Non Tuberculous Mycobacterial Infection
From Oscar Wilde to Gene Sequencing

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Key words:
- Non Tuberculous Mycobacterial Infection
- NTBM
- Diagnosis

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A 70-year old female, lifetime non-smoker was admitted to our outpatient clinic complaining of mild productive cough, dyspnea on exertion and general fatigue. During the last ten years she reported multiple lower respiratory tract infections and was diagnosed with bronchiectasis based on compatible HRCT findings 5 years ago. Four years ago, P*seudomonas Aeruginosa* was isolated from her sputum and was treated with oral ciprofloxacin for 21 days. During the last three years she reported no hospitalizations and was self-prescribing antibiotics during worsening of her symptoms.

On admission she was afebrile, thin and pthysic and had mild kyphoscoliosis. Her clinical examination revealed: SaO_2: 95%, (FiO_2: 21%), heart rate: 90 bpm, respiratory rate: 12/min, and inspiratory squeaks on auscultation, mainly localized on lower lobes. She had no clubbing or ankle edema. She reported no Raynaud's phenomenon or other symptoms of arthritis (arthralgias, morning stiffness) or myositis. Her high resolution computed tomography revealed multiple cystic bronchiectases and nodular tree-in-bud opacities (Figure 1). A complete etiologic investigation of non-cystic

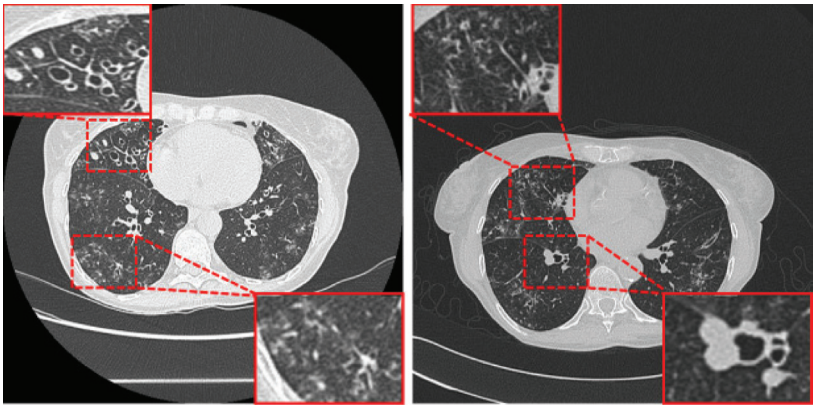

FIGURE 1. High-resolution chest computed tomography shows extensive bronchiectatic lesions and tree-in-bud opacities with linear branching pattern and nearly uniform distribution in the right middle and lower lobe, as well as the lingula and the left lower lobe, indicating architectural distortion and terminal airway mucous impaction with adjacent peribronchiolar inflammation. Insets are showing bronchiectatic lesions and tree-in-bud opacities (left panels and right upper panel) as well as signet-ring shaped bronchiectasis (right lower panel).

fibrosis bronchiectasis was performed.

The patient was immunocompetent based on quantitative serum immunoglobulin and general blood tests, revealed no history compatible with pertussis infection at infancy or childhood and her serum immunologic profile was negative. She was HIV and hepatitis B and C negative. Her tuberculin skin test was 8 mm and the interferon-gamma release assay (IGRA-QuantiferonTB-gold) was negative. Her sputum smears (n=3), PCR assays and solid-medium (Lowenstein Jensen) culture were negative for *mycobacterium tuberculosis (MTB)*. Solid medium cultures of sputum specimens revealed colonies of non-tuberculous *mycobacterium avium complex (MAC)*. She was commenced treatment with a thrice-weekly regimen consisted of: rifampicin – 600 mg (qid), clarithromycin - 1000 mg (bid) and ethambutol-1000 mgr (qid). Two months later the patient reported significant improvement of her dyspnea, fatigue and cough as well as sputum purulence and volume.

The **isolation of non-tuberculous mycobacterial** (NTM) remains a clinical dilemma. Because NTM naturally exist in the environment, isolation of NTM from a non-sterile respiratory specimen does not necessary mean infection. NTM pulmonary infection develops commonly in structural lung disease such as chronic obstructive pulmonary disease (COPD), bronchiectasis, cystic fibrosis, pneumonoconiosis, prior tuberculosis, pulmonary alveolar proteinosis and esophageal motility disorders[1]. In addition, clinicians should be highly aware and raise suspicion for NTM infection in cases of recurrent respiratory infections in immunocompetent individuals with radiological features of bronchiectasis.

More than 20 years ago, NTM pulmonary infection has been described in the context of ***Lady Windermere's syndrome*** which typically consists of the phenotype of a thin, well-mannered elderly woman with voluntarily cough suppression, mainly middle-lobe bronchiectasis and pulmonary *mycobacterium avium* complex infection[2,3]. The fastidious nature and reticence to expectorate are believed to be the main predisposing factors for lung infection by allowing secretions to collect into airways, particularly in the right middle lobe which has the longest and narrowest structure among lobar bronchi. The name originates from the lead character in **Oscar Wilde's** play *Lady Windermere's Fan*, which satirizes the strict morals and polite manners typical of the Victorian era in Great Britain.

The **diagnosis** of NTM pulmonary infection can be challenging and to this end clinicians should integrate atypical respiratory symptoms (cough, sputum, dyspnea) and radiological features (cylindrical bronchiectasis, multifocal tree-in bud opacities or cavitary lesions) to highly specific microbiological findings (positive culture for NTM in more than 2 expectorated sputum specimens or one specimen from bronchial lavage or washing) (Table 1)[4]. It is important to note that AFB stains (Kinyoun method seems to be superior to Ziehl-Neelsen) cannot distinguish between NTM and MTB. Nucleic acid amplification (NAA) tests are needed. **Culture** remains the gold standard for confirmation of NTM diagnosis. Culture media are similar to MTB. Both solid (Lowenstein Jensen) and liquid culture (Middlebrook 7H9) platforms are required. Nevertheless, since treatment and outcomes are different among NTM species, precise NTM identification is critical. Sequencing of the **16sRNA gene is the reference method** of choice for NTM discrimination up to the subspecies level. Gene sequencing can also be used to identify Inducible macrolide resistance, especially in mycobacteria with rapidly growing taxonomy, such as *M. abscessus* complex[4].

Macrolides represent the cornerstone of NTM-MAC treatment (Table 1). Management can be difficult and lengthy (at least 12 months) and should be individually tailored based on the NTM species, disease symptoms, radiological extent and patients' preferences[1]. On the other hand, current guidelines suggest similar to MTB therapeutic regimens (except for pyrazinamide) for the treatment of *M. Kansasii*, which is a relatively treatable pathogen. The therapy for *M. Abscessus* still remains a bottleneck for physicians and researchers. Guidelines suggest an oral macrolide and two parenteral agents such as amikacin, imipenem, tygecycline, cefoxitin and linezolid for several months. Bedaquiline, tigecycline, linezolid and clofazimine (an anti-leprosy drug) represent therapeutic agents used for MDR-TB infections[5-9].

In NTM **refractory cases**, debulking surgery of the most affected area of the lung may be helpful in selected number of patients[10]. In general, except from *M. Kansasii*, NTM infection is difficult to eradicate with anti-microbial therapy alone and is characterized by frequent relapses. Clinical trials enrolling patients with refractory NTM infection are sorely needed. Multiple **combination therapies** involving both surgical and anti-microbial interventions with novel therapeutic agents may hold promise for the future. Early referral to a **reference center** of excellence and multidisciplinary approaches are mandatory for optimal therapeutic decisions.

TABLE 1. Diagnostic Criteria and Therapeutic Approach for Non Tuberculous Mycobacterial (NTM)- MAC *(Mycobacterium Avium complex)* lung Disease

Category	Criteria
Clinical	Pulmonary symptoms 1. Cough 2. Expectoration 3. Exclusion of alternative diagnoses
Radiological	1. CXR – Nodular or cavitary opacities 2. HRCT – Multifocal bronchiectatic lesions with multiple small nodules
Microbiologic	1. Positive culture in at least 2 sputum samples and AFB negative 2. Positive culture in at least 1 bronchial wash or lavage 3. TBB or other lung biopsy with granulomatous inflammation and positive culture for NTM and one positive culture in bronchial wash or lavage
Additional considerations	1. Clinical and radiological criteria are both required for diagnosis 2. Expert referral and consultation for diagnosis and treatment 3. Diagnosis does not necessitate treatment. Treatment should be individually tailored
Treatment	A. Daily regimen: 1. Macrolides (azithromycin 250 mgr or Clarithromycin 1000 mg/day) 2. Rifamycin (rifampin or rifabutin) – 600 mg/day 3. Ethambutol 15 mg/kg/day B. Thrice weekly regimen: 1. Macrolides (azithromycin 500 mg or Clarithromycin 1000 mg) 2. Rifampin 600 mg 3. Ethambutol 25 mg/kg
Duration	1. 18-24 months 2. At least 12 months after culture negativity

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