Original Article

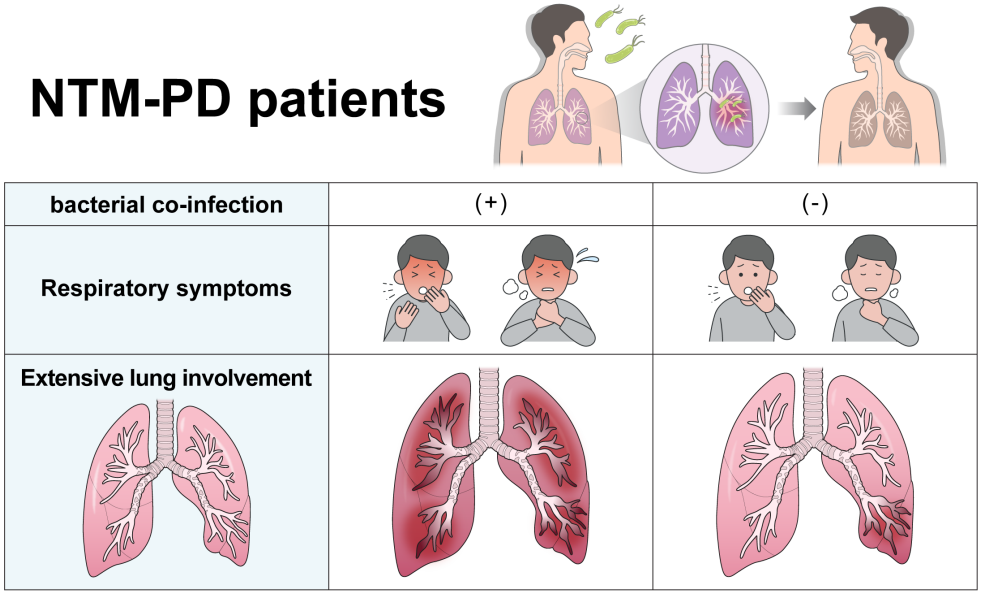
trd Tuberculosis & Respiratory Diseases

Exploring the Association of Bacterial Coinfections with Clinical Characteristics of Patients with Nontuberculous Mycobacterial Pulmonary Disease

https://doi.org/10.4046/trd.2024.0003
ISSN: 1738-3536(Print)/
2005-6184(Online)
Tuberc Respir Dis 2024;87:505-513

Seong Mi Moon, M.D., Ph.D.[1,2], Hyunkyu Cho, M.D., Ph.D.[1] and Beomsu Shin, M.D., Ph.D.[3]

[1]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, [2]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Chungbuk National University Hospital, Chungbuk National University College of Medicine, Cheongju, [3]Department of Allergy, Pulmonology and Critical Care Medicine, Gachon University Gil Medical Center, Gachon University College of Medicine, Incheon, Republic of Korea



Address for correspondence
Beomsu Shin, M.D., Ph.D.
Department of Allergy, Pulmonology and Critical Care Medicine, Gachon University Gil Medical Center, Gachon University College of Medicine, 21 Namdong-daero 774beon-gil, Namdong-gu, Incheon 21565, Republic of Korea
E-mail bsshin83@gmail.com
Received Jan. 7, 2024
Revised Mar. 17, 2024
Accepted Apr. 24, 2024
Published online Apr. 30, 2024

Abstract

Background: Clinical data for bacterial coinfection of the lower respiratory tract in patients with nontuberculous mycobacterial pulmonary disease (NTM-PD) are scarce. This study aims to assess the prevalence of bacterial coinfection and clinical features in NTM-PD patients.

Methods: This retrospective study screened 248 patients with NTM-PD who underwent bronchoscopy between July 2020 and July 2022, from whom newly diagnosed NTM-PD patients were analyzed. Bacterial culture using bronchial washing fluid was performed at the time of NTM-PD diagnosis.

Results: In the 180 patients (median age 65 years; 68% female), *Mycobacterium avium* complex (86%) was the most frequent NTM isolated. Bacterial coinfections were detected in 80 (44%) patients. Among them, the most common bacterium was *Klebsiella*

pneumoniae (n=25/80, 31.3%), followed by *Pseudomonas aeruginosa* (n=20/80, 25%) and *Staphylococcus aureus* (n=20/80, 25%). Compared with NTM-PD patients without bacterial coinfections, patients with bacterial coinfections showed more frequent extensive lung involvement (33% vs. 1%, p<0.001). Additionally, compared with NTM-PD patients without *P. aeruginosa* infection, those with *P. aeruginosa* infection were older (74 years vs. 64 years, p=0.001), had more frequent respiratory symptoms (cough/excessive mucus production 70% vs. 38%, p=0.008; dyspnea 30% vs. 13%, p=0.047), and had extensive lung involvement (60% vs. 9%, p<0.001).

Conclusion: Less than half of patients with newly diagnosed NTM-PD had bacterial coinfections, linked to extensive lung involvement. Specifically, *P. aeruginosa* coinfection was significantly associated with older age, more frequent respiratory symptoms, and extensive lung involvement.

Keywords: Nontuberculous Mycobacteria; Bacterial Coinfection; Bronchiectasis; Bronchiectasis Severity; Pseudomonas Aeruginosa

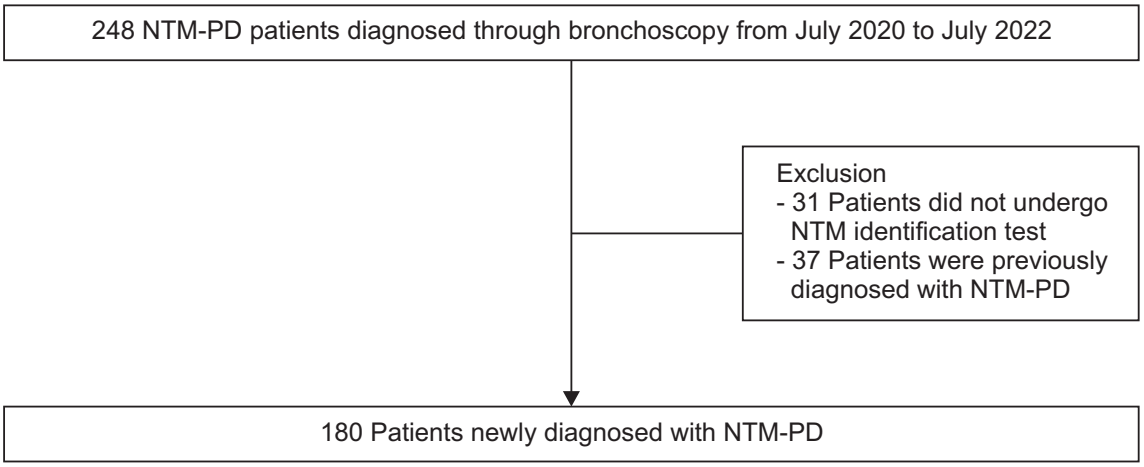
Introduction

Nontuberculous mycobacteria (NTM), a diverse group of organisms excluding *Mycobacterium leprae* and *Mycobacterium tuberculosis* complex, are ubiquitous environmental inhabitants found in soil and water[1]. While often harmless, NTM can cause various human diseases, with pulmonary disease being the most common manifestation[2]. Currently, there is a global trend of increasing incidence in nontuberculous mycobacterial pulmonary disease (NTM-PD), particularly in developed countries including South Korea[3,4].

NTM-PD presents in a variety of clinical manifestations over a long time period after NTM acquisition[1,5]. One of these manifestations is that the bronchi become more susceptible to chronic, slowly progressive inflammation, potentially compromising their structural integrity[6]. Furthermore, the compromised airways in NTM-PD patients are more susceptible to secondary infections, and previous studies have indicated that 12% to 45% of patients with *Mycobacterium avium* complex pulmonary disease (MAC-PD) concurrently exhibit bacterial infections[7-9]. Although prior investigations have consistently identified coinfection with bacterial pathogens alongside NTM acquisition or disease course, these studies have been limited by the use of sputum culture-based bacterial detection, small numbers of patients, and differences in research objectives and testing timing depending on study design[7-10].

This study aims to investigate the prevalence and

Figure 1. Patient flow chart. NTM-PD: nontuberculous mycobacterial pulmonary disease; NTM: nontuberculous mycobacteria.



clinical significance of coinfection with pathogenic microorganisms obtained through bronchoscopy on the initial diagnosis of NTM-PD.

Materials and Methods

1. Study population

We retrospectively reviewed patients who were diagnosed with NTM-PD by bronchoscopic evaluation at Samsung Changwon Hospital (a 761-bed tertiary referral hospital in Changwon, South Korea), between July 2020 and July 2022. A total of 248 NTM-PD patients was initially considered. Among them, 68 were excluded due to absence of NTM identification test (n=31) or previous diagnosis of NTM-PD (n=37). Consequently, 180 patients newly diagnosed with NTM-PD were included in this study (Figure 1). All patients met the clinical and radiologic criteria (nodular or cavitary opacities on chest radiograph, or computed tomography [CT] scan showing bronchiectasis with multiple small nodules) for the diagnosis of NTM-PD with positive culture results from at least one bronchial washing[2].

2. Bronchoscopic procedures

All bronchoscopy procedures were performed by attending physicians. Sedatives including 0.05 to 0.07 mg/kg of midazolam and/or 0.2 to 0.5 μg/kg of fentanyl were administered intravenously to achieve adequate sedation prior to the procedure[11,12]. All procedures were performed transnasally or transorally under local anesthesia. In all patients, complete airway inspection was performed, and bronchial washing was conducted at any involved lesion that was suggestive of NTM-PD on chest CT prior to bronchoscopy evaluation. Sterile normal saline (20 to 40 mL) was injected into the involved lesion, and the specimen was aspirated via bronchoscope.

3. Microbiologic evaluation

All bronchial washing specimens were examined for both mycobacterial and bacterial pathogens. All bronchial washing specimens were cultured using both solid (3% Ogawa medium; Korean Institute of Tuberculosis, Cheongju, Korea) and liquid (BACTEC 960 Mycobacterial Growth Indicator Tube, Becton Dickinson, Sparks, MD, USA) media[13].

All specimens were also examined for bacterial pathogens using the Gram stain method, and bacterial identification was performed using automatic VITEK 2 diagnostic systems (bioMérieux Inc., Hazelwood, MO, USA).

4. Data collection

We retrospectively collected patient data including age, sex, body mass index (BMI), smoking history, respiratory symptoms of NTM-PD (e.g., cough, excessive mucus production, dyspnea, or hemoptysis), presence of underlying pulmonary disease, and bronchial washing specimen results. The laboratory findings, including white blood cell count, C-reactive protein as an inflammatory marker, and albumin as an indicator reflecting overall nutritional status, were assessed.

Radiological phenotypes of NTM-PD were assessed and classified into fibrocavitary form, nodular bronchiectatic form, and unclassifiable form[14]. We defined

Table 1. Baseline characteristics of nontuberculous mycobacterial pulmonary disease patients who underwent bronchoscopy (n=180)

Characteristic	Value
Age, yr	65 (56–74)
Female sex	122 (67.8)
Body mass index, kg/m^2	20.4 (18.9–22.4)
Smoking history	
Non-smoker	139 (77.2)
Comorbidity	
Diabetes mellitus	22 (12.2)
Respiratory symptoms*	
Cough with or without excessive mucus production	75 (41.7)
Dyspnea	26 (14.4)
Hemoptysis	23 (12.8)
Asymptomatic	82 (45.6)
Radiological phenotypes	
Nodular bronchiectatic form	151 (83.9)
Fibrocavitary form	22 (12.2)
Unclassifiable	7 (3.9)
Underlying pulmonary disease*	
Bronchiectasis	169 (93.9)
Tuberculous-destroyed lung	22 (12.2)
Emphysema	12 (6.7)
Interstitial lung disease	4 (2.2)
Laboratory findings	
White blood cell, /μL	5,760 (4,753–6,863)
C-reactive protein, mg/dL	1.4 (0.4–6.4)
Albumin, g/dL	4.4 (4.1–4.7)

Values are presented as median (interquartile range) or number (%).
*Cases are duplicated.

coinfection as the detection of pathogenic microorganisms other than NTM in at least one bronchial washing bacterial culture. Extensive lung involvement was defined if three or more lobes are involved, as assessed by CT scan, as previously reported[15].

5. Ethics

This study was approved by the Institutional Review Board of Samsung Changwon Hospital (reference no. 2024-01-002). The requirement for informed consent was waived due to the retrospective nature of the study and the data were anonymized and de-identified prior to analysis.

6. Statistical analysis

Data are presented as median with interquartile range (IQR) or number (%) of patients. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test[16]. Continuous variables were compared using the Mann-Whitney U test[17]. A p-value <0.05 was considered statistically significant. Statistical analyses were conducted using SPSS version 23.0 for Windows (IBM Co., Chicago, IL, USA)[18].

Results

1. Baseline characteristics of NTM-PD patients

A total of 180 patients was included, and the baseline characteristics are shown in Table 1. The median age of patients was 65 years (IQR, 56 to 74), 122/180 (67.8%) were female, and the median BMI was 20.4 kg/m^2 (IQR, 18.9 to 22.4). Most patients were non-smokers (n=139/180, 77.2%) and 22/180 patients (12.2%) had diabetes mellitus as a comorbidity. The most common predominant symptoms were cough with or without excessive mucus production (n=75/180, 41.7%), followed by dyspnea (n=26/180, 14.4%); however, 82/180 patients (45.6%) were asymptomatic at the time of diagnosis. Regarding radiological phenotypes of NTM-PD, nodular bronchiectatic form (n=151/180, 83.9%) was most often observed, followed by fibrocavitary form (n=22/180, 12.2%) and unclassifiable form (n=7/180, 3.9%). Almost all patients (n=169/180, 93.9%) had bronchiectasis, and tuberculous-destroyed lung, parenchymal destruction due to previous tuberculosis infection, was found in 22/180 patients (12.2%). Median white blood cell, C-reactive protein, and albumin levels

Table 2. NTM species cultured from bronchial washing fluid (n=180)

NTM species	No. (%)
Mycobacterium avium complex	
M. avium	36 (20.0)
M. intracellulare	115 (63.9)
M. abscessus	
M. abscessus subspecies *abscessus*	7 (3.9)
M. abscessus subspecies *massiliense*	11 (6.1)
M. kansasii	6 (3.3)
M. mucogenicum	1 (0.5)
Mixed infection	
M. avium+M. intracellulare	2 (1.1)
M. avium+M. kansasii	1 (0.5)
M. intracellulare+M. abscessus subspecies *massiliense*	1 (0.5)

NTM: nontuberculous mycobacteria.

Table 3. Bacteria species cultured from bronchial washing fluid (n=180)

Variable	No. (%)
Patients with detected bacteria	80 (44.4)
Single bacterium	73
Multiple bacteria	7
Bacteria species*	80
Klebsiella species	29 (36.3)
Klebsiella pneumoniae	25
Klebsiella oxytoca	4
Pseudomonas species	22 (27.5)
Pseudomonas aeruginosa	20
Pseudomonas fluorescens	1
Pseudomonas putida	1
Staphylococcus aureus	20 (25.0)
MSSA	16
MRSA	4
Enterobacter species	5 (6.3)
Enterobacter cloacea	3
Enterobacter aerogenes	2
Haemophilus influenzae	3 (3.8)
Escherichia coli	2 (2.5)
Streptococcus pneumoniae	2 (2.5)
Acinetobacter baumannii	2 (2.5)
Morganella morganii	1 (1.3)
Enterococcus species	1 (1.3)

*Cases are duplicated.
MSSA: methicillin-sensitive *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*.

were 5,760/μL, 1.4 mg/dL, and 4.4 g/dL, respectively.

2. NTM species cultured from bronchial washing fluid

Table 2 shows the NTM species cultured. The most common species was *Mycobacterium intracellulare* (n=115/180, 63.9%), followed by *M. avium* (n=36/180, 20.0%) and *Mycobacterium abscessus* (n=18/180, 10.0%). Mixed infections were found in 4/180 patients (2.2%) and included *M. avium*/*M. intracellulare* (n=2), *M. avium*/*Mycobacterium kansasii* (n=1), and *M. intracellulare*/*M. abscessus* subspecies *massiliense* (n=1).

3. Bacterial culture from bronchial washing fluid

Of the 180 patients with newly diagnosed NTM-PD by bronchial washing, 80/180 (44.4%) had bacteria cultured from bronchial washing fluid, and the species of bacteria are summarized in Table 3. Most (n=73/80, 91.2%) had a single bacterium, and 7/80 (8.8%) had multiple bacteria species. The most commonly identified species were *Klebsiella* species (n=29/80, 36.3%) including 25 cases of *Klebsiella pneumoniae*, followed by *Pseudomonas* species (n=22/80, 27.5%) including 20 cases of *Pseudomonas aeruginosa*, and then by *Staphylococcus aureus* (n=20/80, 25.0%).

Table 4. Comparison of clinical characteristics of nontuberculous mycobacterial pulmonary disease patients with or without bacteria cultured from bronchial washing fluid

Characteristic	Bacteria cultured		p-value
	Yes (n=80)	No (n=100)	
Age, yr	67 (58–75)	64 (56–73)	0.110
Female sex	53 (66.3)	69 (69.0)	0.749
Body mass index, kg/m^2	20.8 (19.0–23.3)	20.4 (18.4–21.7)	0.201
Non-smoker	60 (75.0)	79 (79.0)	0.593
Comorbidity			
Diabetes mellitus	11 (13.8)	11 (11.0)	0.650
Underlying pulmonary disease*			
Emphysema	4 (5.0)	8 (8.0)	0.552
Interstitial lung disease	1 (1.3)	3 (3.0)	0.630
Bronchiectasis	77 (96.3)	92 (92.0)	0.350
Tuberculous-destroyed lung	10 (12.5)	12 (12.0)	>0.999
Respiratory symptoms*			
Cough with or without excessive mucus production	38 (47.5)	37 (37.0)	0.173
Dyspnea	13 (16.3)	13 (13.0)	0.670
Hemoptysis	9 (11.3)	14 (14.0)	0.657
Asymptomatic	32 (40.0)	50 (50.0)	0.228
Radiological phenotypes			
Nodular bronchiectatic form	71 (88.8)	80 (80.0)	0.153
Fibrocavitary form	5 (6.3)	17 (17.0)	0.038
Unclassifiable	4 (5.0)	3 (3.0)	0.702
No. of involved lobes ≥3	26 (32.5)	1 (1.0)	<0.001
Laboratory findings			
White blood cell, /μL	6,050 (4,725–6770)	5,610 (4,778–6,948)	0.491
C-reactive protein, mg/dL	1.3 (0.4–6.8)	1.4 (0.4–5.9)	0.901
Albumin, g/dL	4.4 (4.2–4.6)	4.5 (3.9–4.7)	0.963

Values are presented as median (interquartile range) or number (%).
*Cases are duplicated.

4. Comparison of clinical characteristics of NTM-PD patients according to bacteria

Table 4 shows the comparison of clinical characteristics of NTM-PD patients with or without bacteria identified from bronchial washing fluid. Compared with NTM-PD patients without bacteria, those with bacteria were more likely to have extensive disease with three or more lobes involved (n=26 vs. 1, p<0.001). Patients with the fibrocavitary form of NTM-PD tended to be without bacterial coinfection (p=0.038).

Compared with NTM-PD patients without *P. aeruginosa*, those with *P. aeruginosa* were older (74 years vs. 64 years, p=0.001), tended to be male (60.0 % vs. 28.8%, p=0.009), were ever-smokers (55.0% vs. 18.8%, p<0.001), and had respiratory symptoms including cough with or without excessive mucus production (70.0% vs. 38.1%, p=0.008), dyspnea (30.0% vs. 12.5%, p=0.047), and extensive lung disease (60.0% vs. 9.4%, p<0.001) (Table 5). In contrast, patients with *K. pneumoniae* had no differences compared with those without *K. pneumoniae* except in C-reactive protein level (Supplementary Table S1). Also, patients with *S. aureus* did not differ from those without *S. aureus* (Supplementary Table S2).

Table 5. Comparison of clinical characteristics of nontuberculous mycobacterial pulmonary disease patients with or without *Pseudomonas aeruginosa* cultured from bronchial washing fluid

Characteristic	*Pseudomonas aeruginosa*		p-value
	Yes (n=20)	No (n=160)	
Age, yr	74 (65–80)	64 (56–73)	0.001
Female sex	8 (40.0)	114 (71.3)	0.009
Body mass index, kg/m^2	21.0 (18.9–23.3)	20.4 (19.0–22.0)	0.757
Non-smoker	9 (45.0)	130 (81.3)	<0.001
Comorbidity			
Diabetes mellitus	3 (15.0)	19 (11.9)	0.716
Underlying pulmonary disease*			
Emphysema	3 (15.0)	9 (5.6)	0.134
Interstitial lung disease	1 (5.0)	3 (1.9)	0.378
Bronchiectasis	19 (95.0)	150 (93.8)	>0.999
Tuberculous-destroyed lung	2 (10.0)	20 (12.5)	>0.999
Respiratory symptoms*			
Cough with or without excessive mucus production	14 (70.0)	61(38.1)	0.008
Dyspnea	6 (30.0)	20 (12.5)	0.047
Hemoptysis	2 (10.0)	21 (13.1)	>0.999
Asymptomatic	6 (30.0)	76 (47.5)	0.159
Radiological phenotypes			
Nodular bronchiectatic form	18 (90.0)	133 (83.1)	0.746
Fibrocavitary form	1 (5.0)	21 (13.1)	0.475
Unclassifiable	1 (5.0)	6 (3.8)	0.568
No. of involved lobes ≥3	12 (60.0)	15 (9.4)	<0.001
Laboratory findings			
White blood cell, /μL	6,310 (5,180–7,180)	5,690 (4,710–6,815)	0.139
C-reactive protein, mg/dL	2.2 (1.1–9.8)	1.1 (0.4–5.6)	0.118
Albumin, g/dL	4.3 (4.0–4.5)	4.5 (4.1–4.7)	0.235

Values are presented as median (interquartile range) or number (%).
*Cases are duplicated.

Discussion

In this study, we evaluated the microorganisms in lower respiratory tracts of patients with NTM-PD using bronchoscopic washing fluid analysis. Most NTM-PD patients had nodular bronchiectatic form (n=151/180, 83.9%), and *M. intracellulare* was the most frequent cause of NTM-PD (n=115/180, 63.9%). Less than half of NTM-PD patients (n=80/180, 44.4%) had accompanying bacteria, among which the most common species was *K. pneumoniae* (n=25/80, 31.3%), followed by *P. aeruginosa* (n=20/80, 25.0%) and *S. aureus* (n=20/80, 25.0%). Patients with NTM-PD with extensive involvement tended to have accompanying bacteria including *P. aeruginosa*. In particular, NTM-PD patients with *P. aeruginosa* were older, predominantly male and ever-smokers, with extensive lobe involvement and tended to complain of respiratory symptoms such as cough with or without excessive mucus production or dyspnea.

Although there are several studies regarding the microbiome in bronchiectasis[19,20], data regarding microorganisms in the lower respiratory tract of NTM-PD patients are limited[21]. In a study based on analysis of repeated sputum samples, Fujita et al.[8] suggested that patients with MAC-PD frequently had chronic coinfections of *S. aureus* and *P. aeruginosa* (124/275, 45.1%). Yamasaki et al.[22] evaluated the microbiome from bronchial alveolar lavage samples in bronchiectasis patients and found that anaerobes were abundant in patients with NTM-PD compared to those without NTM-PD. Sulaiman et al.[23] assessed microbiomes in subset of patients with bronchiectasis and found no significant differences in bacterial load or diversity between those with NTM and without. However, these studies analyzed microbiome composition and did not assess the clinical aspects of NTM-PD. Although our study analyzed the bacteria with conventional culture techniques, we also systematically evaluated lower respiratory bacteria obtained through bronchoscopy in a relatively large number of NTM-PD patients; this work suggests that NTM-PD and bacterial coinfection associated with extensive lung disease and *P. aeruginosa*-NTM-PD coinfection is associated with poorer clinical conditions.

In patients with NTM-PD, bronchiectasis is the most relevant comorbid respiratory disease, and nodular bronchiectatic form was the most common phenotype[4,14,24]. In the present study, almost the whole NTM-PD population (93.9%) had bronchiectasis. In contrast, NTM was reported in a wide range of bronchiectasis cohorts (8.8% to 63.0%)[25-27]. *Pseudomonas* infection is a biomarker of severity in bronchiectasis[15,28]. Although NTM and *Pseudomonas* infections are frequent and important in bronchiectasis populations, there are limited data of the impact of *Pseudomonas* infection on the clinical course of NTM-PD. A Taiwanese study suggested association of concomitant NTM and *P. aeruginosa* infection with poorer clinical outcome including the greatest decline in pulmonary function and worst disease severity with more frequent exacerbations[29]. A Japanese study also showed impaired quality of life in patients with NTM- *P. aeruginosa* coinfection in a population with MAC-PD[30]. Our study results consistently demonstrated that NTM- *P. aeruginosa* coinfection was distinctively associated with more extensive lung disease with worse respiratory symptoms compared with NTM-PD without *P. aeruginosa* coinfection. In contrast, NTM-*K. pneumoniae* and NTM-*S. aureus* coinfections did not have a clinical effect on NTM-PD patients.

Interestingly, this study showed male and ever-smoker predominance in *P. aeruginosa* infection among NTM-PD patients. However, Vidaillac et al.[31] evaluated gender differences in bronchiectasis and suggested *P. aeruginosa* to be the predominant pathogen in females rather than males. Additionally, a recent Chinese study found that the prevalence of *P. aeruginosa* infection was greater in females than males in elderly patients with bronchiectasis[32]. Because our study only analyzed NTM-PD patients who could undergo bronchoscopy, a selection bias could exist; the differences in study populations (bronchiectasis patients vs. NTM-PD patients) could account for the discrepancies between previous studies and our study. However, there is much evidence that smoke exposure is associated with increased virulence of *P. aeruginosa* and impaired bacterial clearance after *P. aeruginosa* infection[33,34]. Smoking is also a predisposing factor for NTM-PD[35], and there is a possibility that smoking is associated with increasing risk of *P. aeruginosa* coinfection in patients with NTM-PD.

This study has several limitations. First, the history of antibiotics administration before bronchoscopic procedure was not evaluated and could affect bacterial culture. Second, this retrospective study was conducted at a single center, which might limit its generalizability. Multi-center prospective studies of prevalence and the clinical impact of bacterial coinfections with NTM-PD are warranted. Third, this study only enrolled NTM-PD patients who underwent bronchoscopy, and there could be a selection bias leading to an unrepresentative NTM-PD patient population.

In conclusion, less than half of NTM-PD patients had bacterial coinfections, and they had more extensive

lung involvement. Additionally, patients with NTM-*P. aeruginosa* coinfection had significantly more frequent respiratory symptoms and extensive lung involvement. Further studies with larger cohorts and a prospective design are needed to validate and expand upon these findings.

Authors' Contributions

Conceptualization: Shin B. Methodology: Moon SM, Shin B. Formal analysis: Moon SM, Shin B. Data curation: all authors. Funding acquisition: Moon SM. Project administration: Shin B. Validation: all authors. Investigation: all authors. Writing - original draft preparation: Moon SM, Shin B. Writing - review and editing: all authors. Approval of final manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

This study was presented at the 2023 European Respiratory Society International Congress.

Funding

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. 2021R1G1A1093908).

The authors thank MID (Medical Illustration & Design) for providing excellent support with medical illustration.

Supplementary Material

Supplementary material can be found in the journal homepage (http://www.e-trd.org).

Supplementary Table S1. Comparison of clinical characteristics of NTM-PD patients with or without *Klebsiella pneumoniae* cultured from bronchial washing fluid.

Supplementary Table S2. Comparison of clinical characteristics of NTM-PD patients with or without *Staphylococcus aureus* cultured from bronchial washing fluid.

References

1. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 2007;175:367-416.
2. Daley CL, Iaccarino JM, Lange C, Cambau E, Wallace RJ Jr, Andrejak C, et al. Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline. Eur Respir J 2020;56:2000535.
3. Brode SK, Daley CL, Marras TK. The epidemiologic relationship between tuberculosis and non-tuberculous mycobacterial disease: a systematic review. Int J Tuberc Lung Dis 2014;18:1370-7.
4. Ko RE, Moon SM, Ahn S, Jhun BW, Jeon K, Kwon OJ, et al. Changing epidemiology of nontuberculous mycobacterial lung diseases in a tertiary referral hospital in Korea between 2001 and 2015. J Korean Med Sci 2018;33:e65.
5. Moon SM, Jhun BW, Baek SY, Kim S, Jeon K, Ko RE, et al. Long-term natural history of non-cavitary nodular bronchiectatic nontuberculous mycobacterial pulmonary disease. Respir Med 2019;151:1-7.
6. Fujita J, Ohtsuki Y, Suemitsu I, Shigeto E, Yamadori I, Obayashi Y, et al. Pathological and radiological changes in resected lung specimens in Mycobacterium avium intracellulare complex disease. Eur Respir J 1999;13:535-40.
7. Urabe N, Sakamoto S, Sano G, Ito A, Homma S. Characteristics of patients with bronchoscopy-diagnosed pulmonary Mycobacterium avium complex infection. J Infect Chemother 2018;24:822-7.
8. Fujita K, Ito Y, Hirai T, Kubo T, Togashi K, Ichiyama S, et al. Prevalence and risk factors for chronic co-infection in pulmonary Mycobacterium avium complex disease. BMJ Open Respir Res 2014;1:e000050.
9. Urabe N, Sakamoto S, Shimanuki Y, Kanokogi T, Motohashi T, Anzai N, et al. Impact of chronic co-infection in pulmonary Mycobacterium avium complex disease after treatment initiation. BMC Pulm Med 2022;22:157.
10. Wang G, Stapleton JT, Baker AW, Rouphael N, Creech CB, El Sahly HM, et al. Clinical features and treatment outcomes of pulmonary Mycobacterium avium-intracellulare complex with and without coinfections. Open Forum Infect Dis 2022;9:ofac375.
11. Kim SB, Lee WY, Lee JH, Lee SJ, Lee MK, Kim SH, et al. A variety of bacterial aetiologies in the lower respiratory tract at patients with endobronchial tuberculosis. PLoS One 2020;15:e0234558.
12. Minami D, Takigawa N, Watanabe H, Ninomiya T, Kubo T, Ohashi K, et al. Safety and discomfort during bronchoscopy performed under sedation with fentanyl and midazolam: a prospective study. Jpn J Clin Oncol 2016;46:871-4.
13. Leylabadlo HE, Kafil HS, Yousefi M, Aghazadeh M, Asgharzadeh M. Pulmonary tuberculosis diagnosis:

where we are? Tuberc Respir Dis (Seoul) 2016;79:134-42.
14. Koh WJ, Moon SM, Kim SY, Woo MA, Kim S, Jhun BW, et al. Outcomes of Mycobacterium avium complex lung disease based on clinical phenotype. Eur Respir J 2017;50:1602503.
15. Chalmers JD, Goeminne P, Aliberti S, McDonnell MJ, Lonni S, Davidson J, et al. The bronchiectasis severity index: an international derivation and validation study. Am J Respir Crit Care Med 2014;189:576-85.
16. Bewick V, Cheek L, Ball J. Statistics review 8: qualitative data: tests of association. Crit Care 2004;8:46-53.
17. Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. Ann Math Stat 1947;18:50-60.
18. Masuadi E, Mohamud M, Almutairi M, Alsunaidi A, Alswayed AK, Aldhafeeri OF. Trends in the usage of statistical software and their associated study designs in health sciences research: a bibliometric analysis. Cureus 2021;13:e12639.
19. Tunney MM, Einarsson GG, Wei L, Drain M, Klem ER, Cardwell C, et al. Lung microbiota and bacterial abundance in patients with bronchiectasis when clinically stable and during exacerbation. Am J Respir Crit Care Med 2013;187:1118-26.
20. Rogers GB, van der Gast CJ, Cuthbertson L, Thomson SK, Bruce KD, Martin ML, et al. Clinical measures of disease in adult non-CF bronchiectasis correlate with airway microbiota composition. Thorax 2013;68:731-7.
21. Thornton CS, Mellett M, Jarand J, Barss L, Field SK, Fisher DA. The respiratory microbiome and nontuberculous mycobacteria: an emerging concern in human health. Eur Respir Rev 2021;30:200299.
22. Yamasaki K, Mukae H, Kawanami T, Fukuda K, Noguchi S, Akata K, et al. Possible role of anaerobes in the pathogenesis of nontuberculous mycobacterial infection. Respirology 2015;20:758-65.
23. Sulaiman I, Wu BG, Li Y, Scott AS, Malecha P, Scaglione B, et al. Evaluation of the airway microbiome in nontuberculous mycobacteria disease. Eur Respir J 2018;52:1800810.
24. Loebinger MR, Quint JK, van der Laan R, Obradovic M, Chawla R, Kishore A, et al. Risk factors for nontuberculous mycobacterial pulmonary disease: a systematic literature review and meta-analysis. Chest 2023;164:1115-24.
25. Aksamit TR, O'Donnell AE, Barker A, Olivier KN, Winthrop KL, Daniels ML, et al. Adult patients with bronchiectasis: a first look at the US Bronchiectasis Research Registry. Chest 2017;151:982-92.
26. Suska K, Amati F, Sotgiu G, Gramegna A, Mantero M, Ori M, et al. Nontuberculous mycobacteria infection and pulmonary disease in bronchiectasis. ERJ Open Res 2022;8:00060-2022.
27. Faverio P, Stainer A, Bonaiti G, Zucchetti SC, Simonetta E, Lapadula G, et al. Characterizing non-tuberculous mycobacteria infection in bronchiectasis. Int J Mol Sci 2016;17:1913.
28. Vidaillac C, Chotirmall SH. Pseudomonas aeruginosa in bronchiectasis: infection, inflammation, and therapies. Expert Rev Respir Med 2021;15:649-62.
29. Hsieh MH, Lin CY, Wang CY, Fang YF, Lo YL, Lin SM, et al. Impact of concomitant nontuberculous mycobacteria and Pseudomonas aeruginosa isolates in non-cystic fibrosis bronchiectasis. Infect Drug Resist 2018;11:1137-43.
30. Kamata H, Asakura T, Suzuki S, Namkoong H, Yagi K, Funatsu Y, et al. Impact of chronic Pseudomonas aeruginosa infection on health-related quality of life in Mycobacterium avium complex lung disease. BMC Pulm Med 2017;17:198.
31. Vidaillac C, Yong VF, Jaggi TK, Soh MM, Chotirmall SH. Gender differences in bronchiectasis: a real issue? Breathe (Sheff) 2018;14:108-21.
32. Zhou YY, Wang YH, He SQ, Wang WY, Wang XY, Li DS, et al. Gender differences in clinical characteristics of patients with non-cystic fibrosis bronchiectasis in different age groups in northern China. Clin Respir J 2023;17:311-9.
33. Chien J, Hwang JH, Nilaad S, Masso-Silva JA, Ahn SJ, McEachern EK, et al. Cigarette smoke exposure promotes virulence of Pseudomonas aeruginosa and induces resistance to neutrophil killing. Infect Immun 2020;88:e00527-20.
34. Drannik AG, Pouladi MA, Robbins CS, Goncharova SI, Kianpour S, Stampfli MR. Impact of cigarette smoke on clearance and inflammation after Pseudomonas aeruginosa infection. Am J Respir Crit Care Med 2004;170:1164-71.
35. Chin KL, Sarmiento ME, Alvarez-Cabrera N, Norazmi MN, Acosta A. Pulmonary non-tuberculous mycobacterial infections: current state and future management. Eur J Clin Microbiol Infect Dis 2020;39:799-826.