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CASE REPORT

Non-Tuberculous Mycobacterial Pulmonary Disease with Detection of Mycobacterium Tuberculosis in Pleural Fluid

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SUMMARY

Background: **Tuberculous pleurisy (TP) is one of the most common types of extrapulmonary tuberculosis, often secondary to tuberculosis (TB). Clinical and imaging manifestations of non-tuberculous mycobacterial pulmonary diseases (NTM-PD) are usually similar to those of tuberculosis. Because of their similarity and the high incidence of tuberculosis, non-tuberculous mycobacterial infections are often overlooked for a long time. Especially in people without immunodeficiency.**
Methods: **Mycobacterium tuberculosis (MTB) in pleural effusion was found by metagenomic next-generation sequencing (mNGS). During anti-tuberculosis treatment, mNGS of lung tissue by ultrasound-guided percutaneous lung puncture revealed that this patient had combined NTM-PD.**
Results: **Mycobacterium chelonae (M. chelonae) was detected by mNGS, and after anti-NTM treatment, the patient's chest CT showed that the inflammation was absorbed more than before, and the patient's symptoms improved.**
Conclusions: **When TB is poorly treated with standardized anti-tuberculosis therapy, comorbid non-tuberculous mycobacterial infections may be considered, and mNGS may complement traditional pathogenetic testing.**
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KEYWORDS

Mycobacterium tuberculosis, tuberculous pleurisy, non-tuberculous mycobacterial pulmonary diseases, mNGS

CASE REPORT

Pleural effusions in tuberculous pleurisy (TP) are caused by a combination of direct MTB infection of the pleura and/or pleural hypersensitivity reactions. Thus, pleural effusions are "less bacterial" in nature, with lower MTB levels and positivity rates. NTM is a general term for a large group of mycobacteria other than the mycobacterium tuberculosis complex (MTBC) and mycobacterium leprae. Mycobacterium avium complex (MAC) is the most common pathogenic species worldwide, and M. chelonae has been reported in the literature in a relatively small percentage of cases [1]. The differential diagnosis of MTB and NTM infections is a major challenge in clinical practice, with similar clinical features but significant treatment differences [2]. In this

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paper, we will report the case of a TP patient who was found to have comorbid NTM-PD (M. chelonae) during anti-tuberculosis treatment.

A middle-aged male, 41 years old, was first admitted to the hospital on September 15, 2022, for intermittent cough and sputum, left-sided chest pain for more than 20 days, and fever for 7 days. Chest CT from another hospital 5 days before admission showed a left lower pneumonic lesion and left pleural effusion. At that hospital, he was treated with antibiotics and expectorants, which did not work well. He had a history of diabetes mellitus for more than 1 year; denied of history of tuberculosis and AIDS; had a history of self-reported "penicillin" allergy. On examination: T: 36.1℃, P: 96 beats/minute, R: 19 beats/minute, BP: 116/69 mmHg, respiratory sounds in the left lower lung were low, and the rest of the cardiopulmonary and abdominal examination showed no significant abnormality. Post-admission erythrocyte sedimentation rate (ESR) 52 mm/hour positive tuberculin (PPD) test; negative antacid staining of sputum smear; mycobacterium tuberculosis antibody positive. Chest ultrasound suggests a small amount of pleural effusion bilaterally, and drainage of the left thoracic cavity was performed by thoracentesis. Pleural fluid routine appearance is yellow and slightly cloudy; pleural fluid biochemistry shows adenosine deaminase (ADA) 58.8 U/L; pleural fluid negative for mycobacterium tuberculosis and rifampicin resistance gene test (X-pert); MTBC detected by mNGS in pleural fluid. In summary, the patient was diagnosed with tuberculous pleurisy. Symptoms improved after thoracentesis for fluid extraction and anti-tuberculosis treatment. He was discharged on September 20, 2022. After six months of standardized anti-tuberculosis treatment, the symptoms did not improve. Review chest CT findings: no significant change in multiple small nodular foci in both lungs; no significant change in the amount of pleural effusion; left lower lung hyperdense shadow more progressive than before (Figure 1). Therefore, he was admitted to the hospital again on April 12, 2023. The patient still has an intermittent cough and left-sided chest pain without fever since his last discharge from the hospital and is currently in the consolidation phase of anti-tuberculosis treatment (oral isoniazid, rifampicin). On examination: 36℃, P: 76 beats/minute, R: 19 beats/minute, BP: 140/87 mmHg. Respiratory sounds in the left lower lung were low, and the rest of the cardiopulmonary and abdominal examination showed no significant abnormality; ultrasound-guided percutaneous lung puncture for characterization of left lung lesions. Punctured tissue was sent for pathology which suggested interstitial fibrosis; no tumor cells were seen, and no acid-fast bacilli were found on acid-fast staining. To further define the infectious agent, lung puncture tissue was sent for mNGS detection: 479 M. chelonae, 43 pseudomonas aeruginosa, 1 MTBC, and 3 human herpesvirus 4. No drug-resistance genes were detected. Based on the clinical symptoms, laboratory test results, and chest CT, the final diagnosis of the patient in this case was Tuberculous pleurisy, pulmonary tuberculosis, and NTM-PD (M. chelonae). Based on anti-tuberculosis treatment, oral azithromycin 0.5 g 1/day was given in the initial phase, and oral linezolid 600 mg 1/day anti-NTM in the continuation phase was added as recommended by the guidelines. Chest CT was reviewed (Figure 2), and inflammatory absorption was observed.

DISCUSSION

Up to now, more than 190 species of non-tuberculous mycobacteria have been found. Only a few have the possibility of causing disease and belong to the conditional pathogenic bacteria. It rarely causes disease in people with normal immune function. NTM are widely found in the environment, such as soil, water, and hospital settings, and can infect lymph nodes, skin and soft tissues, bones, joints, and lungs, with lung disease being the most common manifestation [3]. Especially in individuals who already have chronic underlying lung disease (e.g., COPD, bronchiectasis, cystic fibrosis, previous tuberculosis, etc.), it can lead to exacerbation of pre-existing lung disease [4]. A study suggests that prior treatment for tuberculosis is a significant independent predictor of NTM-PD [5]. M. chelonae belongs to one of the NTM, classified in Runyon's classification as Rapidly Growing Mycobacteria (RGM) IV. It was classified as a species in 1992. Like most rapidly growing mycobacterium, it is resistant to chlorine and some detergents. Immunocompromised patients, pedicures, foot baths, tattoo parlors, advanced age, etc., are risk factors for infection with this strain of bacteria [6].

NTM infection is strongly similar to TB in terms of clinical features. Clinical symptoms of NTM-PD are usually nonspecific, with the majority of patients presenting with a chronic cough with or without sputum production or hemoptysis and slowly progressive fatigue or malaise. Systemic symptoms of toxicity are less common, occurring in 30% to 50% of patients [7]. NTM-PD chest CT reported two typical presentations: fibrocavitary and nodular bronchiectasis. It is not easy to associate imaging findings with NTM-PD in the clinical setting, especially when the fibrous cavitary type occurs with TB. In the patient, the chest CT showed a cavitary lesion with unilateral pleural effusion with low-grade fever, which was easily considered an MTB infection. Still, the acid-fast staining was negative, and further investigation of the pleural fluid mNGS detected the MTBC. After six months of standardized anti-tuberculosis treatment with no improvement and even progression of the lesions in chest CT, we considered the presence of co-infection and only detected co-infection with NTM by sending lung tissue for mNGS. Because tuberculous pleurisy has the same treatment regimen as pulmonary tuberculosis, based on the pathogens identified in the pleural fluid and the imaging of the chest CT, we considered the lung lesion to be tuberculosis without further clarification of the lung pathogens. Therefore, it

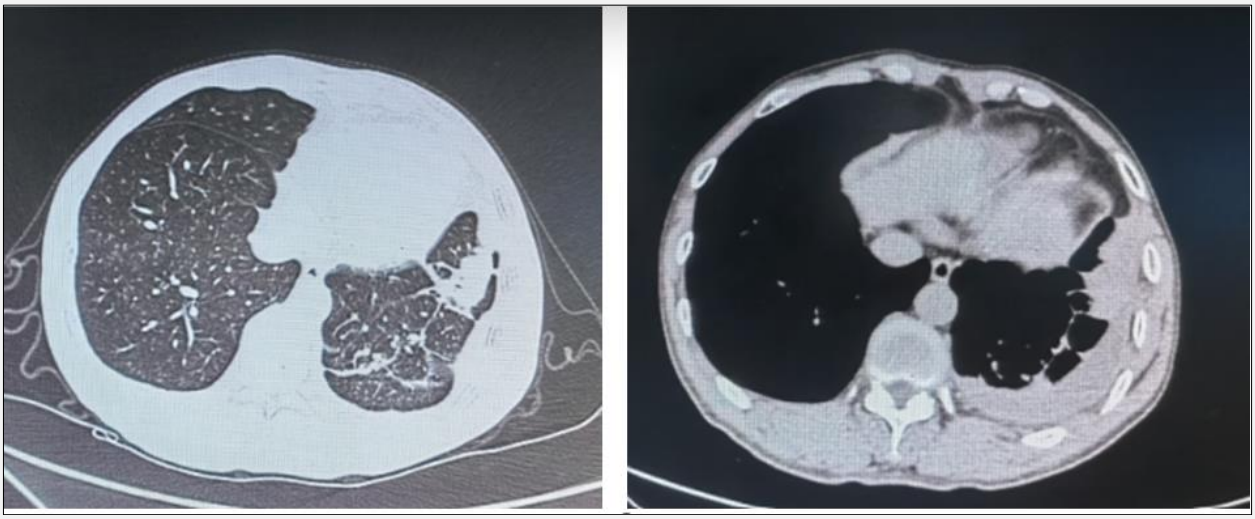

Figure 1. Chest CT scan image from April 12, 2023: High-density shadow in the left lower lung with multiple thick-walled cavitary shadows and left pleural effusion.

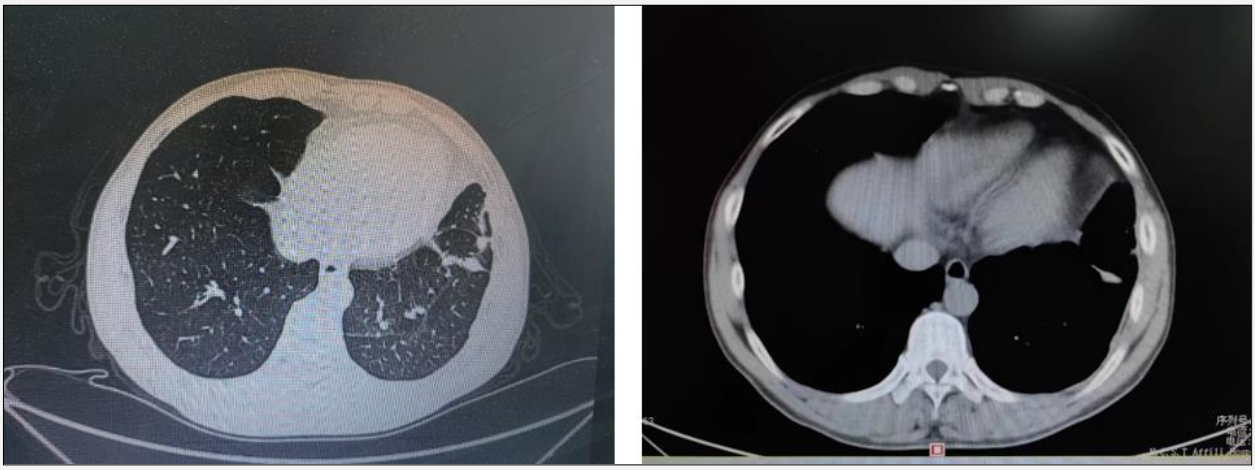

Figure 2. November 16, 2023 CT scan image of the lungs: left pleural effusion absorption and inflammatory lesion absorption.

could not be determined whether the NTM infection was present at the first admission or the during the first anti-tuberculosis treatment. In either case, clinicians are reminded that the incidence of NTM is on the rise year after year and that NTM infections are susceptible to long-term misdiagnosis of tuberculosis and even drug-resistant TB [8].

In general, the diagnosis of TB is made based on the patient's relevant clinical presentation, TB antibodies, PPD test, T-cell spot test for TB infection (T-SPOT), and routine biochemistry of the pleural fluid in combination with the results of a CT scan of the chest, which is routinely done in many TB-endemic countries. Mycobacterium smears and cultures are primary diagnostic

tools. Acid-fast bacilli (AFB) smear microscopy of sputum samples or bronchoscopic lavage is a simple and commonly used method for diagnosing mycobacterium. Still, the positivity rate of the smears is low [9]. AFB smear microscopy could not distinguish between intrapulmonary and extrapulmonary, MTB, and NTM infections. Culture can significantly increase the positive rate of MTB and NTM detection and further the identification of the organism and susceptibility testing. Identifying specific NTM species in clinical specimens is critical because of significant differences in treatment regimens between NTM strains. However, the cultivation period is long and cannot meet the clinical needs [2]. Various molecular biology methods have emerged as platforms for rapidly diagnosing tuberculosis because of the shortcomings of both culture- and microscopy-based diagnostic techniques. Unlike TB, the methods used to diagnose NTM disease, distinguish MTB from NTM, and differentiate NTM species are complex and underdeveloped. Therefore, mNGS plays an essential role in this case report. mNGS uses high-throughput sequencing technology to analyze the nucleic acid content of patient samples to detect and characterize microbial DNA and/or RNA [10]. This unbiased microbiological assay has the advantages of being rapid, having a high positive rate, and being complete. It facilitates the timely detection of pathogenic infections and the detection of drug-resistance genes of pathogens for precise treatment. However, there are no standardized interpretation criteria for NGS reports, and it is difficult for clinicians to consider the pathogenic species in conjunction with the clinical situation.

Our patient was a middle-aged male with standardized anti-tuberculosis treatment for six months, and the diagnosis of comorbid NTM-PD was confirmed by mNGS, with no common resistance genes detected. U.S. guidelines recommend starting treatment rather than watchful waiting in cases of cavitary NTM-PD [11]. Following the expert management consensus on M. chelonae in foreign countries and China [12], azithromycin and linezolid were given as anti-NTM therapy. At the same time, we monitored the adverse effects of the drugs during treatment.

CONCLUSION

This case demonstrates the effectiveness of percutaneous lung puncture combined with mNGS in facilitating and improving the clinical diagnosis of NTM infection. This method is essential for diagnosing long culture cycles, complex cultures, mixed infections, and rare pathogens. We propose combining NGS with traditional diagnostic techniques to identify pathogens in the early stages of disease to implement precision therapies. Clinicians are also reminded that the incidence of NTM-PD has an upward trend that warrants attention.

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Ethical Approval:
The ethics committee of North China University of Science and Technology Affiliated Hospital approved this study. Ethical standards were followed in all procedures performed in the studies. Informed consent was obtained.

Declaration of Interest:
No conflicts of interest.

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