COMPARISON OF CLINICAL AND LABORATORY FINDINGS BETWEEN THOSE WITH PULMONARY TUBERCULOSIS AND THOSE WITH NONTUBERCULOUS MYCOBACTERIAL LUNG DISEASE

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Abstract. In tuberculosis endemic areas, patients with sputum positive for acid-fast bacilli (AFB) are usually diagnosed and treated for pulmonary tuberculosis. The diagnosis of nontuberculous mycobacteria (NTM) lung disease is often ascertained only after lung disease progression occurs, increasing the risk of severe morbidity and mortality. We conducted a matched case-control study among a prospective cohort of 300 patients with newly diagnosed AFB-positive sputum in Thailand during 2010-2012. We compared clinical and laboratory parameters and outcomes among patients with pulmonary tuberculosis, NTM lung disease and NTM colonization. A mycobacterial culture was performed in all patients. Ten patients with NTM lung disease were compared to 50 patients with pulmonary tuberculosis and 10 patients with NTM colonization. The presence of diabetes mellitus or human immunodeficiency virus infection, were associated with NTM lung disease ($p = 0.030$). Patients with NTM lung disease had a significantly lower body weight prior to treatment ($p = 0.021$), a higher body weight change from baseline ($p = 0.038$), and were more likely to have cavitations on chest radiograph ($p = 0.033$) than those with NTM colonization. In tuberculosis endemic areas, mycobacterial identification should be performed among patients with impaired immune function. NTM lung disease treatment should be considered in patients with NTM sputum isolates who have a history of significant weight loss or cavitations on chest radiography.

Keywords: matched case-control study, NTM colonization, sputum

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INTRODUCTION

Nontuberculous mycobacterial (NTM) infections are caused by mycobacterial species other than *Mycobacterium tuberculosis* complex or *M. leprae*; they are acquired through ingestion or inhalation

of contaminated soil or water, or through inoculation (Griffith *et al*, 2007; Taiwo and Glassroth, 2010). More than 125 NTM species have been identified causing four clinical syndromes in humans: lung disease, cutaneous disease, disseminated disease and lymphadenitis (Griffith *et al*, 2007). NTM diseases have been increasing worldwide over the past few decades, possibly due to the increasing prevalence of acquired immunodeficiency syndrome, increasing awareness of NTM diseases and advanced methods for detecting NTM infections (Thomson and Yew, 2009). The incidence of NTM lung disease among patients with acid-fast bacilli (AFB)-positive sputum varies widely, ranging from 0-49%, due to differences in study populations and locations (Levy *et al*, 1989; Jeon *et al*, 2005).

Studies from Thailand have found the lungs to be a common site for infection due to *M. avium* complex, *M. scrofulaceum*, *M. kansasii* and *M. gordonae* (Wongwatana and Sriyabhaya, 1992; Ratanasuwan *et al*, 2002; Chetchotisakd *et al*, 2007). The diagnosis of NTM lung disease relies on identification of mycobacterium species on culture, which is available only at Thai university hospitals and some national institutes for treating pulmonary tuberculosis. Mycobacterial cultures are time consuming, laborious, expensive and may not give accurate results. Repeat sputum samples are needed for mycobacterial culture and follow-up is required before treatment of NTM lung disease can be initiated (Griffith *et al*, 2007; Thomson and Yew, 2009). A study from India, where tuberculosis is endemic, found 65% of patients in whom respiratory NTM were isolated had clinically significant NTM lung disease, but 49% of those patients did not fulfill the American Thoracic Society (ATS) criteria for NTM lung disease (Shenai *et al*, 2010). However, a study from Israel found that 90% of patients in whom NTM were isolated had colonization without infection (Braun *et al*, 2013).

NTM empiric therapy is not recommended for all NTM positive cases due to adverse drug effects and the finding that spontaneous remission may occur following adequate chest physiotherapy and postural drainage in some patients (Griffith *et al*, 2007; Thomson and Yew, 2009). The clinical symptoms and signs in patients with NTM lung disease are non-specific and similar to those found in pulmonary tuberculosis (Griffith *et al*, 2007; Thomson and Yew, 2009). In areas endemic for tuberculosis, particularly those with limited resources for obtaining a culture or performing molecular diagnosis of mycobacteria, patients with AFB-positive sputum results are usually diagnosed and treated as having pulmonary tuberculosis. The diagnosis and management of NTM lung disease is often only considered when the lung disease progresses, leading to higher morbidity and mortality. The case-fatality rate of patients with NTM lung disease can be as high as 20% (Evans *et al*, 1996a).

Clinical and laboratory findings are essential to diagnose and treat NTM lung disease in order to prevent disease progression. Few reports are published regarding the clinical and laboratory findings of patients with NTM lung disease. Therefore, we conducted a matched case-control study at Queen Savang Vadhana Memorial and Chonburi Hospitals to determine the clinical and laboratory findings suggestive of NTM lung disease, pulmonary tuberculosis, and NTM colonization during 2010-2012.

MATERIALS AND METHODS

Ethical considerations

This study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University in Bangkok, and Queen Savang Vadhana Memorial and Chonburi Hospitals. Written informed consent was obtained from all patients before participation in the study.

Study design

A matched case-control study was conducted at Queen Savang Vadhana Memorial and Chonburi Hospitals during 2010-2012. Patients with NTM lung disease (cases) and patients with *M. tuberculosis*-positive sputum by culture (controls) were identified from a prospective cohort of 300 patients newly identified as having AFB-positive sputum. New patients were defined as those who had never received treatment for tuberculosis or had taken anti-tuberculosis drugs for <1 month according to the World Health Organization (WHO, 2010) guidelines for treating tuberculosis. Inclusion criteria were: adults aged ≥14 years who had ≥2 pulmonary or constitutional symptoms, including cough, dyspnea, chest pain, hemoptysis, fever, fatigue, malaise, weight loss, and night sweats, who had an abnormal chest radiographic finding of reticular, interstitial, nodular infiltrate or cavitation and who had ≥1 sputum smear positive for AFB. Patients who had other bacterial infection were excluded from the study. Sputum cultures for mycobacteria were performed at the National Tuberculosis Reference Laboratory in Bangkok, Thailand prior to treatment and at 2 and 5 months after starting treatment. All patients received care and regular follow-up at Queen Savang Vadhana Memorial or Chonburi Hospitals.

NTM lung disease cases were diagnosed using the 2007 ATS criteria with modifications (Griffith *et al*, 2007). Cases were defined as patients with at least two separate NTM-positive sputum samples or with one NTM-positive sputum sample and clinical improvement after receiving NTM treatment and had received appropriate investigations to exclude other diagnoses. Controls (case:control ratio of 1:5) were randomly selected and matched with cases by age and gender. Patients with NTM colonization were defined as patients who had a NTM-positive sputum culture but did not meet the criteria for NTM lung disease. Demographic data, clinical and laboratory findings were collected and recorded.

Of the 300 initial patients with AFB-positive sputum smears, 273 (91%) had a *M. tuberculosis*-positive sputum culture, 19 (6.3%) had negative results on sputum culture, and 8 (2.7%) had a NTM-positive sputum culture. All eight patients with a NTM-positive sputum culture were classified as having NTM lung disease because of clinical improvement following NTM treatment. The NTM regimen included clarithromycin, isoniazid, rifampicin and ethambutol. Among the 273 patients with a *M. tuberculosis*-positive sputum culture, 12 (4.4%) had a NTM-positive sputum culture during anti-tuberculosis treatment. Of these, 2 (16.7%) met the criteria for NTM lung disease due to two separate NTM-positive cultures and clinical improvement after clarithromycin was added to the anti-tuberculosis treatment regimen. Thus, 20 patients with sputum culture positive for NTM were included in the study, of which 10 met criteria for NTM lung disease and the other 10 were categorized as having NTM colonization because of negative NTM sputum cultures

on subsequent specimens during the follow-up period without any alteration in the anti-tuberculosis treatment regimen, and no changes in the chest radiograph during the follow-up period. Cases and controls had median (inter-quartile range, IQR) follow-up of 290 (265–344) and 273 (266–311) days, respectively. Ten cases and 50 controls were compared with eachother and 10 additional patients with NTM colonization were compared with the 10 cases (Fig 1).

Sputum smears and mycobacterium cultures

Sputum samples were collected for smear and culture before treatment and at 2 and 5 months after the onset of treatment. Three early-morning sputum samples were collected on different days in sterile, disposable, leak-proof laboratory-approved containers. Sputum smears were performed at the central laboratory of Queen Savang Vadhana Memorial and Chonburi Hospitals using the Ziehl-Neelsen method (ATS, 2000). The results of the AFB stain were interpreted by a grading system (WHO, 1998) as follows: <1+ = 1–9 AFB per 100 oil immersion fields (OIF), 1+ = 10–99 AFB per 100 OIF, 2+ = 1–10 AFB per OIF, and 3+ = >10 AFB per OIF.

The sputum sample for each patient with the highest grade (WHO, 1998) was transported at 4ºC to the National Tuberculosis Reference Laboratory in Bangkok, Thailand to perform mycobacterial culture. Both Loewenstein-Jensen solid medium and an automated liquid media culture system (MGIT 960, BACTEC, Sparks, MD) were used according to manufacturer's instructions.

Statistical analysis

Microsoft Excel and SPSS for Windows version 18.0 (SPSS, Chicago, IL) were used for data analysis. Categorical variables were summarized as frequencies and percentages and then analyzed with the chi-square test or the Fisher's exact test, where appropriate. Numerical variables were tested for normality using the Kolmogorov-Smirnov test. Variables with non-normal distribution had the median and IQR calculated and were compared with the Mann-Whitney *U* test for two group comparisons. A p-value <0.05 was considered statistically significant.

RESULTS

The majority of baseline characteristics in cases (NTM lung disease, $n = 10$) and controls (pulmonary tuberculosis, $n = 50$) were similar, including age, male gender, marital status, smoking history, and alcohol consumption (Table 1). The cases had a significantly higher proportion with underlying medical illnesses than controls. Among 7 cases (70%) with underlying medical illnesses, diabetes mellitus (4/7, 57.1%), human immunodeficiency virus (HIV) infection (2/7, 28.6%) and thalassemia (1/7, 14.3%) were observed. Among 15 controls (30.6%) with underlying medical illnesses, diabetes mellitus (9/15, 60.0%), coronary artery disease (3/15, 20.0%), HIV infection (1/15, 6.7%), liver disease (1/15, 6.7%) and rheumatoid arthritis (1/15, 6.7%) were observed (data not shown).

The clinical presentations in cases and controls were similar in terms of symptom duration, cough, dyspnea, chest pain, fever, fatigue, night sweats, hemoptysis, body weight prior to treatment, body weight change from baseline and presence of lung crepitations (Table 1). Laboratory investigations showed no differences in sputum AFB 3+, presence of cavitations on chest radiograph, hemoglobin (Hb)

Table 1
Baseline characteristics, clinical findings and outcomes among patients with nontuberculous mycobacterium (NTM) lung disease and those with pulmonary tuberculosis.

Characteristic	NTM lung disease			Pulmonary tuberculosis			*p*-value
	n	Median (IQR)	No. (%)	*n*	Median (IQR)	No. (%)	
Baseline characteristics							
Age (years)	10	45.0 (35.5-51.8)		50	46.0 (34.0-52.2)		0.677
Male gender	10		9 (90.0)	50		45 (90.0)	1.000
Married	10		8 (80.0)	49		41 (83.7)	0.673
Smoking history	10		8 (80.0)	50		43 (86.0)	0.637
Alcohol consumption	10		10 (100)	48		38 (79.2)	0.184
Medical illness	10		7 (70.0)	49		15 (30.6)	0.030
Clinical presentation							
Duration (days)	10	45.0 (14.0-180.0)		50	40.0 (30.0-150.0)		0.579
Cough	10		10 (100)	47		45 (95.7)	1.000
Productive cough			8 (80.0)			43 (91.5)	
Dry cough			2 (20.0)			2 (4.3)	
Dyspnea	10		7 (70.0)	48		34 (70.8)	1.000
Chest pain	10		6 (60.0)	47		25 (53.2)	0.741
Fever	10		5 (50.0)	48		29 (60.4)	0.726
Fatigue	10		5 (50.0)	47		28 (59.6)	0.727
Night sweats	10		4 (40.0)	48		18 (37.5)	1.000
Hemoptysis	10		2 (20.0)	47		15 (31.9)	0.706
Physical examination							
BW prior treatment (kg)	10	45.1 (43.0-51.8)		50	51.5 (45.0-69.1)		0.097
BW change (%)	10	-7.4 (-19.8-2.4)		50	-6.9 (-11.1-29.3)		0.240
Lung crepitations	10		4 (40.0)	50		9 (18.0)	0.201
Laboratory findings							
Sputum AFB 3+	10		1 (10.0)	50		14 (28.0)	0.426
Cavity on CXR	10		5 (50.0)	47		14 (29.8)	0.275
Hemoglobin (g/dl)	8	11.8 (10.7-12.4)		34	11.8 (10.3-13.4)		0.810
WBC ($x10^3/mm^3$)	8	8.8 (7.3-12.3)		34	10.6 (7.0-12.2)		0.654
Outcome							
Death	10		2 (20.0)	48		1 (2.1)	0.074

NTM, nontuberculous mycobacterium; IQR, interquartile range; BW, body weight; AFB, acid-fast bacilli; CXR, chest radiograph; WBC, white blood cell count.

levels and white blood cell (WBC) counts between cases and controls. The proportions of cases who did not survive were not significantly different from each other in cases and controls (Table 1).

The baseline characteristics among the 10 patients with NTM lung disease and 10 patients with NTM colonization were similar in terms of age, male gender, marital status, smoking history and underlying medical illness. Among 5 patients (50%) with NTM colonization who

Table 2
Baseline characteristics, clinical findings and outcomes among patients with nontuberculous mycobacterium (NTM) lung disease and those with NTM colonization.

Characteristic	NTM lung disease			NTM colonization			*p*-value
	n	Median (IQR)	No. (%)	*n*	Median (IQR)	No. (%)	
Baseline characteristic							
Age (years)	10	45.0 (35.5-51.8)		10	47.5 (41.2-53.5)		0.623
Male gender	10		9 (90.0)	10		9 (90.0)	1.000
Married	10		8 (80.0)	10		9 (90.0)	1.000
Smoking history	10		8 (80.0)	10		8 (80.0)	1.000
Alcohol consumption	10		10 (100)	10		10 (100)	NA
Medical illness	10		7 (70.0)	10		5 (50.0)	0.650
Clinical presentation							
Duration (days)	10	45.0 (14.0-180.0)		10	30.0 (13.0-60.0)		0.341
Cough	10		10 (100)	10		8 (80.0)	0.474
Productive cough			8 (80.0)			6 (75.0)	
Dry cough			2 (20.0)			2 (25.0)	
Dyspnea	10		7 (70.0)	10		3 (30.0)	0.180
Chest pain	10		6 (60.0)	10		4 (40.0)	0.655
Fever	10		5 (50.0)	10		1 (10.0)	0.141
Fatigue	10		5 (50.0)	10		1 (10.0)	0.141
Night sweats	10		4 (40.0)	10		1 (10.0)	0.303
Hemoptysis	10		2 (20.0)	10		1 (10.0)	1.000
Physical examination							
BW prior treatment (kg)	10	45.1 (43.0-51.8)		10	59.5 (45.8-66.8)		0.021
BW change (%)	10	-7.4 (-19.8-2.4)		10	-1.6 (-5.2-7.5)		0.038
Lung crepitation	10		4 (40.0)	10		2 (20.0)	0.628
Laboratory findings							
Sputum AFB 3+	10		1 (10.0)	10		1 (10.0)	1.000
Cavity on CXR	10		5 (50.0)	10		0	0.033
Hemoglobin (g/dl)	8	11.8 (10.7-12.4)		7	11.2 (10.9-13.1)		0.908
WBC ($x10^3/mm^3$)	8	8.8 (7.3-12.3)		7	9.2 (6.3-15.3)		0.817
Outcome							
Death	10		2 (20.0)	10		0	0.474

NTM, nontuberculous mycobacterium; IQR, interquartile range; BW, body weight; AFB, acid-fast bacilli; CXR, chest radiograph; WBC, white blood cell count; NA, not applicable.

had underlying medical illness, diabetes mellitus (3/5, 60%), HIV infection (1/5, 20%), and liver disease (1/5, 20%) were observed (data not shown). All patients with NTM lung disease and with NTM colonization had a history of alcohol consumption (Table 2).

The clinical presentations of patients in both groups were similar, including duration of symptoms, cough, dyspnea, chest pain, fever, fatigue, night sweats, hemoptysis and lung crepitations. However, patients with NTM lung disease had a lower body weight prior to treatment

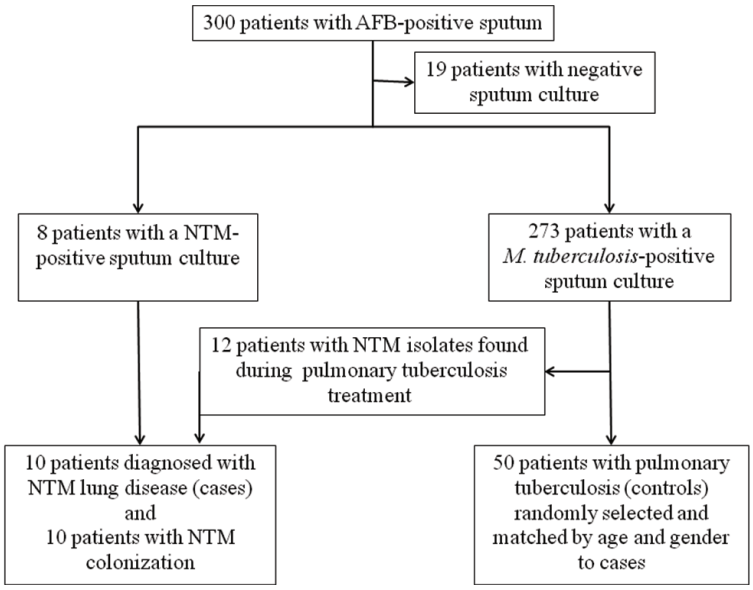

Fig 1–Flow diagram of study subjects.

and a higher change in body weight from baseline than those with NTM colonization (Table 2). Laboratory investigations showed no significant differences in sputum AFB 3+, Hb levels and WBC counts between the two groups. Of the 10 patients with NTM lung disease, 5 (50.0%) had cavitations on chest radiograph but none of the patients with NTM colonization had cavitations on chest radiograph (p = 0.033). Nodular infiltration was seen on chest radiography in 5 patients (50%) with NTM lung disease, but all patients with NTM colonization had fibronodular infiltrations on chest radiography. Two patients with NTM lung disease (20%) died during treatment but no patients with NTM colonization died during treatment (p = 0.474) (Table 2). The causes of death in the 2 patients with NTM lung disease were: out-of-hospital sudden cardiac arrest and esophageal cancer with liver metastases.

DISCUSSION

Over the past few decades, the number of patients with NTM lung disease particularly in Asia has been increasing and the common lung pathogens have been: *M. avium* complex, *M. kansasii* and *M. fortuitum* (Griffith *et al*, 2007; Chen *et al*, 2012; Lee *et al*, 2012). Patients with pre-existing lung disease, patients on immunosuppressive therapy, those with genetic defects or cell-mediated immunity have been at risk for NTM lung disease (Sexton and Harrison, 2008). In our study, the majority of patients with NTM lung disease were male, had underlying medical illness, particularly diabetes mellitus, a history of smoking, and all the patients had a history of alcohol consumption. Previous studies also found NTM lung disease occurred predominately in males, particularly in those with chronic obstructive pulmonary disease (COPD) or previous pulmonary tuberculosis (Sonnenberg *et al*, 2000; Thomsen *et al*, 2002; Van Ingen *et al*, 2009). A more recent study found NTM lung disease can occur in elderly female patients with bronchiectasis (Winthrop *et al*, 2010).

Patients with NTM lung disease usually present with chronic pulmonary symptoms including productive cough, dyspnea, chest pain, hemoptysis, and other constitutional symptoms, such as fever, fatigue, malaise and weight loss (Griffith *et al*, 2007), similar to our findings. Lung rales were common on chest auscultation in our study, but this finding is non-specific and only reflects underly-

ing pathology (Griffith *et al*, 2007). The radiographic findings of NTM lung disease depend on pre-existing lung disease and usually present as fibrocavitation or nodules and bronchiectasis (Griffith *et al*, 2007; Taiwo and Glassroth, 2010). In our study, the most common radiographic finding in patients with NTM lung disease was the presence of cavitations on chest radiograph, which is classically seen in middle-aged or elderly males, those with a history of smoking, pre-existing lung disease or previous pulmonary tuberculosis (Taiwo and Glassroth, 2010). Previous reports found cavitations on chest radiograph in patients with NTM lung disease were characterized by thin-walled cavities with less surrounding parenchymal opacity, less bronchogenic spread and extensive pleural thickening (Evans *et al*, 1996a,b; Taiwo and Glassroth, 2010).

The recovery rate from NTM in our study was 2.7%, which is lower than that reported in another study from Asia (Jeon *et al*, 2005). The recovery rate of NTM among patients with AFB-positive sputum varies widely due to differences in study populations: 7-49% in the United States (Stone *et al*, 1997; Wright *et al*, 1998; Maiga *et al*, 2012), 8.1% in Korea (Jeon *et al*, 2005) and none in South Africa (Levy *et al*, 1989). Of the 273 patients with pulmonary tuberculosis in our study, 12 (4.4%) had a NTM-positive sputum culture during anti-tuberculosis treatment. This figure is lower than a previous report from Korea of 7.1% (Jun *et al*, 2009). We found 2 of 12 patients with pulmonary tuberculosis and a positive culture for NTM (16.7%) developed NTM lung disease during anti-tuberculosis treatment, similar to a previous finding of 16.1% (Grubek-Jaworska *et al*, 2009).

In Thailand, where tuberculosis is endemic, we found patients with AFB-positive sputum who also had underlying medical illness, such as diabetes mellitus or HIV infection, also had NTM lung disease. Clinical and laboratory parameters were not significantly different between patients with NTM lung disease and those with pulmonary tuberculosis, similar to a previous report (Griffith *et al*, 2007). In contrast, data from a region with a low tuberculosis incidence showed having a birthplace outside the United States, age and the presence of COPD could be used to differentiate patients with NTM lung disease from those with pulmonary tuberculosis (Kendall *et al*, 2011). In our study, we found patients with a NTM-positive sputum culture with significant weight loss or cavitations on chest radiograph had NTM lung disease rather than NTM colonization. The case-fatality rate in patients with NTM lung disease in our study was 20%, higher than in patients with pulmonary tuberculosis (2%), but the difference was not statistically significant. None of the patients with NTM colonization died during the follow-up period in our study. A previous report found patients with NTM lung disease had a case-fatality rate of 20%, not significantly different from those with pulmonary tuberculosis (Ratanasuwan *et al*, 2002).

In conclusion, NTM lung disease should be considered in patients with AFB-positive sputum who have an underlying medical illness, especially diabetes mellitus or HIV infection. Patients with a NTM-positive sputum culture who have significant weight loss or the presence of cavitations on chest radiograph are more likely to have NTM lung disease rather than NTM colonization. These findings may help clinicians in early management of patients with NTM lung disease in order to prevent disease progression.

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