvement in bilateral nodular and reticulonodular densities. **C,** Chest CT scan cut at comparable level to A and B 18 mo after starting *M avium* complex therapy with 12 mo of negative sputum cultures for *M avium* complex but with sputum consistently culture positive for *M abscessus* subspecies *abscessus*.

course of MAC therapy. We did not include patients with isolation of other closely related mycobacterial isolates such as *M abscessus* subspecies *massiliense* (MAM), as these organisms appear to have a different prognostic significance than MAA and there were too few patients with these isolates for a separate analysis.^{16,17} The patients fell into two relatively easily identifiable groups. Most patients without evidence of progressive MAA lung disease had MAA isolated from sputum only once, whereas those patients with symptomatic and radiographic evidence of disease progression had multiple and repeated isolations of MAA. The implications of this finding include the following: (1) the isolation of MAA during the course of MAC therapy is common, and determining the significance of MAA isolates requires long-term clinical and microbiologic follow-up of the patient with serial collection of sputum for AFB analysis; (2) the emergence of a new or enlarging cavity while on MAC therapy in association with MAA isolation strongly suggests progressive MAA disease; and (3) a single isolation of MAA, which is known to be found in municipal water, is usually clinically insignificant and may be due to specimen contamination. Consistent with prior reports, not all patients diagnosed with MAA disease required specific MAA therapy and successful MAA lung disease therapy remains difficult regardless of the circumstance.^{1,3,4}

Although, it has also been recognized for many years that NTM are recovered from patients undergoing TB therapy, because of the complexity of NTM lung disease diagnosis, no studies thus far have been completely successful at determining the significance of NTM isolation during TB therapy.⁵⁻¹⁰ Two studies found that patients with cavitary TB were more likely to have co-isolation of NTM and one study suggested that *M abscessus* co-isolation with TB was more clinically significant than MAC co-isolation with TB.⁶⁻⁸ The major shortcomings of most studies so far are either a too-brief follow-up period or the lack of detailed clinical follow-up information for individual patients. A retrospective study from Canada found NTM co-isolation with TB occurred in 11% of 369 patients, 23% of whom went on to have two or more NTM-positive cultures, which would meet contemporary NTM microbiologic diagnostic criteria.¹⁰ However, clinical and radiographic information about these patients was not available.

Findings from this study reinforce the concept that there is currently no substitute for close clinical follow-up of patients with concomitant MAC and MAA isolation to determine the clinical significance of the MAA isolates.

Patients with only one MAA isolate during MAC therapy, who are otherwise clinically responding well, do not require initiation of MAA therapy. As demonstrated in these patients, the diagnosis of MAA lung disease in this scenario is generally presaged by the isolation of multiple, positive respiratory specimens for MAA over a long time period associated with clinical and radiographic manifestations of progressive mycobacterial disease. Certainly, not all patients with co-isolation of MAC and MAA will fall into the tidy and convenient microbiologic and clinical dichotomy that we found, which only reinforces the lack of a substitute for ongoing and tenacious patient scrutiny during and after MAC lung disease therapy.

The findings in this study add another layer of complexity to the evaluation of patients with MAC lung disease who have frequent microbiologic recurrence MAC isolates during and after MAC therapy, also necessitating clinical reassessment similar to that proposed for patients with co-isolation of MAA and MAC.¹² Based on combined data from this and our previous study, patients with NB MAC lung disease have a > 50% chance of requiring reassessment for either MAC lung disease recurrence (relapse or reinfection) or new MAA infection.¹²

It is unclear whether the observations in this study can be extrapolated to other mycobacterial disease treatment scenarios, such as co-isolation of NTM species other than MAA from patients with MAC lung disease or co-isolation of MAA for patients with cavitary MAC disease. However, at least for those with NB MAC lung disease, the risk of some type of NTM lung disease appears to be an indefinite risk requiring indefinite clinical follow-up.

There is an urgent need for better tools to help differentiate between true NTM disease and NTM isolation not associated with disease progression. Study data suggest that some MAA variable number tandem repeat (VNTR) patterns are more associated with progressive disease than others.^{18,19} In one study, a total of 53 MAA and 38 MAM isolates were assembled into three clusters based on their VNTR loci genotyping.¹⁹ The patients in cluster A were more likely to have stable NB disease, as 100% of patients with MAA and 96% of those with MAM in this cluster were followed without antibiotic treatment of > 24 months after diagnosis. In contrast, patients in cluster B were more likely to have progressive NB disease as 96% of those with MAA and 81% of patients with MAM started antibiotic treatment within

24 months after diagnosis. All patients in cluster C had fibrocavitary disease and started antibiotic treatment immediately after diagnosis. Because VNTR is relatively easy to accomplish and less expensive than traditional strain-typing procedures, it could prove useful for judging the potential for MAA disease progression, although the technique requires further validation before widespread use in this context. Identification of specific genotypes may also be predictive of pathogen virulence for disease sites including lung and skin and soft tissue infection.^{19,20}

The future for evaluating NTM isolates will likely be identification of specific genotypes associated with disease progression and/or identification of virulence factors. At this juncture, however, clinical follow-up is indispensable and still the most important method for

determining the clinical significance of co-isolated mycobacterial species.

Conclusions

Isolation of MAA from the sputum of patients with MAC lung disease who are on therapy is common. Currently, there is no rapid way to determine the significance of these isolates, so long-term follow-up with serial sputum AFB analysis is necessary to make that determination. Based on this single-center study from an NTM disease referral center, patients who have a single MAA respiratory isolate will probably not require therapy for MAA lung disease. Some patients will have multiple respiratory cultures positive for MAA associated with progressive symptoms and radiographic findings, especially cavitation, and will require MAA lung disease therapy.

Acknowledgments

Author contributions: D. E. G. served as principal author, had full access to all of the data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis. D. E. G., J. V. P., B. A. B.-E., J. L. B., S. S., D. Y., and R. J. W. contributed to study conception and design, or acquisition of data, or analysis and interpretation of data; drafting or revision of the manuscript; and approval of the final version to be published.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following conflicts of interest: Dr Philley has served on an advisory board and participated in previous in vitro minimum inhibitory concentration studies and clinical trials for Insmad Inc. Dr Wallace has served as laboratory director of a clinical laboratory that served as the mycobacterial identification and susceptibility laboratory (including some DNA sequencing) for a double-blind trial of the liposomal, inhaled amikacin, Arikace (Insmad Inc) for treatment failure of nontuberculous mycobacterial disease (2012-2014). The remaining authors have reported that no potential conflicts of interest exist with any companies/organizations whose products or services are discussed in this article.

Role of sponsors: The sponsors had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

References

- Griffith DE, Girard WM, Wallace RJ Jr. Clinical features of pulmonary disease caused by rapidly growing mycobacteria. An analysis of 154 patients. *Am Rev Respir Dis*. 1993;147(5):1271-1278.
- Griffith DE, Aksamit T, Brown-Elliott BA, et al; ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007;175(4):367-416.
- Jarand J, Levin A, Zhang L, Huitt G, Mitchell JD, Daley CL. Clinical and microbiologic outcomes in patients receiving treatment for *Mycobacterium abscessus* pulmonary disease. *Clin Infect Dis*. 2011;52(5):565-571.
- Jeon K, Kwon OJ, Lee NY, et al. Antibiotic treatment of *Mycobacterium abscessus* lung disease: a retrospective analysis of 65 patients. *Am J Respir Crit Care Med*. 2009;180(9):896-902.
- Epstein MD, Aranda CP, Bonk S, Hanna B, Rom WN. The significance of *Mycobacterium avium* complex cultivation in the sputum of patients with pulmonary tuberculosis. *Chest*. 1997;111(1):142-147.
- Jun HJ, Jeon K, Um SW, Kwon OJ, Lee NY, Koh WJ. Nontuberculous mycobacteria isolated during the treatment of pulmonary tuberculosis. *Respir Med*. 2009;103(12):1936-1940.
- Huang CT, Tsai YJ, Shu CC, et al; Tami Group. Clinical significance of isolation of nontuberculous mycobacteria in pulmonary tuberculosis patients. *Respir Med*. 2009;103(10):1484-1491.
- Kendall BA, Varley CD, Hedberg K, Cassidy PM, Winthrop KL. Isolation of non-tuberculous mycobacteria from the sputum of patients with active tuberculosis. *Int J Tuberc Lung Dis*. 2010;14(5):654-656.
- Hwang SM, Lim MS, Hong YJ, et al. Simultaneous detection of *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria in respiratory specimens. *Tuberculosis (Edinb)*. 2013;93(6):642-646.
- Damaraju D, Jamieson F, Chedore P, Marras TK. Isolation of non-tuberculous mycobacteria among patients with pulmonary tuberculosis in Ontario, Canada. *Int J Tuberc Lung Dis*. 2013;17(5):676-681.
- Wallace RJ Jr, Brown-Elliott BA, McNulty S, et al. Macrolide/azalide therapy for nodular/bronchiectatic *Mycobacterium avium* complex lung disease. *Chest*. 2014;146(2):276-282.
- Wallace RJ Jr, Glassroth J, Griffith DE, Olivier KN, Cook JL, Gordin F. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. This official statement of the American Thoracic Society was approved by the Board of Directors, March 1997. *Am J Respir Crit Care Med*. 1997;156(2 pt 2):S1-S25.
- Wallace RJ Jr, Brown BA, Griffith DE, et al. Initial clarithromycin monotherapy for *Mycobacterium avium*-intracellular complex lung disease. *Am J Respir Crit Care Med*. 1994;149(5):1335-1341.
- Wallace RJ Jr, Brown BA, Griffith DE, Girard WM, Murphy DT. Clarithromycin regimens for pulmonary *Mycobacterium avium* complex. The first 50 patients. *Am J Respir Crit Care Med*. 1996;153(6 pt 1):1766-1772.
- Telenti A, Marchesi F, Balz M, Bally F, Böttger EC, Bodmer T. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *J Clin Microbiol*. 1993;31(2):175-178.
- Harada T, Akiyama Y, Kurashima A, et al. Clinical and microbiological differences between *Mycobacterium abscessus* and *Mycobacterium massiliense* lung diseases. *J Clin Microbiol*. 2012;50(11):3556-3561.
- Koh WJ, Jeon K, Lee NY, et al. Clinical significance of differentiation of *Mycobacterium massiliense* from *Mycobacterium abscessus*.

- Am J Respir Crit Care Med.* 2011;183(3):405-410.
18. Wong YL, Ong CS, Ngeow YF. Molecular typing of *Mycobacterium abscessus* based on tandem-repeat polymorphism. *J Clin Microbiol.* 2012;50(9):3084-3088.
 19. Shin SJ, Choi GE, Cho SN, et al. Mycobacterial genotypes are associated with clinical manifestation and progression of lung disease caused by *Mycobacterium abscessus* and *Mycobacterium massiliense*. *Clin Infect Dis.* 2013;57(1):32-39.
 20. Cheng A, Liu YC, Chen ML, et al. Extrapulmonary infections caused by a dominant strain of *Mycobacterium massiliense* (*Mycobacterium abscessus* subspecies *bolletii*). *Clin Microbiol Infect.* 2013;19(10):E473-E482.