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Review Article

Clinical relevance and diagnosis of nontuberculous mycobacterial pulmonary disease in populations at risk

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The lungs are the most common disease site of nontuberculous mycobacteria (NTM). However, the isolation of NTM in a respiratory specimen does not indicate lung disease (LD). Differentiation between NTM colonization and NTM-LD remains challenging. In this brief review, we summarize the clinical impact of NTM-LD on morbidity and mortality in high-risk populations. The diagnosis criteria for NTM-LD—including clinical features, radiological presentations, and microbiological evidence—are also reviewed, according to the latest American Thoracic Society (ATS)/Infectious Disease Society of America (IDSA) guideline and the British Thoracic Society (BTS) guideline. However, the diagnosis of NTM-LD does not necessitate the initiation of anti-NTM treatment. Both environmental, host, and bacterial factors should be considered to identify patients that require NTM-LD treatment.

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Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous microorganisms widely dispersed in natural and man-made environments, especially in soil and water.[1,2] More than 190 NTM species have been identified, although only a few have been linked to human diseases. Although, NTM can cause diseases at any site within the human body and even disseminate disease in an immunocompromised host, the lungs are the most common disease site. Despite a high probability of colonization of the respiratory tract, NTM-lung disease (NTM-LD) can lead to considerable risk of morbidity and mortality, especially in high-risk populations. In this review, we address the clinical relevance of NTM-LD and current recommendations for the diagnosis of NTM-LD.

Populations at risk of NTM-LD

Several risk factors for NTM-LD have been reported, and they jointly indicate that both host and environment factors should be considered. Environmental sources of NTM include soil, dust from agriculture and gardening, indoor swimming pools, hot tubs, and aerosols from indoor humidifiers.[3,4] Host factors associated with NTM-LD include comorbidities that impair immunity and diseases associated with structural change in the lungs. Impaired immunity—such as that in people with human immunodeficiency virus (HIV) infection[5]; malignancies[6]; and exposure to immunosuppressants, including biological agents and oral and inhaled corticosteroids—is a well-recognized risk factor for NTM-LD.[7,8] Adult-onset immunodeficiency, a recently identified immunodeficiency disorder characterized by anti-interferon-gamma autoantibodies, has been reported to result in a high risk of disseminated NTM diseases, including those with lung involvement.[9–11] Patients with structural lung disorders usually have impaired mucociliary clearance in their airway, which leads to increased susceptibility to NTM-LD. Therefore, patients with bronchiectasis, chronic obstructive pulmonary disease, and previous pulmonary *Mycobacterium tuberculosis* (MTB) infection are also risk populations for NTM-LD.[12,13]

NTM colonization in respiratory tract

A positive NTM culture in a respiratory specimen, unlike a positive MTB culture, does not necessarily indicate a pulmonary infection that requires treatment. The possibilities of NTM contamination of specimen during collection, contamination of reagents used in specimen processing, or contamination of medical tools should be considered.[14,15] Transient or persistent colonization of the respiratory tract by NTM is also possible and should be carefully distinguished from true infection, which indicates tissue invasion and progressive nature. Several factors may affect the clinical status of NTM isolates in respiratory specimen. The percentages that a positive NTM isolate from respiratory specimens that represent true NTM-LD varies in different NTM species and some species have higher pathogenic potential.[16–18] The detailed information is discussion below in the section of "Clinical relevance of NTM species". Patients with higher NTM burden and in respiratory specimens are more likely to develop progressive change, which indicate a true disease.[19] The characteristics of hosts also play important roles as a multiple centers study in Taiwan indicated that patients with low body mass index and radiographic nodular-bronchiectatic pattern were correlated with progressive disease nature.[20] Although a well-established follow-up protocol remains unavailable, patients with NTM isolates in respiratory specimens and concomitant risk factors deserve a close observation, if aggressive treatment is not indicated initially.

Clinical relevance and impact of NTM-LD

However, the impact of NTM-LD on morbidity and mortality should never be overlooked. Studies have reported inconsistent mortality rates of NTM-LD. A single-center study conducted in Taiwan that included 40 patients with disseminated NTM disease (52.5% of cases were of NTM-LD) reported an overall mortality rate of 30%.[21] A recent nationwide population-based study conducted in South Korea reported a 5-year mortality of 17.8% in the population of those with NTM infection; this was a mortality ratio of 2.16 compared with the general population.[22] A Japanese study that included HIV-negative patients with *Mycobacterium avium* complex (MAC)-LD reported an all-cause-mortality rate of 25.2% over a median follow-up period of 4.7 years.[23] A population-based study conducted in Canada reported a hazard ratio of 1.47 for death relative to controls. The 5-year mortality was 26.6% among patients with NTM isolation from the respiratory tract and 36.9% among patients with NTM-LD.[24] Regarding the risk factors associated with mortality in patients with NTM-LD, the commonly reported risk factors have included male sex, older age, hemoptysis, fibrocavitary diseases, and pulmonary hypertension.[25–28]

The effects of NTM-LD on the outcomes of special populations, especially those with high risk of NTM-LD, also deserve our attention. A retrospective study conducted in US included HIV-infected patients with *Mycobacterium kansasii* in respiratory specimens from 1989 to 2002 reported a mortality rate of 53%.[29] A US database study reported that the percentage of NTM-related deaths decreased from 33% in 1999 to 4% in 2014, although mortality was found to increase in patients with NTM but without HIV infection.[30] According to a retrospective study conducted in a medical center in Taiwan, in 5.8% of patients admitted to intensive care units, NTM was isolated from respiratory specimens.[31] The intensive care unit mortality rate among patients with NTM-LD and NTM lung colonization was 47% and 8%, respectively. A case—control study conducted in Taiwan enrolled patients with rheumatoid arthritis who were receiving antirheumatic medications and reported 50 (0.56%) cases of NTM disease among 8927 patients with rheumatoid arthritis; NTM-LD was discovered in 39 cases.[8] Patients with rheumatoid arthritis and NTM-LD were found to have a higher hospitalization rate than those without NTM disease did (76.9% vs. 31.4%). In 39 patients with rheumatoid arthritis and NTM-LD, 6 (15.4%) died during the study period. The average time

between onset of overall NTM disease and death was 1.12 ± 0.87 years.

Diagnosis of NTM-LD

The diagnosis of NTM-LD remains clinically difficult and is frequently complicated by NTM colonization of the airway and nonspecific clinical presentation. The isolation of NTM in respiratory specimens does not confirm the diagnosis of NTM-LD. In general, NTM-LD should be diagnosed using a variety of clinical features, microbiological studies, and radiographic manifestations. The most commonly used diagnostic criteria are the American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) guideline published in 2007 and the British Thoracic Society (BTS) guideline published in 2017.[32,33] According to these guidelines, NTM-LD can be diagnosed only when all the clinical, radiological, and microbiological criteria are met, and other diagnoses have been appropriately excluded (Table 1).

The current diagnostic criteria in guidelines of IDSA and BTS are generally similar. Due to lack of robust clinical evidences, the diagnostic criteria cannot be further clearly defined. However, clinicians should be aware of some controversial issues in applying the criteria in clinical management. In clinical features, the criteria ask an appropriate exclusion of other diseases, for example some pulmonary diseases. However, we feel that NTM-LD in patients with concomitant chronic pulmonary disorders, such as chronic obstructive pulmonary disease, asthma, bronchiectasis, chronic pulmonary aspergillosis, or even active tuberculosis (TB), remains possible. It would be clinically difficult to differentiate the symptoms from NTM-LD or concomitant pulmonary diseases. The presence of concomitant pulmonary disorders does not exclude the need to initiate NTM-LD treatment in our opinions. In a TB endemic area like Taiwan, concomitant infection of PTB and NTM-LD would be a critical issue requires further clarification. In microbiological evidences, the criteria ask for more than two positive culture from sputum samples without a clear statement of the time interval between the two samples. A time interval of two days or two months between the two samples positive for NTM would have different clinical implication. Further studies would be needed to clarify these issues.

Clinical features

The symptoms of NTM-LD are nonspecific and occur in subjects with pre-existing respiratory diseases as well as in those with NTM-LD. Distinguishing the symptoms of NTM-LD from those caused by underlying pulmonary conditions is difficult. Additionally, the symptoms reported by studies have varied considerably. The most commonly reported symptoms are cough (21%—100%) and sputum production (12%—100%). Other less common symptoms are fever (11%—58%), hemoptysis (11%—37%), and weight loss (4%—38%).[23,34—46]

Microbiological studies

Sample collection

Respiratory specimens—including expectorated sputum, induced sputum, tracheal aspirates, bronchial washings, and bronchoalveolar lavage fluid—are often obtained to detect NTM-LD.[32] Transbronchial biopsy may demonstrate granulomatous inflammation pathologically for definite diagnosis of NTM-LD in addition to microbiological evaluation, but it clearly has an associated procedural risk.[47] There have been no systematic comparisons of these methods of NTM detection to date. However, expectorated sputum is by far the most often used specimen for microbiological diagnosis. The ATS and BTS guidelines recommend for an early-morning sample to be obtained on three different days.[32,33] This recommendation was derived from a study reporting that 97% of subjects with radiographic evidence of NTM-LD had two or more isolations of NTM from the first three of their collected respiratory samples.[19] Some authors have recommended that subjects brush the teeth, rinse their mouth with water and dry it with tissue paper, then proceed with spitting saliva and finally cough up sputum. The value of brushing teeth or rinsing the mouth

Table 1 Diagnostic criteria of NTM-LD.[a]

Clinical criteria	Manifestations	Compatible symptoms[b]
	Radiological presentations	1. Nodular or cavitary opacities on chest radiograph or 2. Multifocal bronchiectasis with small nodules on HRCT
	Appropriate exclusion of other diseases	
Microbiological criteria	1. Positive culture from ≥2 expectorated sputum samples or 2. Positive culture from ≥1 bronchial wash or bronchoalveolar lavage fluid or 3. Compatible histologic features in lung or transbronchial biopsy[c], and ≥1 positive culture from lung biopsy or sputum or bronchial wash	

NTM-LD, nontuberculous mycobacteria—lung disease; HRCT, high-resolution computed tomography.

[a] Modified from 2007 ATS/IDSA guidone[32] and 2017 BTS guideline.[33]

[b] Compatible symptoms include recurrent cough, sputum production, fatigue, malaise, dyspnea, fever, hemoptysis, chest pain, and weight loss.

[c] Compatible histologic features include granulomatous inflammation and presence of acid-fast bacilli.

has not been extensively proven, though.[48] Pleural fluid and tissue samples from pleural biopsies and lung biopsies may also be used to help diagnose NTM-LD in appropriate clinical settings.[32,49,50] Thoracentesis and pleural biopsy could be considered in patients with pleural effusion. Biopsies from pleura and lung also help the differential diagnosis. Positive culture from pleural fluid or demonstration of granuloma in tissue pathology would suggest these microorganisms cause true disease rather than colonized in the airways. Sample processing should ideally be completed within 24 h of collection to prevent overgrowth of bacteria, which could increase the likelihood of decontamination and increase the NTM's viability.[51] If a processing delay of longer than 24 h is anticipated, freezing the samples at −20 °C was demonstrated to improve the detection rate of MTB without preservatives needing to be added.[52]

Smear microscopy

Similar to MTB, NTM may be visualized directly in clinical samples by microscopy using fluorochrome (auramine phenol), Ziehl–Neelsen, or Kinyoun staining. Microscopic examination provides a useful adjunct to cultures and should be routinely performed. Fluorochrome staining has higher sensitivity than Ziehl–Neelsen staining does.[53] Smear positivity can alert the clinician at an early stage to the presence of mycobacteria, which may serve as a prognostic factor for progression to active disease.[46] In a systematic review, fluorochrome staining was demonstrated to have approximately 10% higher sensitivity than Ziehl–Neelsen staining does.[53] In addition, fluorochrome staining has comparable sensitivity for the detection of NTM as for that of MTB.[54] The sensitivity of microscopy is strongly influenced by sample quality, the mycobacterial species, and laboratory experience; thus, microscopy should not be used as the sole indicator of bacterial load. Additionally, NTM cannot be distinguished from MTB by using microscopy. Mycobacterial culture must be used to confirm NTM.[53,55]

Molecular detection

Direct molecular detection is useful for rapidly differentiating MTB complex from NTM in smear-positive specimens.[56] Nucleic acid amplification tests such as the Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) may be valuable for the identification of MTB in this clinical scenario.[57] However, modern commercially available molecular techniques—such as polymerase chain reaction (PCR) restriction analysis, sputum *rpoB* gene analysis, and the GenoType Mycobacteria Direct test—are costly and less sensitive than the conventional acid-fast bacilli culture is.[58–60] No conclusive evidence has yet been obtained regarding their role in routine clinical practice.

Mycobacterial culture

The most sensitive and reliable method of detecting NTM in a respiratory specimen is through mycobacterial culture, although culture of NTM can be difficult and may not be routinely optimized. In the published guidelines,[32,33] optimal media selection, culture duration and temperature, and method of decontamination have yet to be clearly defined, and it may be varied in different NTM species. Before inoculation for mycobacterial culture, nonsterile specimens must be decontaminated to prevent the overgrowth of mycobacterial cultures. Several protocols, including 1%–2% N-acetyl L-cysteine (Nalc)–NaOH and 5% oxalic acid or 1% chlorhexidine, may be used for this purpose.[33,57] A two-step method for processing respiratory samples can be used to enhance the decontamination protocol and thus mitigate the adverse effects on NTM viability.[61] Most European clinical microbiology laboratories use Nalc–NaOH for decontamination before mycobacterial culture.[33] The use of a second decontamination step involving oxalic acid has been demonstrated to achieve NTM recovery from persistently contaminated samples at the cost of decreased sensitivity.[62] In general use, two main culture methods are employed: automated liquid culture systems and solid media. Automated liquid culture systems, such as Mycobacteria Growth Indicator Tube (BD Biosciences, Sparks, MD, USA), save time and are more sensitive than are solid media culture methods, such as Löwenstein–Jensen or Middlebrook 7H10 and 7H11 media.[33] By contrast, the major advantages of solid media culture are that colony morphology can be evaluated, multiple species and mixed infections can be identified, and NTM can be detected even in cases of inadequate sample decontamination.[63] Some studies have indicated that the Löwenstein–Jensen medium may have higher sensitivity to NTM than the Middlebrook 7H11 medium does.[64,65] The highest recovery rates have been obtained using a combination of liquid and solid culture media, and a 100% recovery rate was reported when all culture systems were utilized.[66] Culture is usually performed at 35 °C, which is the optimal recovery temperature for MTB. However, the optimal temperature for various NTM species differs. For example, *Mycobacterium abscessus* grows best at 28°C-30 °C, whereas *Mycobacterium xenopi* does so at 45 °C.[32] Furthermore, some NTM species require additional conditions, such as supplemental media for *Mycobacterium genavense* and *Mycobacterium haemophilum* and an extended incubation period for *Mycobacterium malmoense*.[33] Most pathogenic NTM will grow within 6 weeks, but the optimal duration of incubation has yet to be determined. An extension to 12 weeks is recommended in the BTS guidelines.[33]

Species identification

Another review article titled "Nontuberculous mycobacterial epidemiology in Taiwan: A systematic review" in this review series explains the details of species identification in NTM.

Clinical relevance of NTM species

NTM species vary in their clinical relevance. Clinical relevance can be defined as the percentage of patients with positive NTM culture from a respiratory sample meeting the ATS/BTS diagnostic criteria of NTM-LD.[32,33] Huang et al. performed a retrospective multicenter study in Taiwan to evaluate the epidemiology of NTM pulmonary infection.[16] They reported that the most common isolates were MAC (2395 cases, 27.6%), followed by *M. abscessus* complex (1467 cases, 16.9%), and *Mycobacterium fortuitum* (1430 cases, 16.5%). Isolation of *M. kansasii* (31.3%), *M. abscessus* complex (27.8%), or MAC (24.1%) was associated with a higher probability of NTM-LD, indicating the higher clinical

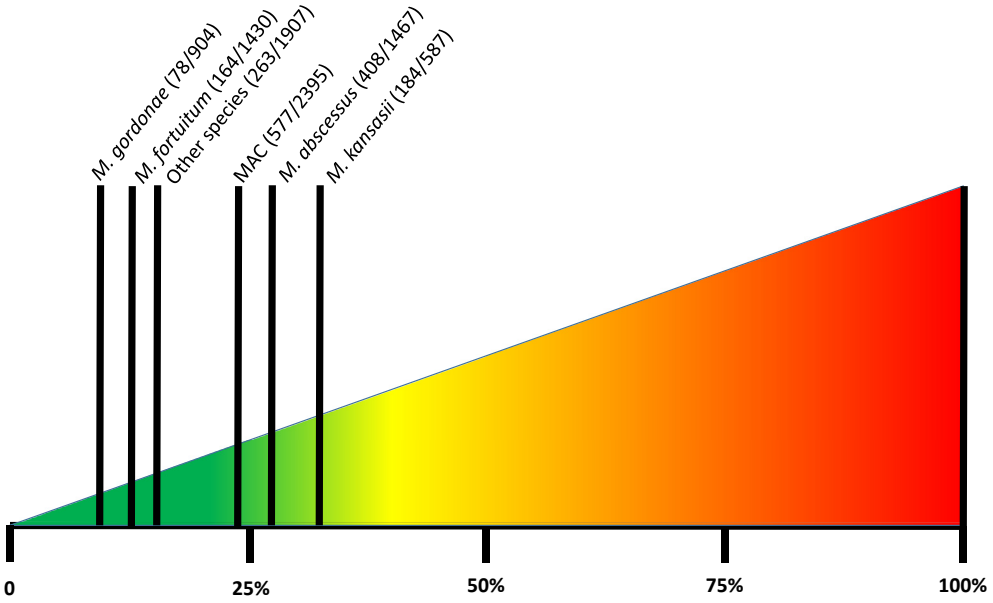

Figure 1 Clinical relevance of nontuberculous mycobacteria (NTM) isolated from respiratory samples in Taiwan. Data were modified from Huang et al.[54]

relevance of these three species. *M. fortuitum* and *Mycobacterium gordonae* were usually found to be a result of colonization rather than of a true infection. The clinical relevance of NTM species may also differ by geography. Although a South Korean study obtained a similar distribution of causative pathogens as that found in Taiwan,[35] epidemiological data from the Netherlands and United States differ from such data from East Asia.[17,18] Fig. 1 illustrates the clinical relevance of the various NTM species in Taiwan. Percentage of NTM-LD varies in different NTM species. It depicts that NTM species have different pathogenic potential.

Radiological manifestations

Radiographic abnormalities are one of the clinical diagnostic criteria for NTM-PD. Chest radiography is generally the first imaging tool employed for evaluating thoracic lesions. Nodular and cavitary opacities are easily recognized on a chest radiograph. They raise suspicion about not only infectious disease but also other disease entities such as malignancies. In individuals with underlying lung parenchymal disease, interpretation of chest radiographs can be challenging. As a conjunctive tool to conventional radiography, high-resolution computed tomography (HRCT) has powerful ability to detect subtle lesions and mediastinal abnormalities and can image the entire picture of NTM-LD involvement.[67–69] HRCT is also recommended for evaluating treatment response[33,70,71]: interobserver agreement has been moderate to high in the diagnosis and assessment of treatment-induced changes,[70–72] and HRCT should thus be routinely performed when NTM-LD is suspected.

Radiological manifestations are of two major types: nodular bronchiectatic and fibrocavitary patterns (Fig. 2A and B).[33] Nodular bronchiectatic disease is characterized by dilated bronchi, poorly defined nodules, and tree-in-bud opacities. A predilection for the middle and ligula lobes is more common in NTM than MTB. The nodular bronchiectatic pattern is common in white, postmenopausal, tall, and thin women, and is named Lady Windermere syndrome in this population.[73,74] These women do not usually have a history of previous pulmonary disorders. Investigations of the immune system of this specific population have obtained mixed results and are ongoing.[12,75] The fibrocavitary pattern is often associated with a long history of smoking and pre-existing diseases, such as chronic obstructive pulmonary disease and previous pulmonary TB with residual sequelae. Pulmonary cavities are mostly small and thin-walled, with upper lung predominance. In addition to the presence of cavities, lung volume loss, pleural thickening, fibrotic changes, and bronchiectactic lesions with tree-in-bud patterns may also occur.[76]

Other radiologic patterns, such as consolidation, mass-like lesions, and diffuse infiltration, are less common.[38,40] The overall prevalence of these unusual presentations are unknown because of no available general investigations. In some retrospective studies, mostly with small sample sizse, the percentage of these pattern varied: consolidation (12%—63%), mass-like lesion (3.6%—33%) and the diffuse infiltrative form (36%).[38,40,77,78] Owing to absence of standard definition regarding to these image patterns, the real prevalence and clinical significance remained controversial. In some cases, NTM-LD can also present as asymptomatic incidental pulmonary nodules.[79] The overlap of these features is prevalent, and these features are of limited diagnostic value when determining the specific NTM

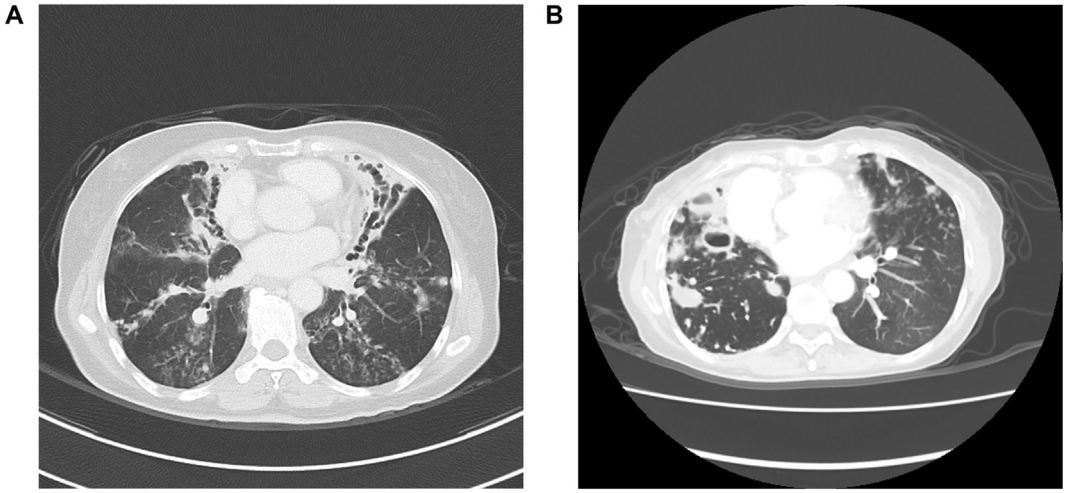

Figure 2 Two major types of radiological manifestation of NTM—lung disease: A) nodular bronchiectatic disease caused by *Mycobacterium avium* complex; B) fibrocavitary disease caused by *Mycobacterium kansasii*.

species and when discriminating between MTB and NTM.[77,80] However, a recent multicenter retrospective study conducted in Taiwan suggested that *M. kansasii*—LD is more likely to exhibit a fibrocavitary pattern and multifocal involvement than are MAC and *M. abscessus* complex.[16] This suggests that the radiologic characteristics of the various NTM species may have geographic differences. Two retrospective cohort studies have suggested that pleural effusion and basal pleura disease are rarer in NTM-LD (prevalence of 1.2%—1.4%) than in pulmonary TB.[81,82] Therefore, pleural effusion is highly suggestive of diseases other than NTM. In a single-center retrospective study in Taiwan, the researchers enrolled 35 cases of NTM isolated from pleural fluid, within the study period of 8 years.[83] They reported that the patients with NTM pleurisy were less likely to have lung involvement than those without pleurisy were. They also discovered that MAC was the most common pathogen.

In immunocompromised patients other than those with HIV, the computed tomographic (CT) findings were discovered to be similar to those in immunocompetent hosts; bronchiectasis and ill-defined nodular opacities without cavities were the most common findings in patients with MAC-LD.[71] In patients with HIV, however, NTM can lead to disseminated diseases other than LD, and a normal chest radiograph is not rare.[84,85] Nonspecific image findings including nodular infiltrates and adenopathy have also been reported in patients with HIV. Coexistence of other pulmonary infections can interfere with interpretation and diagnosis. In a Taiwanese study enrolling 15 patients with HIV and disseminated MAC disease, six patients had pulmonary involvement with presentations of pulmonary infiltrates, mass-like lesion, and hilar enlargement.[86] Investigations of image manifestations in diagnosis and prognosis for immunocompromised patients are few, and further research is warranted.

Recently, evidence has been accumulating for the association between radiological manifestations and prognosis. When investigating MAC-LD and *M. kansasii*-LD, several studies have reported poor response to treatment and unfavorable outcomes in patients with cavitary lesions.[23,40,46,87] Consolidation has also been reported to be an indicator of poor outcome.[28,40] In clinical practice, radiographic manifestations provide important information to physicians in making the decisions of NTM treatment initiation and further assessment.

Histology and serology

The most common and characteristic histological finding in NTM disease is necrotizing granulomatous inflammation, which is also found in MTB. Other histological findings in lung biopsy include bronchocentric chronic inflammation, bronchiectasis, granulomatous inflammation, and cavitation.[88,89] The presence of acid-fast bacilli is occasionally reported, but it cannot be used to differentiate NTM from MTB.

The role of serology tests in NTM-PD diagnosis remains limited. A commercial kit for measuring anti glycopeptidolipid immunoglobulin A is available for the diagnosis of MAC disease. A single-center study conducted in Taiwan and including patients with NTM-LD and pulmonary TB reported serology tests to have a sensitivity of 60% and specificity of 91% when used for MAC-LD diagnosis.[90] A meta-analysis of 14 studies reported a moderate sensitivity of 69.6% and specificity of 90.6% in the diagnosis of MAC disease.[91] A test of this level of sensitivity cannot generally be used to exclude MAC-LD or distinguish MAC-LD from LD caused by a rapid-growth mycobacterium.

Conclusions

The lungs are the most common disease site in NTM disease. Studies have demonstrated the adverse effects of NTM-LD on the morbidity and mortality of special populations, such as patients with HIV, immunocompromised hosts, and patients with critical illness. The diagnosis of NTM-LD is based on the collection of clinical symptoms, microbiological evidence, and characteristic radiological findings. The roles of

histology and serology in the diagnosis of NTM-LD remain uncertain. Knowing that the isolation of NTM in the respiratory tract usually represents airway colonization and does not indicate NTM-LD is crucial. Clinicians should also keep in mind that NTM-LD diagnosis does not necessitate the initiation of NTM treatment; clinical judgement is critical. The environment component, host issues, and microorganism factors should be considered when making the final decision to commence treatment.

Declaration of Competing Interest

The authors have no conflicts of interest relevant to this article.

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