

# Bronchoscopic Microsampling for Bacterial Colony Counting in Relevant Lesions in Patients with Pulmonary *Mycobacterium avium* Complex Infection

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## Abstract

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**Objective** The incidence of pulmonary *Mycobacterium avium* complex (MAC) infections with nodular/bronchiectasis lesions is increasing. However, factors determining deterioration are unknown. In the present study, we investigated quantitative MAC cultures obtained through bronchoscopic microsampling (BMS) from patients with pulmonary MAC infection and analyzed the relationship between MAC culture and the short-term natural history. We also assessed chest computed tomography (CT) findings for the deteriorating factors.

**Design** For this prospective study, MAC was collected from peripheral lung lesions by BMS through endobronchial ultrasonography. MAC colonies were counted on Middlebrook 7H11 agar. We compared the number of MAC colonies with laboratory data and chest CT findings.

**Patients** We studied 26 patients with pulmonary MAC infection.

**Results** The patients were divided into 2 groups: 11 patients in the non-deteriorated group and 15 patients in the deteriorated group. The number of MAC colonies was significantly correlated with deterioration of MAC infection ( $p < 0.001$ ). In the non-deteriorated group, chest CT scans showed nodular/bronchiectasis lesions in 8 patients (73%) and consolidated lesions in 3 patients (27%). In the deteriorated group, chest CT scans showed nodular/bronchiectasis lesions in 1 patient (7%), consolidated lesions in 6 patients (40%), and cavitary lesions in 8 patients (53%).

**Conclusion** The number of MAC colonies in relevant lesions investigated by BMS was significantly larger in the deteriorated group than in the non-deteriorated group. Cavitary and consolidated lesions observed from chest CT scans are thought to indicate a high risk of progression of pulmonary MAC infection.

**Key words:** non-tuberculous mycobacteriosis, *Mycobacterium avium* complex, bronchoscopic microsampling, endobronchial ultrasonography

(Intern Med 50: 1287-1292, 2011)

(DOI: 10.2169/internalmedicine.50.5034)

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## Introduction

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Pulmonary *Mycobacterium avium* complex (MAC), which includes *M. avium* and *M. intracellulare* infection, occurs in patients without predisposing lung diseases. The number of cases has risen in recent years (1-3). Chest computed tomography (CT) scans of MAC infection show the character-

istic findings, such as multiple small nodules, bronchiectasis, consolidation and cavitary lesions distributed mainly in the upper, middle, and lingular lobes (2-4). Early studies have shown that pulmonary MAC infections in symptomatic (and some apparently asymptomatic) patients can be fatal (5). On the other hand, Thomson and Yew documented cases where disease remitted spontaneously (6). Wallace et al. found that semiquantitative (0-4+) cultures of MAC colony counts on

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Received for publication December 20, 2010; Accepted for publication March 1, 2011

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solid media are useful for assessing responses to therapy (7). However, it is unclear if this approach is useful for predicting the short-term natural history of pulmonary MAC infection.

A bronchoscopic microsampling (BMS) probe was developed to sample biochemical constituents of the local bronchial epithelial lining fluid (ELF) (8). BMS is performed while observing the bronchial lumen using a bronchofiberscope. When the target site is reached, the inner probe is advanced slowly into the distal airway to absorb bronchial ELF. BMS is useful for detecting the biochemical constituents in acute respiratory distress syndrome (8, 9) and respiratory tract infection (10, 11) and for detecting antibiotic concentrations (12, 13), tumor markers of small peripheral lung carcinoma (14), and gene expression in pulmonary nodules (15). In addition, Sasabayashi et al. reported that BMS can be used for quantitative sampling of bacteria *in vitro* (16). Recently developed small-caliber ultrasound probes have enabled a wider clinical application of ultrasonography (US) for evaluating tracheal and bronchial lesions. Endobronchial US (EBUS) has been used for imaging guidance in transbronchial approaches to peripheral pulmonary lesions (17). Thus, utilizing EBUS with a guide sheath (EBUS-GS) increases the ability to endoscopically diagnose peripheral pulmonary lesions (18).

In the present study, we used BMS guided EBUS-GS to assess the bacteria number in relevant lesions in patients with MAC infection and analyzed the correlation of the number of MAC colonies with deterioration and chest CT findings.

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## Study Population and Methods

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### 1) Patients

We studied 26 patients with pulmonary MAC infection, the diagnosis of which was based on criteria of the American Thoracic Society/Infectious Disease of America (19). All patients visited our hospital because of cough and/or sputum and abnormal findings on chest radiographs and CT scans, such as multiple centrilobular small nodules, bronchiectasis, consolidation, and/or cavities suggesting MAC infection. All patients had nodular/bronchiectasis disease (19). None of the patients had previously received treatment for pulmonary MAC infection. Written informed consent was obtained from all patients before inclusion in the study, which was approved by the Ethics Committee of the Shinshu University School of Medicine.

### 2) Laboratory data and chest CT scan

Clinical data, including age, body mass index (BMI), and laboratory tests, including those for white blood cell count (WBC), lymphocyte count (LYM), total protein (TP), albumin (ALB), C-reactive protein (CRP), and the erythrocyte sedimentation rate (ESR), were collected for each patient. We also used high-resolution CT scans to assess all patients.

All tables represent data collected at baseline, when pulmonary MAC infection was suspected.

### 3) EBUS-GS and BMS procedures

After routine premedications, a flexible 1T-260 fiberoptic bronchoscope (Olympus, Tokyo, Japan) was inserted beyond the vocal cords so that all segments of the bronchial tree were visualized. Based on the radiographic findings, a curette that had been introduced into the guide sheath was maneuvered into the bronchus of interest. After the angulated curette was advanced into the bronchus leading to the lesions, a fluorescent radiograph was taken to confirm that the curette had reached the lesions. The curette was then withdrawn, leaving the guide sheath in place.

An ultrasound probe (20 MHz, mechanical-radial type, UM-S30-20R; Olympus) connected to an endoscopic ultrasound system (EU-M2000; Olympus) was introduced into the guide sheath until the tip of the probe extended to the far end of the guide sheath to confirm that the BMS probe had been inserted into the relevant lesion to collect ELF from the same site.

A polyethylene probe (length, 30 mm; diameter, 1.2 mm) attached to the tip of the BMS device (BC-401C; Olympus) was introduced into the guide sheath, used to absorb samples from relevant lesions, and stored back in the sheath after 10 seconds. The BMS device was then removed from the guide sheath, and the probe was cut with disinfected scissors and placed in 1 mL normal saline in an Eppendorf tube. This sampling procedure was repeated thrice.

Following EBUS-GS and BMS sampling, bronchial washing was performed by instilling 20 mL of 0.9% normal saline at room temperature through the internal channel of the bronchoscope and aspirated into a trap connected to suction tubing. On average, 3-5 mL fluid was retrieved in the suction trap.

The patients were monitored using continuous pulse oximetry and electrocardiography during the bronchoscopy.

### 4) MAC culture

The Eppendorf tube was stirred for 1 minute using a vortex. Serial dilutions (1:10) were prepared and inoculated (100  $\mu$ L) on Middlebrook 7H11 agar (Becton Dickinson, Sparks, MD, USA). MAC was cultured at 37°C in 5% CO<sub>2</sub> for 4 weeks before the colonies were counted.

### 5) Estimation of MAC deterioration

Deterioration of infection was estimated mainly on the basis of chest CT scans. Chest CT was performed at intervals of 4 months after BMS without treatment. The CT scans were evaluated by 2 chest radiologists who were unaware of the patients' clinical course. After a mean observation period of 11 $\pm$ 3 months (4 to 12 months), 26 patients were divided into 2 groups: the deteriorated group and the non-deteriorated group. Deterioration was defined as follows: appearance of new lesions, increase in the number of the nodules, progression of bronchiectasis, or expansion of

**Table 1. Clinical Features of Patients with Pulmonary MAC Infection**

	Data are mean ± SD		
	Non-deteriorated group (n = 11)	Deteriorated group (n = 15)	p
Age (y)	67 ± 10	67 ± 9	NS
Sex (female/male)	9/2	14/1	NS
BMI (kg/m <sup>2</sup> )	20.0 ± 2.2	18.7 ± 1.7	NS
WBC (cells/μL)	5464 ± 1608	6938 ± 2882	NS
Lymphocyte (cells/μL)	1517 ± 410	1472 ± 643	NS
Total protein (g/dL)	7.3 ± 0.7	7.5 ± 0.4	NS
Albumin (g/dL)	4.3 ± 0.2	4.0 ± 0.4	NS
CRP (mg/dL)	0.20 ± 0.26	1.23 ± 2.00	NS
ESR (mm/h)	19 ± 14	38 ± 29	NS

MAC: *Mycobacterium avium* complex; BMI: body mass index; WBC: white blood cell; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; NS: not significant

**Table 2. Clinical Findings and the Number of MAC Colonies in the Deteriorated Group of Patients with Pulmonary MAC Infection**

Patient No.	Age (y)	Sex	Symptoms	Chest CT findings	EBUS findings	BMS MAC (CFU/mL)	Washing MAC (CFU/mL)
1	76	M	sputum	cavitary	low and iso	1.7 × 10 <sup>4</sup>	ND
2	55	F	sputum	cavitary	low and iso	1.3 × 10 <sup>5</sup>	ND
3	68	F	bloody sputum	consolidation	iso	1.1 × 10 <sup>5</sup>	2.0 × 10 <sup>5</sup>
4	56	F	cough	consolidation	iso	1.0 × 10 <sup>4</sup>	5.8 × 10 <sup>4</sup>
5	59	F	sputum	consolidation	iso	6.0 × 10 <sup>3</sup>	1.8 × 10 <sup>4</sup>
6	71	F	cough	consolidation	iso	3.0 × 10 <sup>4</sup>	5.3 × 10 <sup>4</sup>
7	76	F	cough	cavitary	low and iso	3.1 × 10 <sup>6</sup>	2.4 × 10 <sup>3</sup>
8	66	F	cough	cavitary	low and iso	1.7 × 10 <sup>5</sup>	2.7 × 10 <sup>5</sup>
9	59	F	bloody sputum	cavitary	low and iso	8.6 × 10 <sup>4</sup>	7.0 × 10 <sup>3</sup>
10	71	F	cough	cavitary	low and iso	3.7 × 10 <sup>5</sup>	8.4 × 10 <sup>4</sup>
11	83	F	bloody sputum	cavitary	low and iso	5.0 × 10 <sup>4</sup>	1.5 × 10 <sup>5</sup>
12	79	F	cough	cavitary	low and iso	2.9 × 10 <sup>6</sup>	3.3 × 10 <sup>4</sup>
13	58	F	sputum	consolidation	iso	3.4 × 10 <sup>4</sup>	4.0 × 10 <sup>3</sup>
14	73	F	sputum	consolidation	iso	2.5 × 10 <sup>5</sup>	6.9 × 10 <sup>6</sup>
15	59	F	bloody sputum	nodular/bronchiectasis	heterogeneous	7.1 × 10 <sup>3</sup>	1.8 × 10 <sup>4</sup>

CT: computed tomography; BMS: bronchial microsampling; MAC: *Mycobacterium avium* complex; CFU: colony forming unit; EBUS: endobronchial ultrasonography; ND: not done

consolidation or cavity. If a chest CT scan showed improvement for 1 lesion and progression for the other lesions, we defined the case as not deteriorated.

## 6) Statistical analysis

All results, except for the number of MAC colonies, were expressed as mean ± SD. The number of MAC colonies was expressed as median figures and ranges. Clinical features, laboratory data, and the number of MAC colonies between the 2 groups were compared using an unpaired *t*-test. The difference in sex and symptoms between the 2 groups was compared using Fisher's exact test. Dunnett's test was used for comparison of the number of MAC colonies among three groups. Correlation between deterioration and the number of MAC colonies was assessed by Fisher's exact test. Correlations between the number of MAC colonies collected by BMS and those collected by bronchial washing and clinical features were examined using Pearson's product-moment correlation coefficient. A *p* value of <0.05 indicated a significant difference.

## Results

### 1) Patient characteristics and laboratory data

We studied 26 patients who had no underlying disease. The patients were divided into 2 groups based on chest CT findings: 11 patients (9 females) in the non-deteriorated group and 15 patients (14 females) in the deteriorated group. Laboratory data for all patients did not change at the end of deterioration. Laboratory data were not significantly different between the 2 groups (Table 1). There were significantly more patients with bloody sputum in the deteriorated group (Table 2).

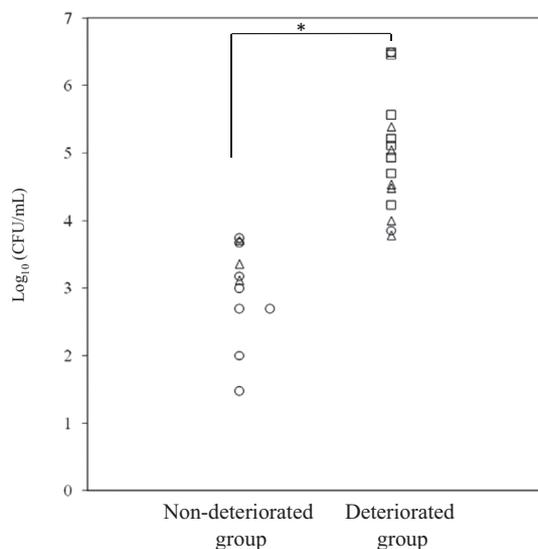
### 2) Number of MAC colonies

The number of MAC colonies collected by BMS is shown in Table 2, 3. There was no correlation between the number of MAC colonies collected by BMS and clinical features (age, BMI, WBC, LYM, TP, ALB, CRP, and ESR). The number of MAC colonies collected by BMS was significantly larger in the deteriorated group (6.0 × 10<sup>3</sup>-3.1 × 10<sup>6</sup>; me-

**Table 3. Clinical Findings and the Number of MAC Colonies in The-non Deteriorated Group of Patients with Pulmonary MAC Infection**

Patient No.	Age (y)	Sex	Symptoms	Chest CT findings	EBUS findings	BMS MAC (CFU/mL)	Washing MAC (CFU/mL)
1	83	M	cough	consolidation	iso	$5.1 \times 10^3$	ND
2	51	F	cough	nodular/bronchiectasis	heterogeneous	$5.5 \times 10^3$	$2.8 \times 10^3$
3	76	M	sputum	nodular/bronchiectasis	heterogeneous	$5.0 \times 10^2$	$1.0 \times 10^3$
4	72	F	cough	nodular/bronchiectasis	heterogeneous	$1.0 \times 10^2$	$1.1 \times 10^4$
5	75	F	sputum	consolidation	iso	$2.3 \times 10^3$	$3.4 \times 10^2$
6	59	F	cough	nodular/bronchiectasis	heterogeneous	$3.0 \times 10$	$1.0 \times 10$
7	56	F	cough	nodular/bronchiectasis	heterogeneous	$5.0 \times 10^2$	$3.0 \times 10^2$
8	72	F	cough	nodular/bronchiectasis	heterogeneous	$1.5 \times 10^3$	$3.9 \times 10^3$
9	63	F	sputum	consolidation	iso	$1.3 \times 10^3$	$2.8 \times 10^3$
10	61	F	sputum	nodular/bronchiectasis	heterogeneous	$4.7 \times 10^3$	$1.0 \times 10$
11	66	F	sputum	nodular/bronchiectasis	heterogeneous	$1.0 \times 10^2$	$2.3 \times 10^3$

CT: computed tomography; BMS: bronchial microsampling; MAC: *Mycobacterium avium* complex; CFU: colony forming unit; EBUS: endobronchial ultrasonography; ND: not done

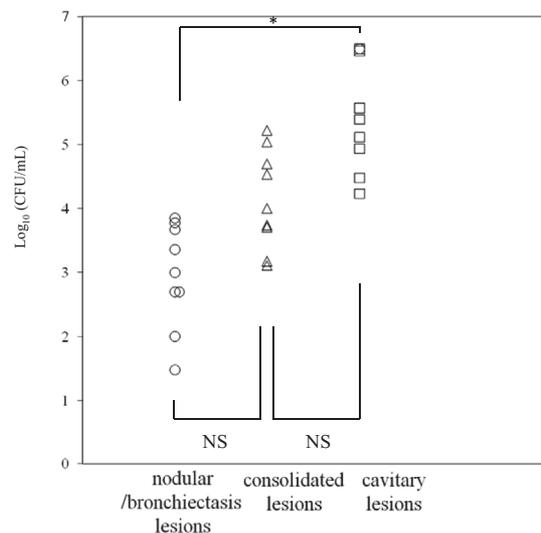


**Figure 1.** The number of MAC colonies in ELF collected by BMS under EBUS-GS. The number of MAC colonies in the deteriorated group (n=15) was significantly larger than that in the non-deteriorated group (n=11). \*p<0.001. Circle, nodular/bronchiectasis lesion; triangle, consolidated lesion; square, cavitary lesion. Fisher's exact test showed a significant correlation between the number of MAC colonies and the deteriorated group (p<0.001).

dian:  $8.6 \times 10^4$  colony forming units (CFU)/mL) than in the non-deteriorated group ( $3.0 \times 10^3$ - $5.5 \times 10^3$ ; median:  $1.3 \times 10^3$  CFU/mL), as shown in Fig. 1 (p<0.001).

The number of MAC colonies collected by BMS was significantly larger in cavitary lesions ( $1.7 \times 10^4$ - $3.1 \times 10^6$ ; median:  $1.5 \times 10^5$  CFU/mL) than in nodular/bronchiectasis lesions ( $3.0 \times 10^3$ - $7.1 \times 10^3$ ; median:  $1.0 \times 10^3$  CFU/mL; p<0.05). The difference between the number of MAC colonies collected by BMS in consolidated lesions ( $1.3 \times 10^3$ - $2.5 \times 10^5$ ; median:  $1.0 \times 10^4$  CFU/mL) and those in nodular/bronchiectasis lesions and cavitary lesions was not significant (Fig. 2).

The number of MAC colonies collected by bronchial washing is shown in Table 2, 3. Although we counted no colonies in 3 cases, the number of MAC colonies collected



**Figure 2.** The number of MAC colonies collected by BMS under EBUS-GS. The number of MAC colonies in the cavitary lesions (n=8) was larger than that in the nodular/bronchiectasis lesions (n=9). \*p<0.05. The number of MAC colonies between nodular/bronchiectasis lesions and consolidated lesions (n=9) was not significantly different. The number of MAC colonies collected by bronchial washing among nodular/bronchiectasis lesions, consolidated lesions and cavitary lesions was not significant. Furthermore, the number of MAC colonies collected by bronchial washing did not correlate with that collected by BMS (r=-0.02).

by bronchial washing was significantly larger in the deteriorated group ( $2.4 \times 10^3$ - $6.9 \times 10^6$ ; median:  $5.3 \times 10^4$  CFU/mL) than in the non-deteriorated group ( $1.0 \times 10^3$ - $1.1 \times 10^4$ ; median:  $1.7 \times 10^3$  CFU/mL; p<0.001). The patient recording > $1.0 \times 10^4$  CFU/mL MAC colonies was significantly correlated with the deteriorated group (p<0.05) rather than the non-deteriorated group. However, the difference in the number of MAC colonies collected by bronchial washing among nodular/bronchiectasis lesions, consolidated lesions and cavitary lesions was not significant. Furthermore, the number of MAC colonies collected by bronchial washing did not correlate with that collected by BMS (r=-0.02).

### 3) Chest CT scan findings and EBUS

The chest CT scans displayed 3 patterns. The first pattern consisted of multiple centrilobular small nodules and bronchiectasis distributed mainly in the upper, middle, and lingular lobes (nodular/bronchiectasis lesions), while EBUS demonstrated heterogeneous internal echoes and unclear margins around the lesions. The second pattern exhibited consolidation and/or collapse (consolidated lesions) with nodular/bronchiectasis lesions, and EBUS recorded iso-echoes and clear margins around the lesions. The third pattern consisted of single or multiple thin-wall cavities (cavitary lesions) with nodular/bronchiectasis, while EBUS demonstrated low-echoes surrounded by iso-echoes.

Chest CT scans in the non-deteriorated group (Table 3) showed nodular/bronchiectasis lesions in 8 patients (73%) and consolidated lesions in 3 patients (27%); cavitary patterns were not included. In the deteriorated group (Table 2), chest CT scans showed nodular/bronchiectasis lesions in 1 patient (7%), consolidated lesions in 6 patients (40%) and cavitary lesions in 8 patients (53%).

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## Discussion

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In the present study, we counted the number of MAC colonies collected from relevant lesions in patients with pulmonary MAC infections using a BMS probe. EBUS imaging of peripheral lung cancer results in a definite hypoechoic or iso-echoic signal that reflects solid tissue (17, 20). Our study showed EBUS that imaging reflected heterogeneous patterns of the relevant lesions. Solid lesions, including small nodules, collapse, infiltration and cavity walls, showed hypoechoic or iso-echoic signals as has been reported. Thus, we concluded that samples from relevant lesions were accurately collected using EBUS.

Bronchoalveolar lavage (BAL) and protected specimen brushes (PSB) were used for collecting pathogenic organisms (21-23). However, BAL may carry the risk of subsequent complications, e.g., transient hypoxia or intrapulmonary dissemination of pathogenic organisms (24, 25). A 10-fold variation in specimen size collected by PSB would yield a difference of only 1 log unit with quantitative bacterial counts (16). Sasabayashi et al. reported that their BMS probe was capable of repeatedly collecting MAC without altering bacterial concentration (16). This may explain the lack of correlation between MAC colony numbers from BAL and BMS collections.

Radiological findings of nodular/bronchiectasis disease showed centrilobular nodules in the outer zone of the middle lobe and lingula fields, associated with dilatation and thickening of the draining small bronchi. Cavitation and consolidation frequently accompany these findings (19, 26, 27). The pathological examination of the only nodular/bronchiectasis lesions showed peribronchial infiltration of mononuclear cells and epithelioid cells surrounding a bronchus or a bronchiole, but the alveolar region was almost nor-

mal (27, 28). On the other hand, the pathological examination of consolidation lesions showed inflammatory thickening of the alveolar walls, loosely grounded granulomas, and/or coalescent inflammatory infiltrates completely replacing the normal alveoli (27) and that of cavitary lesions showed caseous materials, epithelioid cells with multinucleated giant cells, granulation tissue, and a fibrous capsule had replaced the normal alveoli (29). These results suggested that consolidation lesions are more invasive than only nodular/bronchiectasis lesions. Therefore, we classified the CT findings into three patterns (nodular/bronchiectasis lesions).

The number of MAC colonies in relevant lesions investigated by BMS was significantly larger than in the non-deteriorated group. This result suggests that the number of MAC colonies in relevant lesions is useful for predicting the short-term natural history of pulmonary MAC infection. The chest CT scan finding, too, was at least partly useful for predicting the short-term natural history of pulmonary MAC infection. The number of MAC colonies in patients with nodular/bronchiectasis lesions was significantly smaller than that in those with cavitary lesions. However, of 9 patients with consolidated lesions, 3 patients (33%) were from the non-deteriorated group and 6 (67%) were from the deteriorated group. Although consolidated lesions may be progressive, the number of MAC colonies is more useful for predicting deterioration. In fact, patients with consolidated lesions having more than  $6.0 \times 10^3$  CFU/mL MAC colonies underwent deterioration in the present study. Okumura et al reported that fibrocavitary disease demonstrated a high risk for disease progression (26). All 8 patients with cavitary lesions in the present study deteriorated within 12 months. The number of MAC colonies in cavitary lesions was significantly greater than that in nodular/bronchiectasis lesions. Furthermore, the deteriorated patients with consolidated lesions showed high MAC colony numbers. Thus, the number of MAC colonies may predict deterioration more precisely than chest CT findings.

The number of MAC colonies collected by bronchial washing may also be useful for predicting the short-term natural history of pulmonary MAC infection, but in the present patients this was not significantly different among nodular/bronchiectasis lesions, consolidated lesions, and cavitary lesions. We suspect that the samples from peripheral lesions were not accurately collected because the washing fluid was minimal and sample quantity differed for each case. Therefore, the number of MAC colonies collected by bronchial washing does not reflect the difference between each lesion.

Because the natural history of pulmonary MAC infection is thought to vary widely, a diagnosis of pulmonary MAC infection does not necessarily imply a need for immediate treatment. The decision to provide treatment involves an observation period for both radiological and symptomatic progression (6). The present study suggests that cavitary and consolidated lesions that are evident on chest CT scans (especially with a high number of MAC colonies) indicate a

high risk of progression for pulmonary MAC infection. Therefore, we suggest that patients with such chest CT scan findings begin therapy based on their clinical course to prevent further deterioration.

In conclusion, the number of MAC colonies in relevant lesions investigated by BMS was significantly larger in the deteriorated group than in the non-deteriorated group. Cavitory and consolidated lesions evident on chest CT scans displayed large numbers of MAC colonies and are thought to indicate high risk of deterioration. Although the sample size in the present study was small, BMS seems to be a useful tool to predict deterioration of pulmonary MAC infection.

**The authors state that they have no Conflict of Interest (COI).**

#### Acknowledgement

The authors wish to thank the staff from the Department of Laboratory Medicine of Shinshu University Hospital for MAC cultures.

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