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SHORT COMMUNICATION

The Incidence of Non-tuberculous Mycobacterium Lung Disease in Patients with Suspected Pulmonary Tuberculosis

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Abstract Non-tuberculous mycobacterium (NTM) lung disease is increasing in prevalence. We analyzed the frequency of NTM lung disease among patients who are suspected of tuberculosis. NTM was isolated from about one-fourth of the mycobacterium culture-positive patients and about half of these had NTM lung disease. Therefore, NTM isolates should be routinely identified at the species level for adequate treatment.

Keywords Tuberculosis · Nontuberculous mycobacterium · NTM lung disease

Abbreviations

NTM	Nontuberculous mycobacterium
AFB	Acid-fast bacilli
MTB	*Mycobacterium tuberculosis*
REBA	Reverse blot hybridization assay

Nontuberculous mycobacteria (NTM) are ubiquitous in the environment and cause four distinct clinical syndromes: chronic pulmonary disease, lymphadenitis, cutaneous disease and disseminated disease [1]. Because there is no evidence of person-to-person transmission, NTM infections are not reportable diseases and the exact prevalence remains unknown [2]. There is marked geographic variability both in the prevalence of disease and in the responsible mycobacterial species [3]. In countries with high prevalence rates of tuberculosis, patients with acid-fast bacilli (AFB) positive sputum or chest radiographic finding suggest active tuberculosis have been presumed to have pulmonary tuberculosis and were treated empirically with antituberculous drugs. Therefore, many patients with NTM lung disease were treated with inappropriate or unnecessary treatment due to incorrect diagnoses with pulmonary tuberculosis [4]. In addition, when NTM was isolated in respiratory specimen, NTM lung disease was requires differentiation from contamination or colonization [5]. The aim of this study was to evaluate the incidence of NTM pulmonary disease and the prevalence of NTM species among the patients suspected to have tuberculosis in a university hospital in Korea. We analyzed 10,143 sputa from 3975 patients suspected to have pulmonary tuberculosis from July 2008 through April 2010. All patients diagnosed with NTM lung disease met the diagnostic criteria for NTM lung disease according to the American Thoracic Society in 2007 as follows [5]: (1) clinically; pulmonary symptoms, nodular or cavitary opacities on chest radiograph (Fig. 1), or an high resolution computed tomography (HRCT) showing multifocal bronchiectasis with multiple small nodules and appropriate exclusion of other diagnoses and (2) microbiologically; positive culture results from at least two separate expectorated sputum samples, positive culture results from at least one bronchial wash or lavage, or transbronchial or other lung biopsy with mycobacterial histopathologic features and positive culture for NTM or biopsy showing mycobacterial histopathologic features and one or more sputum or bronchial washings that are culture-positive for NTM. Patients were classified

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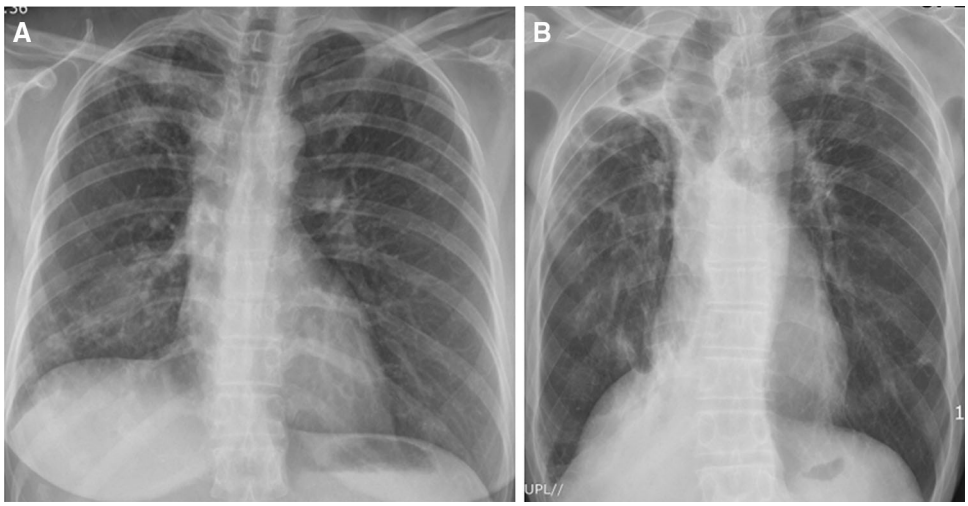

Fig. 1 **a** A 43-years-old women with pulmonary tuberculosis. The chest radiograph shows mass-like opacities on *right* lung and multiple tiny nodules on *right lung* and *left upper lobe*. **b** A 74-year-old men with *Mycobacterium intracellulare* lung disease. The chest radiograph shows atelectasis of *right upper lobe* associated with cavitary lesion. Nodules and fibrosis are seen on both lungs

as having definite, probable, or contaminant/colonization, based on the above criteria. Definite NTM lung disease was diagnosed if the clinical, radiological, and microbiological criteria of the American Thoracic society were fulfilled. Probable NTM lung disease was diagnosed if there was insufficient support for microbiologic criteria (NTM isolated only one time), but suspicious NTM lung infection clinically. NTM contaminant/colonization was diagnosed if the patient fulfilled none of the criteria for either definite or probable NTM lung disease. Patients with definite or probable NTM lung disease were regarded as having clinically significant NTM lung disease. Unidentified isolates were also categorized according to ATS/IDSA criteria. We could not further test for identification these isolates. Sputum specimens were homogenized and decontaminated using the NALC-2 % NaOH method (4 % NaOH, 2.9 % sodium citrate, 0.5 % N-Acetyl-L-Cysteine) and concentrated by centrifugation at 3000*g* for 30 min. Following concentration and resuspension of the sediments in 1.5 mL of phosphate buffer, part of the sediment from each specimen was used for AFB stain and inoculated onto 3 % Ogawa media (Asan pharmaceutical, Seoul, Korea). Smears were stained with the auramine-rhodamine fluorochrome stain and microscopically examined under 200× magnification with >30 fields and auramine-rhodamine-positive smears were confirmed by Ziehl–Neelsen staining [6]. Ziehl–Neelsen stained slides and the results were then assessed under 1000× magnification with >300 fields according to the classification of the Centers for Disease Control [7]: negative, no bacilli in 300 fields; trace, 1-2 bacilli in 300 fields; grade 1, 1-9 bacilli in 100 fields; grade 2, 1-9 bacilli in 10 fields; grade 3, 1-9 bacilli in 1 field; and grade 4, >9 bacilli in 1 field (Fig. 2a). Sputa with trace to grade 4 were considered positive in this study. Specimens were inoculated onto 3 % Ogawa media and then incubated for at least 8 weeks at 37 °C and observed every week. All cultured *Mycobacterium* were tested by Cobas Taqman 48 MTB test (Roche Diagnostics, Mannheim, Germany) and/or TB Ag MPT64 Rapid (Standard Diagnostics, Inc., Kyonggi-do, Korea) to detect the specific antigen for *M. tuberculosis*. All procedures were performed according to the manufacturer's instructions. NTM species were identified using REBA Myco-ID® (M&D, Inc., Wonju, Korea). REBA Myco-ID® uses a reverse blot hybridization assay (REBA) by binding the amplifying *rpo*B gene product to species-specific probes (Fig. 2b).

Among 3975 patients, 271 patients (6.8 %) had positive sputum specimens on *Mycobacterium* culture during the study period. *Mycobacterium tuberculosis* (MTB) was isolated from 199 (73.4 %) and NTM were isolated from 72 (26.6 %) among 271 culture-positive patients. The proportion of NTM lung disease among NTM-positive specimens according to AFB stain results is shown in Fig. 3. The NTM were recovered from 21.8 % (43/197) of patients with smear positive sputum. Among them, the NTM lung disease was diagnosed in 62.8 % (27/43) of patients. The NTM were recovered from 39.2 % (29/74) of patients with smear negative sputum and the NTM lung disease were diagnosed in 41.4 % (12/29) patients. Patients were then grouped into three disease categories based on their likelihood of NTM disease according to the definitions cited above. Of these 72 patients, 33 patients (45.8 %) received a diagnosis of definite NTM lung disease, six patients (8.3 %) had probable disease, and 33 patients (45.8 %) had NTM contamination (Table 1). The most frequently isolated organisms were *Mycobacterium avium* complex (MAC) (n = 38, 52.8 %) which consisted of 24 isolates of *Mycobacterium intracellulare* and 14 isolates of *M. avium*, followed by *Mycobacterium abscessus* (n = 11, 15.3 %), *Mycobacterium fortuitum* (n = 5, 6.9 %), and *Mycobacterium kansasii* (n = 4, 5.6 %). MAC accounted for 20 (52.6 %) of the definite NTM lung disease cases and

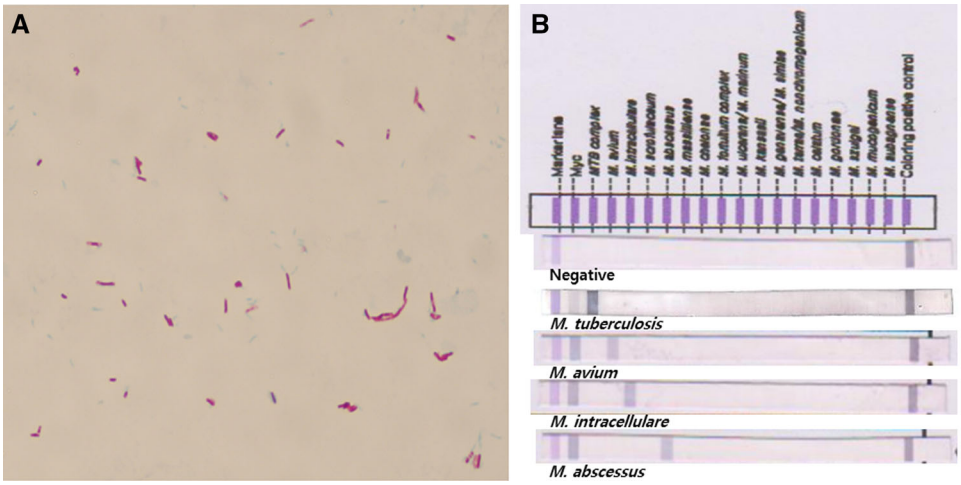

Fig. 2 **a** Ziehl–Neelsen stained smear showed acid fast bacilli appear as purple to red, slightly curved, short or long rods (Ziehl–Neelsen stain, 1000×). **b** Reverse blot hybridization assay (REBA) for identification of *Mycobacterium*. The amplifying *rpo*B gene product hybridized to species-specific probes

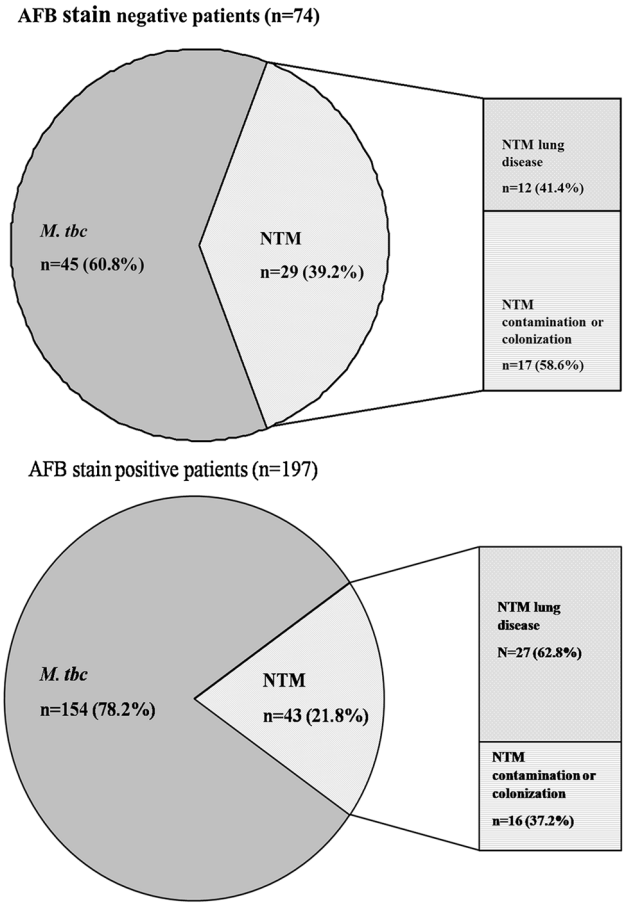

Fig. 3 Number of cases of NTM lung disease among cultures positive for mycobacteria

five (13.2 %) of the probable NTM lung disease cases. *M. abscessus* accounted for six (54.5 %) of the definite NTM lung disease cases and one (9.1 %) of the probable NTM lung disease cases. All five cases of *M. fortuitum* were diagnosed as contaminants. There were co-infections with MTB in 6 patients, 1 *M. abscessus*, 1 *M. fortuitum*, 1 *M. intracellulare*, 1 *M. abscessus*, and 2 unidentified isolates. These isolates were considered contamination/colonizer category due to isolate at once. There were no co-infections with other NTM species. Nineteen of 39 patients (48.7 %) had underlying lung disease (Table 2). Bronchiectasis was the most common condition, which was present in nine patients (23.1 %). Four patients had chronic obstructive pulmonary disease, four had past history of pulmonary tuberculosis, one had lung cancer, and one had gastroesophageal reflux disease. Twenty of 39 patients (51.3 %) did not have any underlying disease. These patients presented only with mild pulmonary symptoms such as cough and sputum production.

The reasons for the recent increase in rate of NTM lung disease are unclear, but the increase may reflect advances in the isolation and identification of NTM and a decline in the incidence of MTB [4]. In this study, NTM were isolated in 72 patients (26.6 %) among 271 *Mycobacterium* culture-positive patients and among them, 39 patients (54.2 %) were diagnosed clinically with significant NTM lung infection. Previous reports have shown that the proportion of NTM as an etiology of lung disease was about 40–50 % among isolated NTM from sputa in USA, Canada, and West Europe [8, 9], and about 10–20 % in East Asian countries, Hong Kong and Japan [10, 11]. In regions where there is a high prevalence of MTB infections, patients positive for AFB stain are typically classified as having tuberculosis, but MTB is not confirmed bacteriologically and is experimentally treated with anti-tuberculosis drugs with frequent therapeutic failure [12]. Therefore, the isolation and identification of the causative organism are necessary for a correct diagnosis in patients with AFB-positive sputum specimens. Rapid and accurate identification of NTM and differentiation from MTB is essential. *M. avium complex* were most frequently recovered in those

Table 1 Frequency and clinical significance of isolated NTM species

	Isolated number (%)	NTM lung disease		
		Definite	Probable	Contamination
MAC				
M. intracellulare type I	24 (33.3)	11 (15.3)	4 (5.6)	9 (12.5)
M. avium	14 (19.4)	9 (12.5)	1 (2.8)	4 (5.6)
M. abscessus	11 (15.3)	6 (33.3)	1 (2.8)	4 (5.6)
M. fortuitum	5 (6.9)	0 (0.0)	0 (0.0)	5 (6.9)
M. kansasii	4 (5.6)	2 (2.8)	0 (0.0)	2 (2.8)
M. mucogenicum	4 (5.6)	0 (0.0)	0 (0.0)	4 (5.6)
M. szulgai	1 (1.4)	1 (1.4)	0 (0.0)	0 (0.0)
M. terrae complex	2 (2.8)	1 (1.4)	0 (0.0)	1 (1.4)
M. lentiflavum	1 (1.4)	0 (0.0)	0 (0.0)	1 (1.4)
Not identified	6 (8.3)	3 (4.2)	0 (0.0)	3 (4.2)
Total	72 (100.0)	33 (45.8)	6 (8.3)	33 (45.8)

NTM non-tuberculous mycobacterium, *MAC Mycobacterium avium* complex

Table 2 Characteristics of patients diagnosed with NTM lung disease

Characteristics	Number (%)
Gender	
Male	16 (41.0)
Female	23 (59.0)
Age (y)	
<20	1 (2.6)
20–40	2 (5.1)
41–60	16 (41.0)
>60	20 (51.3)
Comorbidities and predisposing conditions	
Bronchiectasis	9 (23.1)
COPD	4 (10.3)
Post TB	4 (10.3)
Lung cancer	1 (2.6)
GERD	1 (2.6)
None	20 (51.3)

Including definite and probable NTM lung disease

NTM non-tuberculous mycobacterium, *COPD* chronic obstructive pulmonary disease, *TB* tuberculosis, *GERD* gastroesophageal reflux disease

with NTM lung disease, followed by *M. abscessus*, *M. fortuitum* and *M. kansasii,* frequencies similar to those observed in prior Korean studies. Correct identification is also important to show the different clinically relevant NTM species [13]. Molecular methods were most widely used and among molecular methods, PCR and reverse blot hybridization assay using the *rpoB* gene has been developed in Korea. However, some species (uncommonly encountered species) could not be identified for precise identification of *M. abscessus* complex at subspecies level, other methods, such as sequencing of other genes including *hsp65*, *rpoB*, and 16S-23S rRNA internal transcribed space region are needed [13]. In our study, most patients with NTM lung disease were middle-aged or elderly, which supports the hypothesis that increased age, is a risk factor for NTM lung disease [12]. About 49 % of patients with NTM lung disease had underlying lung disease: bronchiectasis, COPD, prior MTB, lung cancer or gastroesophageal reflux disease. NTM lung disease had underlying disorders commonly include COPD, previous MTB, silicosis, gastroesophageal reflux disease (GERD), and lung cancer [2, 4]. The patients with no previous pulmonary diseases were lower than 30 % in previous report [2, 12], but 51.3 % (20/39) patients did not have underlying lung disease in this study. This data presented that NTM lung disease was increased in patients without underlying disease.

In conclusion, we found that about half of all cases of isolated NTM caused lung disease and that the high incidence rate of NTM lung disease is show in patients with no underlying disease. Differential diagnosis of NTM lung disease from pulmonary tuberculosis is very important for accurate diagnosis and appropriate treatment.

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Compliance with Ethical Standards

Conflict of interest All authors have no conflict of interest.

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