Bronchial Brushing and Diagnosis of Pulmonary Nontuberculous Mycobacteria Infection

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Keywords

Nontuberculous mycobacteria · Bronchoscopy · Bronchial brushing

Abstract

Background: The optimal bronchoscopy procedure for diagnosis of pulmonary nontuberculous mycobacteria (NTM) infection is unclear. **Objective:** This study investigated the usefulness of bronchial brushing in bronchoscopy for diagnosis of pulmonary NTM infection in patients with suspected NTM lung disease and nodular bronchiectasis on chest computed tomography (CT) images. **Methods:** Bronchoscopy was prospectively performed for 69 patients with clinically suspected pulmonary NTM infection on chest CT from December 2017 through December 2019. Before and after bronchial brushing, bronchial washing was performed with 20 or 40 mL of normal sterile saline at the same segmental or subsegmental bronchi. Before and after bronchial brushing, samples of the washing fluid (pre- and postbrushing samples) and brush deposits (brush samples) were obtained and cultured separately. **Results:** NTM was detected in 37 of the 69 (53.6%) patients (*Mycobacterium avium* in 27, *Mycobacte-*

rium intracellulare in 7, *M. abscessus* in 2, and *M. kansasii* in 2). NTM was detected in 34 (49.3%) prebrushing samples, in 27 (39.1%) postbrushing samples, and in 20 (29.0%) brush samples from the 69 patients. In 2 (2.9%) patients, NTM was detected only in postbrushing samples; in 1 (1.4%) patient, NTM was detected only in a brush sample. As compared with bronchial washing only, additional bronchial brushing increased the NTM culture-positive rate by 4.3% (3/69). Bronchial brushing caused bleeding, requiring hemostasis in 5 (7.2%) patients. **Conclusion:** Additional bronchial brushing increased the NTM culture-positive rate by only 4.3% (3/69), as compared with bronchial washing alone. Thus, the usefulness of brushing appears to be limited.

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Introduction

The prevalence of pulmonary nontuberculous mycobacteria (NTM) disease is increasing worldwide [1] and in Japan [2]. Pulmonary NTM disease usually manifests as slowly progressive nodular bronchiectatic lesions and fibrocavitary lesions on chest computed tomography

Fig. 1. Flowchart of the study design. NTM, nontuberculous mycobacteria; MAC, *Mycobacterium avium* complex.

(CT) [3]. According to an American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) statement published in 2007 [4], diagnosis of pulmonary NTM disease (including *Mycobacterium avium* complex [MAC], *M. kansasii*, and *M. abscessus*) requires a positive culture result for at least 2 separate expectorated sputum samples or at least 1 bronchial lavage sample. Bronchoscopy was reported to be useful for diagnosis of pulmonary NTM infection in patients with negative sputum cultures for NTM [5–14]; however, the bronchoscopy procedures for NTM diagnosis are not standardized. In addition, no previous study investigated the diagnostic accuracy of bronchial washing with or without bronchial brushing. The genus *Mycobacterium* including MAC and *M. abscessus* have been reported to form biofilms on a variety of different devices [15]. Another report showed that *Mycobacterium avium* (*M. avium*) strains form biofilms on bronchial epithelium in vitro [16]. We hypothesized that NTM form granulomas and biofilm in bronchioles; thus, damage to granulomas and biofilm caused by brushing might increase the culture positivity rate. We therefore investigated the usefulness of bronchial brushing for bronchoscopy diagnosis of pulmonary NTM infection.

Materials and Methods

Study Design

This single-center prospective interventional study enrolled adults (age, ≥ 18 years) with suspected pulmonary NTM disease and bronchiectasis on chest CT. All patients were required to fulfill the ATS criteria for diagnosis of NTM [4]; recruitment took place from December 2017 through December 2019 at Toho University Omori Medical Center. Patients with positive sputum smears for mycobacteria on any 3 consecutive days were excluded.

Sixty-nine patients with suspected pulmonary NTM disease, with small nodular infiltrates with or without bronchiectasis on chest CT images, prospectively underwent bronchoscopy examination. The bronchoscopy procedure is shown in Figure 1. First, we performed bronchial washing at the most affected segmental or subsegmental bronchi, as determined by chest CT. Second, bronchial brushing of the same lesion was done for 10 s (BC-202D-1210, OLYMPUS, Tokyo, Japan) (Fig. 2). Finally, bronchial washing was repeated. Bronchial washing was done with 20–40 mL of normal sterile saline, and samples of bronchial washing fluid were collected before and after bronchial brushing (pre- and postbrushing samples), as were samples of brush deposits (brush samples). Each sample was examined with Ziehl-Neelsen and Gram stains and cultured for mycobacteria, other bacteria, and fungi. In addition, PCR assays for *M. tuberculosis*, *M. avium*, and *Mycobacterium intracellulare* (*M. intracellulare*) were performed. We used DNA-DNA hybridization to identify non-MAC species.

Fig. 2. **a** The most affected lesion on chest CT imagery. **b** Chest X-ray image of bronchial brushing of the most affected lesion.

The patients were classified as NTM culture-positive (positive group) or NTM culture-negative (negative group), and the 2 groups were compared. NTM culture-positive patients were further classified as prebrushing sample culture-positive (prepositive) patients and pre-brushing sample culture-negative (prenegative) patients, and the clinical characteristics, subjective symptoms, positive rate for glycopeptidolipid (GPL) core serum IgA, and chest CT findings of these groups were compared.

Data Collection

The following patient data were collected: age, sex, BMI, smoking history, comorbidities, CT findings for the chest and paranasal sinuses, and culture results for sputum and bronchoscopy. Chest CT scanning was performed during the period 1 month before bronchoscopy. Most patients underwent COPD assessment testing (CAT) to confirm subjective symptoms at the time of admission for bronchoscopy [17].

Chest CT Score

Chest CT score was defined as previously described [18]. We divided the lungs into 6 zones at the levels of the carina and inferior pulmonary vein. Using high-resolution CT, we categorized the 4 types of MAC lesions (cavity, bronchiectasis, nodule, and infiltration) into 5 stages, according to the occupation rate in each zone (0: no lesion, 1: 1–24% occupied, 2: 25–49% occupied, 3: 50–74% occupied, and 4: 75–100% occupied). Chest CT score was assessed by 2 respiratory specialists.

PCR Assay

PCR of *M. tuberculosis*, *M. avium*, and *M. intracellulare* was performed using the COBAS TaqMan MTB and MAI kit (Roche Diagnostics Inc, Tokyo, Japan). The real-time PCR assay amplified a region of the 16S rRNA gene and detected each specific mycobacterium gene sequences.

GPL-Core Serum IgA Assay

The levels of serum IgA antibody against the GPL-core antigen of MAC were measured using an enzyme immunoassay kit (TAUNS Laboratory Inc, Shizuoka, Japan). This kit measured levels of serum IgA antibody against the GPL-core antigen of MAC by using ELISA. GPL-core IgA antibody was developed by Kitada et al. [19] and has been commercially available since 2011 in Japan.

Statistical Analysis

Data are shown as numbers and percentages of patients. Age, BMI, chest CT score, and CAT score are expressed as average $\pm$ SD. Associations of categorical and continuous variables between patients in the positive and negative groups and in the prepositive and prenegative groups were tested with the χ^2 or Fisher's exact test and the Mann-Whitney U test, respectively. A *p* value of <0.05 was considered to indicate statistical significance. Statistical analyses were performed with SPSS version 22 software (IBM, Endicott, NY, USA).

Results

Patient Characteristics

The characteristics of the 69 patients, who were enrolled during the period from December 2017 through December 2019, are shown in Table 1. The mean age was 67.1 ± 10.4 years, 84.1% were woman, mean BMI was 19.5 ± 2.3 kg/m², and 68.1% were never smokers; the GPL-core serum IgA positive rate was 58%. Comorbidities were rheumatoid arthritis (10.1%), Sjögren syndrome (1.4%), and sinusitis (13%). The underlying pul-

Table 1. Clinical characteristics of NTM culture-positive and culture-negative patients

Characteristic	Total	Positive NTM culture	Negative NTM culture	<i>p</i> value
Patients, <i>n</i>	69	37	32	
Age, mean ± SD, years	67.1±10.4	66.8±10.2	67.6±10.8	0.760
Gender (female), <i>n</i> (%)	58 (84.1)	30 (81.1)	28 (87.5)	0.348
BMI, mean ± SD, kg/m ²	19.5±2.3	19.8±2.5	19.2±2.2	0.270
Smoking (never), <i>n</i> (%)	47 (68.1)	25 (67.6)	20 (62.5)	0.562
Positive result for GPL-core serum IgA, <i>n</i> (%)	40 (58.0)	30 (81.1)	10 (31.3)	<0.001
Comorbidities, <i>n</i> (%)				
RA	7 (10.1)	3 (8.1)	4 (12.5)	0.417
SjS	1 (1.4)	0 (0)	1 (3.1)	1.000
Sinusitis	9 (13.0)	4 (10.8)	5 (15.6)	0.406
Underlying pulmonary disease, <i>n</i> (%)				
Emphysema	5 (7.2)	1 (2.7)	4 (12.5)	0.136
Interstitial pneumonia	2 (2.9)	1 (2.7)	1 (3.1)	0.716
Chest CT imaging results				
Chest CT score, mean ± SD	7.65±4.0	7.68±4.01	7.63±4.06	0.959
Cavitary lesion, <i>n</i> (%)	15 (21.7)	9 (24.3)	6 (18.8)	0.397
Subjective symptoms				
CAT score	7.89±4.91	7.21±4.80	8.69±5.01	0.240
Cough score ^a	1.81±1.51	1.21±1.04	2.50±1.68	0.001
Sputum score ^a	1.61±1.35	1.21±1.09	2.07±1.48	0.012
Bronchial washing location, <i>n</i> (%)				
Right upper lobe	17 (24.6)	9 (24.3)	8 (25.0)	0.584
Left upper segment	2 (2.9)	2 (5.4)	0	0.284
Right middle lobe	36 (52.1)	15 (40.5)	21 (65.6)	0.033
Lingula	8 (11.6)	6 (16.2)	2 (6.3)	0.182
Right lower lobe	2 (2.9)	2 (5.4)	0	0.284
Left lower lobe	4 (5.8)	3 (8.1)	1 (3.1)	0.364
With bronchoscopy, <i>n</i> (%)				
Positive culture of <i>M. avium</i> / <i>M. intra</i> /non-MAC	27 (39.1)/7 (10.1)/4 (5.8)	27 (73.0)/7 (18.9)/4 (10.8)	0	
Positive PCR of <i>M. avium</i> / <i>M. intra</i>	27 (39.1)/7 (10.1)	26 (70.3)/6 (16.2)	1 (3.1)/1 (3.1)	
Positive smear of acid-fast bacilli	15 (21.7)	15 (40.5)	0	
Positive culture of <i>P. aeruginosa</i>	5 (7.2)	0	5 (15.6)	0.018
Positive culture of <i>H. influenzae</i>	6 (8.7)	1 (2.7)	5 (15.6)	0.070
With sputum, <i>n</i> (%)				
Positive culture of <i>M. avium</i> / <i>M. intra</i> /non-MAC	6 (8.7)/1 (1.5)/0	6 (16.2)/1 (2.7)/0	0	
Positive PCR of <i>M. avium</i> / <i>M. intra</i>	9 (13.0)/3 (4.3)	8 (21.6)/3 (8.1)	1 (3.1)/0	
Bleeding complications, <i>n</i> (%)	5 (7.2)	1 (2.7)	4 (12.5)	0.136

NTM, nontuberculous mycobacterial; RA, rheumatoid arthritis; SjS, Sjögren syndrome; CT, computed tomography; CAT, COPD assessment test; GPL, glycopeptidolipid; *M. avium*, *Mycobacterium avium*; *M. intra*, *Mycobacterium intracellulare*; non-MAC, non-*Mycobacterium avium* complex; *P. aeruginosa*, *Pseudomonas aeruginosa*; *H. influenzae*, *Haemophilus influenzae*. ^a Score for subjective symptoms of cough and sputum, contained in CAT score.

monary disease was pulmonary emphysema in 7.2% and interstitial pneumonia in 2.9%. Chest CT score was 7.65 ± 4.0. The CAT score for subjective symptoms was 7.89 ± 4.91, the CAT score for cough was 1.81 ± 1.51, and the CAT score for sputum was 1.61 ± 1.35. The bronchial wash site was the right upper lobe in 24.6% of patients, left upper segment in 2.9%, right middle lobe in 52.1%, left lingula segment in 11.6%, right lower lobe in 2.9%, and left lower lobe in 5.8% of patients. There were significant differences between the positive and negative groups in positive rate of GPL-core serum IgA (81.1 vs.

31.3%; *p* < 0.001), the score for subjective symptoms of cough in CAT score (1.21 ± 1.04 vs. 2.50 ± 1.68; *p* = 0.001), sputum in CAT score (1.21 ± 1.09 vs. 2.07 ± 1.48; *p* = 0.012), the rate of right middle lobe in bronchial washing location (40.5 vs. 65.6%; *p* = 0.033) and the rate of positive culture result for *Pseudomonas aeruginosa* (0 vs. 15.6%; *p* = 0.018).

Microbiological Results of Bronchoscopy

The culture results for acid-fast bacteria were as follows: *M. avium*, 27/38 (71.1%); *M. intracellulare*, 7/38

Table 2. Microbiological results of bronchoscopy

Patient No.	Prebrushing sample			Brush sample			Postbrushing sample			Culture result
	smear	MAC-PCR	culture	smear	MAC-PCR	culture	smear	MAC-PCR	culture	
1	+	+	+	+	+	+	+	+	–	<i>M. avium</i>
2	–	–	+	–	–	–	–	–	–	<i>M. kansasii</i>
3	–	+	+	–	+	+	–	+	+	<i>M. avium</i>
4	–	+	+	+	+	+	+	+	+	<i>M. avium</i>
5	–	–	+	–	–	–	–	–	–	<i>M. avium</i>
6	–	–	–	–	–	–	–	–	+	<i>M. abscessus</i>
7	–	–	+	–	–	+	–	+	+	<i>M. avium</i>
8	–	–	+	–	+	+	–	+	+	<i>M. intracellulare</i>
9	–	–	–	–	–	–	–	+	+	<i>M. avium</i>
10	–	+	+	–	–	–	–	+	+	<i>M. avium</i>
11	–	+	+	–	+	+	–	+	+	<i>M. avium</i>
12	+	+	+	+	+	+	+	+	+	<i>M. intracellulare</i>
13	–	+	+	–	–	–	–	+	+	<i>M. avium</i>
14	+	+	+	+	+	+	+	+	+	<i>M. avium</i>
15	–	+	+	–	+	–	–	+	+	<i>M. intracellulare</i>
16	–	+	+	–	–	–	–	–	–	<i>M. avium</i>
17	–	+	+	–	–	+	–	+	+	<i>M. avium</i>
18	+	+	+	+	+	+	+	+	+	<i>M. avium</i>
19	–	+	+	–	–	–	–	–	–	<i>M. avium</i>
20	+	+	+	–	+	+	–	+	–	<i>M. avium</i>
21	–	–	–	–	+	+	–	–	–	<i>M. avium</i>
22	–	+	+	–	–	–	–	–	–	<i>M. intracellulare</i>
23	+	+	+	+	+	+	+	+	+	<i>M. intracellulare</i>
24	–	+	+	–	–	–	–	+	+	<i>M. avium</i>
25	+	+	+	–	–	–	+	+	+	<i>M. avium</i>
26	+	+	+	+	+	+	+	+	+	<i>M. avium</i>
27	–	–	+	–	–	–	–	–	+	<i>M. kansasii</i>
28	–	+	+	–	–	–	–	+	–	<i>M. avium</i>
29	+	+	+	+	+	+	–	+	–	<i>M. avium</i>
30	+	+	+	+	–	–	+	+	+	<i>M. avium</i>
31	+	+	+	+	+	+	+	+	+	<i>M. avium</i>
32	+	+	+	–	+	–	+	+	+	<i>M. avium</i>
33	+	+	+	+	+	+	+	+	+	<i>M. avium</i>
34	–	–	+	–	–	+	–	–	+	<i>M. abscessus</i>
35	+	+	+	+	+	+	+	+	+	<i>M. avium/intracellulare</i>
36	–	+	+	–	–	–	–	+	+	<i>M. intracellulare</i>
37	–	+	+	–	+	+	–	+	+	<i>M. avium</i>
Total, <i>n</i>	14	27	34	12	19	20	13	28	27	

M. avium, *Mycobacterium avium*; *M. intracellulare*, *Mycobacterium intracellulare*; MAC, *Mycobacterium avium* complex.

(18.4%); *M. kansasii*, 2/38 (5.3%); and *M. abscessus*, 2/38 (5.3%). The microbiological results of bronchoscopy are shown in Tables 2 and 3 and Figure 3. Among the 37 NTM culture-positive patients (*M. avium* and *M. intracellulare* were both detected in one patient), the positive rates for prebrushing samples in smear, MAC-PCR, and NTM culture were 37.8% (14/37), 73% (27/37), and 91.9% (34/37), respectively; the positive rates for brush samples in smear, MAC-PCR, and NTM culture were

32.4% (12/37), 51.4% (19/37), and 54.1% (20/37), respectively; and the positive rates for postbrushing samples in smear, MAC-PCR, and NTM culture were 35.1% (13/37), 75.7% (28/37), and 73.0% (27/37), respectively. Among the 3 patients with culture-negative prebrushing samples, 2 were culture positive only for the postbrushing sample and 1 patient was culture positive only for the brush sample.

Table 3. Microbiological results by sample type

	Prebrushing sample	Brush sample	Postbrushing sample	Total
Smear	37.8% (14/37)	32.4% (12/37)	35.1% (13/37)	40.5% (15/37)
MAC-PCR	73.0% (27/37)	51.4% (19/37)	75.7% (28/37)	83.8% (31/37)
Culture	91.9% (34/37)	54.1% (20/37)	73% (27/37)	100% (37/37)

MAC, *Mycobacterium avium* complex.

Fig. 3. Distribution of culture-positive patients as visualized with a Venn diagram.

Comparison of Patients with Positive and Negative Bronchoscopy Culture Results before Brushing

The 37 patients in the positive group were classified into 2 groups, in accordance with their bronchoscopy culture results: 34 patients were classified as prepositive (mean age, 66.7 ± 10.0 years; 82.4% women) and 3 were classified as prenegative (mean age, 66.8 ± 10.2 years; 81.1% women). The characteristics of these 2 patient groups are shown in Table 4. Chest CT score was significantly higher in the prepositive group than in the prenegative group (7.88 ± 4.12 vs. 5.33 ± 0.58 ; $p = 0.003$).

Safety of Bronchial Brushing

Bronchial brushing resulted in bronchial bleeding requiring hemostatic treatment in 5 patients. However,

no patient developed serious adverse events or complications such as pneumonia, hemoptysis, or pneumothorax.

Discussion/Conclusion

Several studies investigated the diagnostic accuracy of bronchoscopy for NTM [5–14]. Some performed bronchoscopy for patients with chest CT findings suggestive of pulmonary NTM disease, during a defined period [5, 13, 14]; however, the bronchoscopy procedure differed between studies. Tanaka et al. performed transbronchial lung biopsy with bronchial washing and detected NTM in 15 of 26 patients (57.7%) [5]. Kitada et al. performed

Table 4. Clinical characteristics of patients in prepositive and prenegative groups

Characteristic	Prepositive	Prenegative	<i>p</i> value
Patients, <i>n</i>	34	3	
Age, mean ± SD, years	66.7±10	68.0±13.1	0.879
Gender (female), <i>n</i> (%)	28 (82.4)	1 (33.3)	0.477
BMI, mean ± SD, kg/m ²	19.7±2.4	21.0±2.9	0.504
Smoking (never), <i>n</i> (%)	23 (67.6)	2 (66.7)	0.704
Positive result for GPL-core serum IgA, <i>n</i> (%)	28 (82.4)	2 (66.7)	0.477
Comorbidities, <i>n</i> (%)			
RA	3 (8.8)	0	0.770
SjS	0	0	–
Sinusitis	3 (8.8)	1 (33.3)	0.298
Underlying pulmonary disease, <i>n</i> (%)			
Emphysema	1 (2.9)	0	0.919
Interstitial pneumonia	1 (2.9)	0	0.919
Chest CT imaging results			
Chest CT score, mean ± SD	7.88±4.12	5.33±0.58	0.003
Cavitary lesion, <i>n</i> (%)	8 (23.5)	1 (33.3)	0.578
Subjective symptoms			
CAT score	7.15±5.16	7.00±3.46	0.925
Cough score ^a	1.23±1.06	1.0±1.0	0.740
Sputum score ^a	1.23±1.12	1.0±1.0	0.741
BALF location, <i>n</i> (%)			
Right upper lobe	7 (20.6)	2 (66.7)	0.141
Left upper segment	2 (5.9)	0	0.842
Right middle lobe	14 (41.2)	1 (33.3)	0.644
Lingula	6 (17.6)	0	0.579
Right lower lobe	2 (5.9)	0	0.842
Left lower lobe	3 (8.8)	0	0.770
With bronchoscopy, <i>n</i> (%)			
Positive culture of <i>M. avium</i> / <i>M. intra</i> /non-MAC	25 (73.5)/7 (20.6)/3 (8.8)	2 (66.7)/0/1 (33.3)	–
Positive PCR of <i>M. avium</i> / <i>M. intra</i>	24 (70.6)/6 (17.6)	1 (33.3)/0	–
Positive smear of acid-fast bacilli	15 (44.1)	0	–
Positive culture of <i>P. aeruginosa</i>	0	0	–
Positive culture of <i>H. influenzae</i>	1 (2.9)	0	0.919
With sputum, <i>n</i> (%)			
Positive culture of <i>M. avium</i> / <i>M. intra</i> /non-MAC	5 (14.7)/1 (2.9)/0	1 (33.3)/0/0	–
Positive PCR of <i>M. avium</i> / <i>M. intra</i>	7 (20.6)/3 (8.8)	1 (33.3)/0	–
Bleeding complications, <i>n</i> (%)	1 (2.9)	0	0.919

NTM, nontuberculous mycobacterial; RA, rheumatoid arthritis; SjS, Sjögren syndrome; CT, computed tomography; CAT, COPD assessment test; GPL, glycopeptidolipid; *M. avium*, *Mycobacterium avium*; *M. intra*, *Mycobacterium intracellulare*; non-MAC, non-*Mycobacterium avium* complex; *P. aeruginosa*, *Pseudomonas aeruginosa*; *H. influenzae*, *Haemophilus influenzae*. ^a Score for subjective symptoms of cough and sputum, contained in CAT score.

bronchial washing only and detected NTM in 28 of 56 patients (50%) [13]. Our previous study of bronchial brushing with washing detected NTM in 36 of 71 patients (50.7%) [14]. Tanaka et al. [5] reported that among 15 patients with positive NTM cultures, transbronchial lung biopsy detected only 9 with epithelioid granuloma. No characteristic histological findings were obtained from 11 patients with negative NTM cultures. Although the number of patients was small, they concluded that the diag-

nostic rate was lower for transbronchial lung biopsy than for bronchial washing. No previous study compared the diagnostic rate of bronchial washing with or without bronchial brushing in the same patients. We investigated the additional effects of bronchial brushing in 69 patients that underwent bronchial washing before and after bronchial brushing.

In this study, patients in the negative group tended to be infected with *P. aeruginosa* and more severe subjective

symptoms of cough and sputum. This is the same trend as our previous report and suggest that subjective symptoms be milder in patients with pulmonary NTM disease than in those with chronic lower respiratory infection for other pathogenic bacteria, even at the same disease severity in chest CT [14].

During bronchial brushing, the bronchial surface is scraped to obtain cytological and microbiological specimens from inside the airway mucosa or bronchial lesions [20]. This technique is useful as a possible auxiliary method for diagnosis of pulmonary lesions beyond the reach of bronchoscopy. Sensitivity in peripheral lung cancer cytology was reported to be 43% with bronchial washing alone, but this increased to 54% after bronchial brushing [21]. Another study reported that the diagnostic rates of bronchial washing and brushing were 37.3 and 46.4%, respectively, for peripheral lung cancer [22]. According to these reports, use of brushing increased the diagnosis rate for peripheral lung cancer by 9.1–11%. The authors concluded that bronchial brushing improved the diagnostic rate for patients with suspected peripheral lung cancer. In the present study, prebrushing samples resulted in positive NTM culture in 34 of 69 (49.3%) patients, 20 of 69 (29.0%) had a positive NTM culture of brush samples, and 27 of 69 (39.1%) had a positive culture of postbrushing samples. The diagnostic rate was not increased by bronchial brushing.

Pathological studies showed granulomatous inflammation around bronchi and bronchioles in pulmonary MAC patients [23]. Another report showed that *M. avium* strains form biofilms on bronchial epithelium in vitro [16]. We predicted that NTM forms granulomas and biofilm in bronchioles; thus, damaging granulomas and biofilm by brushing might increase culture positivity. However, the culture-positive rate was lower for postbrushing samples than for prebrushing samples, which indicates that NTM attached to bronchial epithelium might be removed during the first wash (prebrushing sample). Therefore, NTM cultures were negative in the second wash (postbrushing sample). Furthermore, bronchial brushing was performed in a relatively peripheral bronchial region, and a 20- to 40-mL lavage solution might not sufficiently reflect the effect of brushing.

Although the culture-positive rates for postbrushing and brush samples were lower than that of prebrushing samples, only the postbrushing sample was culture positive in 2 patients, and only the brush sample was culture positive in 1 patient. Bronchial washing is usually performed only after bronchial brushing. The present overall positive rate of NTM cultures was 53.6% (37/69; includ-

ing the 3 patients added to the 34 culture-positive patients in the prebrushing sample). Thus, bronchial brushing increased the rate of positive NTM cultures by 4.3%, as compared with bronchial washing only.

Bronchial brushing resulted in bronchial bleeding requiring hemostatic treatment in 5 patients. Bleeding improved promptly in all these patients, and no subsequent complications such as pneumonia or hemoptysis were observed. This result suggests that bronchial brushing for pulmonary NTM is relatively safe.

Although bronchial brushing was relatively safe, it increased the rate of positive NTM cultures by only 4.3%, which was lower than the increase for peripheral lung cancer. Furthermore, 91.9% (34/37) of NTM culture-positive cases were detected by bronchial washing alone. Therefore, the usefulness of bronchial brushing in bronchoscopy for diagnosis of pulmonary NTM infection appears to be limited. Although additional bronchial brushing may be regarded as a part of routine procedure, bronchial washing alone is worth performing for diagnosis of pulmonary NTM disease if bronchial brushing is not indicated for patients at risk of bleeding or if fluoroscopy is not available for reasons such as pregnancy.

In this study, chest CT scores were better for prenegative patients than for prepositive patients, which suggests that additional bronchial brushing may be effective in patients with mild disease on chest CT images. However, 13 of 34 patients (38.2%) in the prepositive group had a relatively low chest CT score lower than 5.3 in the prenegative group. This result requires confirmation in a larger study.

This study had several limitations. First, it was a single-center study of a small number of patients. Second, selection bias is a concern because all enrolled patients had chest CT findings suggestive of pulmonary NTM disease, as determined by a respiratory physician.

To our knowledge, this is the first study of the usefulness of additional bronchial brushing for diagnosis of pulmonary NTM infection in bronchoscopy. In conclusion, additional bronchial brushing increased the NTM culture positivity rate by only 4.3%, as compared with bronchial washing alone. Although the procedure was relatively safe, the usefulness of brushing appears to be limited.

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Statement of Ethics

This study protocol was approved by the Ethics Committee of Toho University Omori Medical Center (Approval No. M17310), and the study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All patients provided written informed consent prior to participation.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Naohisa Urabe: conceptualization and methodology. Susumu Sakamoto: formal analysis and writing – original draft preparation. Ai Ito: visualization. Ryo Sekiguchi: data curation. Yui Shimanuki: investigation. Takumi Kanokogi: investigation. Takumi Motohashi: investigation. Nanami Anzai: investigation. Sakae Homma: supervision and validation. Kazuma Kishi: writing – reviewing and editing.