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Original Article

Clinical Outcome of *Mycobacterium abscessus* Infection and Antimicrobial Susceptibility Testing

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BACKGROUND/PURPOSE: *Mycobacterium abscessus* is the most resistant and rapidly growing mycobacterium and causes a wide range of clinical infectious diseases. The relationship between antimicrobial susceptibility and clinical outcome needs to be further evaluated.

METHODS: Forty *M. abscessus* isolates were obtained from clinical specimens of 40 patients at the Taichung Veterans General Hospital from January 2006 to December 2008. Antimicrobial susceptibility testing was performed using the broth microdilution method according to the recommendations of the National Committee for Clinical Laboratory Standards. The clinical manifestations and outcomes were reviewed from medical records.

RESULTS: Twenty-two patients were diagnosed with *M. abscessus* infection. Cough (86.3%), hemoptysis (31.8%) and fever (18.1%) were the most common symptoms. The radiographic findings included reticulonodular opacities (50.0%), consolidation (31.8%) and cavitary lesions (18.1%). The 40 isolates were susceptible to amikacin (95.0%), cefoxitin (32.5%), ciprofloxacin (10.0%), clarithromycin (92.5%), doxycycline (7.5%), imipenem (12.5%), moxifloxacin (22.5%), sulfamethoxazole (7.5%) and tigecycline (100%). The rate of treatment failure was 27.3% at the end of the 12th month after the start of treatment, although these patients were treated with a combination of clarithromycin and other antimicrobial agents.

CONCLUSION: *M. abscessus* is naturally susceptible to clarithromycin and amikacin, variably susceptible to cefoxitin and imipenem, and resistant to most other antimicrobial drugs. Combination therapy with

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clarithromycin, amikacin and other active antimicrobial agents may lead to clinical improvement; however, the rate of treatment failure is still high.

KEYWORDS: *Mycobacterium abscessus*, rapidly growing mycobacteria, susceptibility testing

Introduction

Mycobacterium abscessus belongs to a group of rapidly growing mycobacteria, which are Runyon group IV organisms that usually form colonies on solid media within 7 days.[1] *M. abscessus* is the most resistant mycobacterium of the known pathogenic rapidly growing mycobacteria, and causes a wide spectrum of clinical diseases, including localized soft tissue infections, pulmonary disease and disseminated infections.[2] Soft tissue infection by *M. abscessus* is typically caused by contamination of a wound with infected material, non-sterile surgical procedures, injections, implantations of foreign bodies or in connection with tympanic tubes. *M. abscessus* causes over 80% of rapidly growing mycobacteria chronic respiratory disease.[3] Such pulmonary diseases are especially common in patients with cystic fibrosis. Various types of systemic immunosuppression may also predispose individuals to disseminated *M. abscessus* infections.

Clarithromycin and azithromycin are the only effective oral antimycobacterial agents for *M. abscessus* and should, preferably, be supplemented with other drugs since long-term monotherapy may cause resistance.[1] Amikacin is a major parenteral drug used to treat *M. abscessus* that should also be given in combination with another drug.[2] Because of the variable susceptibility of *M. abscessus* to cefoxitin, imipenem and ciprofloxacin, antibiotic susceptibility testing of all clinically significant isolates is recommended.[4]

To our knowledge, *M. abscessus* infection is a rare disease and so case numbers are limited. Two studies have reviewed the clinical outcomes of 126 patients and 29 patients respectively,[3,5] and the only other related publications are case reports. Because it is not a common disease, the relationship between the clinical outcome of *M. abscessus* infection and its antimicrobial susceptibility has never been evaluated in Taiwan. Hence, we assess the clinical manifestations of *M. abscessus* infections, antimicrobial susceptibility and responses to antimicrobial therapy.

Methods

M. abscessus isolates

Forty *M. abscessus* isolates were obtained from the sputum ($n=35$), blood ($n=4$) and cornea ($n=1$) of 40 patients at Taichung Veterans General Hospital from January 2006 to December 2008.

Antimicrobial susceptibility testing

Susceptibility testing was performed using the broth microdilution method according to the recommendations of the National Committee for Clinical Laboratory Standards.[6] The isolates were grown on blood agar and incubated at 30°C in room air. The minimal inhibitory concentrations (MIC) were read on the 3rd and 4th days. The inocula were prepared from actively growing bacteria in 10 mL of cation-adjusted Mueller-Hinton broth. The strains were then adjusted with cation-adjusted Mueller-Hinton broth to a bacterial cell density of 10^6 colony forming units/mL (cfu/mL), and diluted to a final inoculum of approximately 5×10^4 cfu/well. Standard reference powders of nine antimicrobial agents including amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, sulfamethoxazole, moxifloxacin and tigecycline were obtained from pharmaceutical companies. Antibiotics were serially diluted 2-fold in 50 µL of cation-adjusted Mueller-Hinton broth. The range of antibiotic concentrations was 256–0.016 µg/mL. The final reaction volume was 100 µL (50 µL of antibiotic solution and 50 µL of bacterial suspension). The MIC breakpoints indicating susceptibility, moderate susceptibility and resistance were interpreted according to the tentative guidelines established by the National Committee for Clinical Laboratory Standards and the modified values for tigecycline by Petrini.[2] *Staphylococcus aureus* ATCC 29213 was used as a quality control strain.

Clinical assessment and definitions

The diagnostic criteria for pulmonary *M. abscessus* infection were those outlined in the recommendations of the

American Thoracic Society 2007[4] as follows: (1) clinical pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high resolution computed tomography (CT) scan showing multifocal bronchiectasis with multiple small nodules coupled with the appropriate exclusion of other diagnoses; and (2) one of the following microbiological findings: sputum; ≥2 positive cultures from different samples or bronchial wash/lavage: ≥1 positive culture or lung biopsy: granulomatous inflammation/acid-fast bacilli plus positive culture from biopsy, sputum or bronchial specimen.

The definition of disseminated *M. abscessus* infection was modified from the definition originally developed for the diagnosis of tuberculosis as follows: Isolation of *M. abscessus* from blood or bone marrow, from a liver biopsy specimen, or from specimens from two or more noncontiguous sites such as respiratory tract, lymph node, ascites, pleural effusion, pericardial effusion, joint fluid or cerebral spinal fluid.[7]

Review of radiographs

For the purpose of analysis, we divided each lung into two zones (upper and lower lobes), and each zone was assessed separately. The radiographic patterns were classified as reticulonodular opacities, consolidation and cavitary lesions.

Outcome measurement

Outcome measurement was performed at the end of the 12th month after the start of treatment.[4]

Clinical outcomes

Clinical outcome measurements were obtained from chart review. Clinical cure was defined as the resolution of the index infection (disappearance of acute signs and symptoms related to the infection, or sufficient improvement such that additional antimicrobial therapy was not required). Clinical improvement was defined as improvement of clinical symptoms and radiographic findings. Clinical failure was defined as one of the following: persisting symptoms and signs, recurrent infection or death related to *M. abscessus* infection. Indeterminate outcome was defined as assessment not possible for any reason.

Microbiological outcomes

Microbiological responses were based on the results of the appropriate cultures. Eradication was defined as absence of *M. abscessus* on culture. Persistence was defined as presence of *M. abscessus* in a patient with clinical failure. Indeterminate outcome was defined as assessment not possible for any reason.

Results

The results of antimicrobial susceptibility testing are shown in Table 1. The susceptibility rates of the 40 *M. abscessus* isolates to the nine antimicrobial agents were as follows: amikacin (95.0%), cefoxitin (32.5%), ciprofloxacin (10.0%), clarithromycin (92.5%), doxycycline (7.5%), imipenem

Table 1. In vitro susceptibilities of 40 clinical isolates of *Mycobacterium abscessus* to 9 antimicrobial agents

Antimicrobial agents	MIC (μg/mL)			Susceptibility[a]
	Range	MIC_{50}	MIC_{90}	
Amikacin	0.125–64	4	16	38/40 (95.0)
Cefoxitin	16–256	32	32	13/40 (32.5)
Ciprofloxacin	0.064–64	4	16	4/40 (10.0)
Clarithromycin	0.032–8	0.25	2	37/40 (92.5)
Doxycycline	0.125–256	32	128	3/40 (7.5)
Imipenem	1–256	16	128	5/40 (12.5)
Moxifloxacin	0.064–32	8	32	9/40 (22.5)
Sulfamethoxazole	16–256	256	256	3/40 (7.5)
Tigecycline	0.064–2	0.5	2	40/40 (100)

[a]Data presented as number of positive cases/total cases (%). MIC=minimum inhibitory concentration.

Table 2. Clinical symptoms of 22 patients with *Mycobacterium abscessus* infection

Symptoms	*n* (%)
Cough	19 (86.3)
Hemoptysis	7 (31.8)
Fever	4 (18.1)
Chest pain	2 (9.1)
Dyspnea	1 (4.5)
Body weight loss	1 (4.5)
Skin rash	1 (4.5)

Table 3. Chest radiograph findings of 22 patients with *Mycobacterium abscessus* infection

Chest radiograph	*n* (%)
Location	
Right upper lobe	5 (22.7)
Left upper lobe	1 (4.5)
Right lower lobe	1 (4.5)
Left lower lobe	3 (13.6)
Bilateral	11 (50.0)
Pattern	
Reticulonodular opacities	11 (50.0)
Consolidation	7 (31.8)
Cavitary lesions	4 (18.1)

(12.5%), moxifloxacin (22.5%), sulfamethoxazole (7.5%) and tigecycline (100%).

Twenty-two patients were diagnosed with *M. abscessus* infection. Eighteen *M. abscessus* isolates from 18 patients were considered as colonization. The clinical manifestations are shown in Tables 2 and 3. Symptoms included cough (86.3%), hemoptysis (31.8%), fever (18.1%), chest pain (9.1%), dyspnea (4.5%), body weight loss (4.5%) and skin rash (4.5%). Some of the 22 patients had associated disorders, including previous episodes of tuberculosis (7/22, 31.8%), chronic obstructive pulmonary disease (3/22, 13.6%), diabetes mellitus (5/22, 22.7%), and malignancy (3/22, 13.6%). Of the three patients with *M. abscessus* bacteremia that received antimicrobial therapy, one died due to recurrent bacteremia 6 months later, and the other two patients died from unrelated causes.

The most common radiographic finding was reticulonodular opacities (11 patients, 50.0%), predominantly in the right upper lung. Cavitary lesions (4 patients, 18.1%) were seen in upper the lobes and were predominant in the right upper lobe. Consolidation (7 patients, 31.8%) was distributed in right upper and left lower lobes. One or both upper lobes were involved in 76% of the patients. Multi-lobar (involving 3 or more lobes) involvement was seen in 19% of patients, and bilateral lobe involvement was seen in 52%. Lymph node enlargement was not seen in any of the radiographs.

Table 4. Clinical and microbiologic outcome of 22 patients with *Mycobacterium abscessus* infection 12 months after start of treatment

	n (%)
Clinical outcome	
Cure	5 (22.7)
Improvement	3 (13.6)
Failure	6 (27.2)
Indeterminate	8 (36.3)
Microbiologic outcome	
Eradication	7 (31.8)
Persistence	7 (31.8)
Indeterminate	8 (36.3)

Table 4 lists the clinical and microbiological outcomes. The clinical outcomes at the end of the 12th month after the start of treatment were as follows: cure (5 patients, 22.7%); improvement (3 patients, 13.6%); failure (6 patients, 27.2%); and indeterminate outcome (8 patients, 36.3%). The microbiological outcomes at the end of the 12th month after the start of treatment were as follows: eradication (7 patients, 31.8%); persistence (7 patients, 31.8%); and indeterminate outcome (8 patients, 36.3%).

Discussion

The main clinical symptoms of *M. abscessus* infection in this study included cough, hemoptysis and fever. Cough was also the most common symptom, along with other frequent symptoms such as low-grade fever, hemoptysis, sputum production and weight loss in previous studies.[1,8] In this study, we found that patients had higher rates (40%) of structural lung disease, including previous episodes of pulmonary tuberculosis and chronic obstructive pulmonary disease. Disorders associated with

M. abscessus infection, such as bronchiectasis and prior mycobacterial infection, were also observed in a previous study.[4] Disseminated non-tuberculous Mycobacterial disease is rare in non-human immunodeficiency virus-infected hosts and is associated with various immunosuppressive conditions (e.g. renal or cardiac transplantation, chronic corticosteroid use, and hematologic malignancy).[1,7] In this study, one patient had a history of acute lymphoblastic leukemia and five patients had diabetes mellitus.

Characteristics of the radiographic findings included reticulonodular opacities, consolidation and cavitary lesions. These findings were comparable to those described in previous studies.[3,9] The high resolution CT scan of one patient with *M. abscessus* pulmonary disease showed diffuse bronchiectasis. In contrast, the most common CT findings in a previous study were branching nodular opacities and cylindrical bronchiectasis.[9] In patients with pre-existing pulmonary tuberculosis, the new infiltrate of *M. abscessus* infection generally occurred in the same area as the previous disease.[8]

In this study, the most active antimicrobial agents against the 40 *M. abscessus* isolates were tigecycline (100%), amikacin (95.0%) and clarithromycin (92.5%), while the others were less active. Surveillance data collected by the Multicenter Antimicrobial Resistance in Taiwan (SMART program) from January 2002 to December 2003[10] showed clarithromycin to be less active (52.7%) against 167 *M. abscessus* isolates, whereas ciprofloxacin (35.7%) and imipenem (28.9%) were more active than suggested by the present study. The activity of the other antimicrobial agents in this study was similar to those seen in the SMART program. A Korean study of *in vitro* antimicrobial susceptibility (74 isolates of *M. abscessus* obtained from July 2005 to December 2006) revealed that amikacin (99%), cefoxitin (99%) and clarithromycin (91%) all had excellent activity against most isolates, and that imipenem (55%), moxifloxacin (73%) and ciprofloxacin (57%) had moderate activity.[11] *M. abscessus* is naturally sensitive to amikacin and clarithromycin/azithromycin, variably susceptible to cefoxitin and imipenem,[2] and very resistant to many other chemotherapeutic agents.[1]

Of the 10 patients treated with a combination of clarithromycin and other antimicrobial agents for a period of 4–8 months, seven still had a positive culture at the end of the 12th month after the start of treatment. Although combination therapy with a macrolide (clarithromycin or azithromycin) and one or more parenteral agents (amikacin plus cefoxitin, imipenem) for 2–4 months usually results in clinical and microbiological improvement, no regimens based on *in vitro* susceptibility have been shown to produce long-term sputum conversion in patients with *M. abscessus* pulmonary diseases.[4] This discrepancy between eradication rates and antimicrobial susceptibility may be due to structural lung disease or immunosuppressive conditions. For *M. abscessus* pulmonary disease, radical surgery may be curative when it is possible to perform, but pulmonary operations on this patient group are associated with significant risks.[12]

The combination of tigecycline for 2 weeks plus clarithromycin for 8 months resulted in both cure and eradication in one patient. The new drug tigecycline is promising. It has low MIC values ($\leq$0.03–1 μg/mL) for *M. abscessus*, but needs further clinical evaluation before a definite role in therapy can be established.[13,14] *M. abscessus* is partially susceptible (23%) to linezolid *in vitro*, with an MIC$\leq$8 μg/mL, but its long-term use is limited due to its hematological and neurological side effects, as well as its high price.[15,16]

In summary, *M. abscessus* is naturally susceptible to clarithromycin and amikacin, variably susceptible to cefoxitin and imipenem, and resistant to most antimicrobial drugs. Susceptibility testing is recommended to guide antimicrobial therapy for *M. abscessus* infections. Combination therapy with clarithromycin, amikacin and other active antimicrobial agents may result in clinical improvement; however, the rate of treatment failure is still high. The effect of combination treatment remains to be determined.

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