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Case Report

Mycobacterium paragordonae pulmonary disease with rapidly growing solitary lesions: a case report and literature review

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Abstract

Introduction: Mycobacterium paragordonae (MPG) is a novel and uncommon nontuberculous mycobacterium (NTM). We describe a case of MPG pulmonary disease (MPGPD) with a single, rapidly growing, pulmonary mass, which has rarely been reported.

Case report: A chest CT scan of a 66-year-old woman revealed a rapidly growing solitary mass-like lesion in the upper lobe of the right lung, which was not seen in the previous chest CT scan six months ago. H&E-stained section of the CT-guided percutaneous lung tissue biopsy specimen showed chronic inflammatory changes with epithelioid granulomas. Metagenomic next-generation sequencing (mNGS) of lung tissue biopsy specimen identified MPG with a sequence number of 1617 and a confidence level of 99%. Because the subsequent MPG droplet digital PCR (MPG-ddPCR) test of the lung tissue biopsy was positive, she was eventually diagnosed with MPGPD. She was administered a quadruple oral regimen comprising clarithromycin, levofloxacin, rifampicin, and ethambutol according to the ATS/IDSA protocol for Mycobacterium gordonae (MG) infection. The chest CT scans showed a significant reduction in the lesion one month after the treatment and almost complete resolution four months later.

Conclusions: MPGPD is a rare NTM infection. The imaging manifestations of MPGPD are diverse and may even show rapid development. mNGS of tissue biopsy can enable prompt diagnosis of MPG infection and is a good alternative to routine NTM microbial testing. The ATS/IDSA protocol for MG infection is an effective treatment for MPG infection.

Key words: Mycobacterium paragordonae; nontuberculous mycobacterium; metagenomic next-generation sequencing; lung biopsy.

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Introduction

Mycobacterium paragordonae (MPG) is a clinically uncommon nontuberculous mycobacterium (NTM) that was first isolated and identified from a sputum sample of a lung infection patient in 2014 by Kim *et al*. [1]. MPG is a slow-growing pigmented NTM with rod-shaped, acid-resistant cells and no visible spores or mycelia under a microscope. Its 16S rRNA gene sequence is 99% identical to that of *Mycobacterium gordonae* (MG) [2,3]. Recent research has demonstrated that MPG is ubiquitous in soil and water environments and can be detected in freshwater, pipes, and laboratory taps [4-6]. Kim *et al*. found that MPG grows well at 25°C but not at 37°C and proposed that the optimum temperature for MPG growth is 25–30 °C [1]. While MPG is abundant in the environment, it is only mildly pathogenic to humans, and clinical isolation of this strain should be regarded as contamination or transient colonization.

The literature on MPG disease is very limited, and definitively diagnosed MPG pulmonary disease (MPGPD) is clinically very rare. This report describes a case of MPGPD identified through percutaneous lung biopsy and metagenomic next-generation sequencing (mNGS).

Case presentation

A 66-year-old woman was admitted due to the detection of a new mass in the posterior segment of the right upper lung lobe on chest CT. She had no history of smoking or alcohol consumption. She had a history of bronchial asthma for 40 years; however, her condition had been stable for nearly 20 years, and she had not received any treatment, including inhaled glucocorticoids. In September 2020, she underwent a thoracoscopic apical segmentectomy of the right upper lobe in another hospital after the detection of a “right upper lobe nodule” in a chest CT scan. The

postoperative pathological diagnosis was "micro-invasive adenocarcinoma" with a stage of pT1a(mi)N0M0. After surgery, she received no treatment and was monitored with chest CT scans every 6 months. She underwent three chest CT scans at our hospital between October 2020 and November 2021. A few subpleural fibrous strands and local pleural thickening were observed, which were considered old postoperative changes without new lesions (Figure 1, A1-A3).

A follow-up chest CT scan at our hospital in May 2022 revealed a new mass in the posterior segment of the right upper lobe, following she was admitted. The patient had no symptoms such as cough, sputum, hemoptysis, chest pain, chest tightness, or fever. The results of the blood routine, CRP, PCT, T-SPOT, and various pathogen antibody tests were all normal. The bacterial culture of induced sputum and sputum smears for acid-fast bacilli were negative. HIV antibody was negative, and the absolute lymphocyte counts and CD3+CD4+ lymphocytes were all within the normal range. The results of the autoantibody series P-ANCA and C-ANCA and other vasculitis-related tests were all negative. Tumor-associated antigen test results were within the normal range, including CEA, CA19-9, CA125, NSE, and CYFRA21-1 (Table 1). Contrast-enhanced chest CT scan in June 2022 revealed that the lesion was approximately 58 × 37 mm in size, with smooth edges, no burrs, and no obvious lobulation. The posterior edge showed linear calcification, while the local edge showed ring enhancement (Figure 1, B1-B3). The lesion was new compared to the chest CT scan performed in November 2021.

Fiber-optic bronchoscopy revealed a small amount of secretion in the tracheal lumen, right middle and lower lobe bronchus lumens, and external pressure stenosis in the right main bronchus, with no other obvious abnormalities (Figure 2A). Bronchoscopy brushes tested negative for acid-fast staining, and H&E staining revealed no tumor cells. Bronchoalveolar lavage fluid (BALF) culture was negative for pathogenic bacteria, and mNGS detected sequence number 1 of *Haemophilus mycobacterium*, which was considered to be a contamination. The patient underwent a CT-guided percutaneous lung biopsy in June 2022 (Figure 2B). H&E-stained section of the biopsy specimen showed chronic inflammatory changes with epithelioid granulomas (Figure 2C). The fungi-specific PAS and PAMS stains (Figures 2D and E), the acid-fast stains (Figure 2F), and routine tuberculosis PCR yielded negative results. mNGS of the biopsy detected NTM with a sequence number of 6951 and MPG with a sequence number of 1617 at a confidence level of 99% (Figure 3).

The detailed experimental steps and methods for mNGS were as follows: (1) DNA extraction, library preparation, and sequencing: DNA was extracted from formalin-fixed paraffin-embedded (FFPE) samples using the QIAsymphony DSP DNA Mini Kit following the manufacturer's instructions. The selected DNA was used to construct the library with Nextera XT DNA

Figure 1. Changes in chest CT scan before and after treatment. A1-A3: on admission in November 2021, a few subpleural fibrous bands in the right upper lung lobe and local pleural thickening were observed. B1-B3: A new spherical mass, approximately 58 × 37 mm, with a smooth edge, no burr, and no lobulation was seen in the posterior segment of the upper lobe of the right lung by June 2022; linear calcification (black arrow) can be seen at the trailing edge, and annular enhancement (white arrow) can be seen at local edges. C1-C3: the mass had significantly reduced one month after treatment in July 2022. D1-D3: the mass had almost completely resolved in October 2022, four months after treatment.

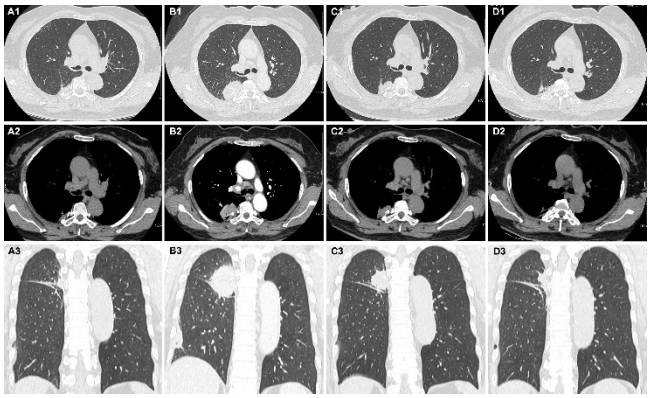
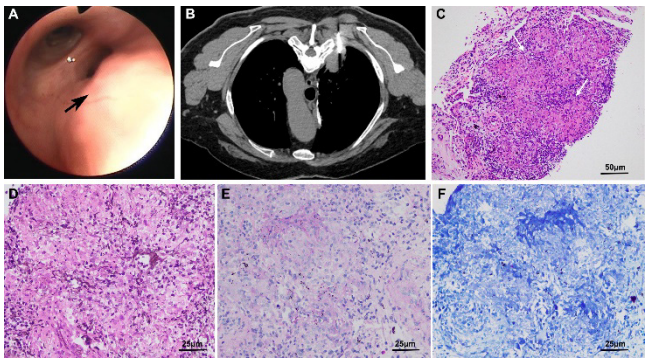


Figure 2. Fibrobronchoscopy and histopathology examination of lung biopsy. A: fiberoptic bronchoscopy revealed a constriction of the right main bronchus due to external pressure (black arrow). B: the patient received CT-guided percutaneous lung biopsy in a prone position, and the cutting needle was placed in the mass. C: H&E-stained section of the lung biopsy specimen showed chronic inflammatory changes, with epithelioid granulomatous inflammation (white arrow). D: Periodic Acid-Schiff (PAS) staining of the lung biopsy was negative. E: Periodic Acid-Silver Methenamine (PAMS) staining was also negative. F: Acid-fast staining of the lung biopsy was negative.



Library Prep Kit (Illumina, San Diego, CA) [7]. The library was quality-controlled using the Qubit dsDNA HS Assay Kit and High Sensitivity DNA kit (Agilent) on an Agilent 2100 Bioanalyzer. Library pools were then loaded onto an Illumina Nextseq CN500 sequencer for 75 cycles of single-end sequencing of 20 million reads for each library. Whole-blood sample from healthy donors was prepared alongside each batch, as a negative control, using the same protocol [8]. (2) Bioinformatics analyses: Trimmomatic [9] was used to remove low-quality reads, adapter contamination, and duplicated reads, as well as those shorter than 50 bp. Human sequence data were identified by mapping to a human reference (hg19) using Burrows-Wheeler Aligner software [10] and excluded. The remaining sequence data were aligned to the current bacterial, viral, fungal, and protozoan databases (NCBI; ftp://ftp.ncbi.nlm.nih.gov/genomes). Unique reads were defined as those with alignment length > 80%, identity with reference sequence > 90%, and a ratio of suboptimal to optimal alignment score < 0.8. A positive detection was reported for a given species or genus if the reads per million (RPM) ratio or RPM-r was ≥ 10, where the RPM-r was defined as the RPMsample/RPMNTC (the RPM corresponding to a given species or genus in the clinical sample divided by the RPM in the NTC) [7]. MPG-specific primers (Forward: CTAGCCAACACCGGCATCT; Reverse: GTTGGTTAGTGGGCCGAGG) designed by Vision Medicals (Guangzhou, China) were further used for droplet digital PCR (ddPCR) detection. The amplified product was absolutely consistent with the sequence alignment of MPG. According to the Tuberculosis Branch of Chinese Medical Association's criteria [11], a positive NTM culture or a positive molecular biology test of the biopsy specimen is confirmative of diagnosis. Therefore, the patient was finally diagnosed with MPGPD.

Because of the lack of any specific guidelines for MPGPD, we followed the ATS/IDSA guidelines for MG infection and prescribed quadruple oral therapy (clarithromycin 0.5 g bid, levofloxacin 0.4 g qd, rifampicin 0.45 g qd, and ethambutol 0.75 g qd). The medication was well tolerated by the patient. Repeat chest CT performed one month after the treatment showed a significant reduction in the lesion (July 2022; Figure 1, parts C1, C2, and C3). Four months later, the lesion had almost completely resolved (October 2022; Figure 1, parts D1, D2, and D3).

A flowchart showing the timeline of diagnosis, treatment, and follow-up is shown in Figure 4.

Discussion

MPG was first isolated from the lungs of a patient

Table 1. Main laboratory data.

Laboratory study	Data	Reference values
Hematology		
WBC count	5.89×10^9/L	$3.5\text{-}9.5 \times 10^9$/L
Neutrophil count	3.44×10^9/L	$1.8\text{-}6.3 \times 10^9$/L
Hemoglobin	130 g/L	$115\text{-}150 \times 10^9$/L
RBC count	4.56×10^{12}/L	$3.8\text{-}5.5 \times 10^{12}$/L
Lymphocyte count	1.87×10^9/L	$1.1\text{-}3.2 \times 10^9$/L
T cell counts		
T helper cells (CD4)	45.8%	28.5-60.5
CD4/CD8	1.4	1-2.5
CD19	6.6%	6.4-22.6
NK cell	8.6%	5.6-30.9
CD3	84.1%	59.4-84.6
Blood biochemistry		
BUN	6.95 mmol/L	2.8-7.6 mmol/L
Albumin	44.6 g/L	35-52 g/L
Inflammation profile		
CRP	8.8 mg/L	0-10 mg/L
PCT	0.05 ng/ml	0-0.05 ng/ml
Tuberculosis and fungal-related tests		
Tuberculosis antibody	Negative	Negative
TB-SPOT	Negative	Negative
Acid-fast staining of sputum	Negative	Negative
G test (serum)	< 37.5 pg/ml	0-69.99 pg/ml
GM test (serum)	0.04 μg/L	0-0.49 μg/L
Cryptococcus capsule antigen	Negative	Negative

WBC: white blood cell; RBC: red blood cell; BUN: blood urea nitrogen; CRP: C-reactive protein; PCT: procalcitonin; G: (1:3)-β-d-glucan; GM: galactomannan.

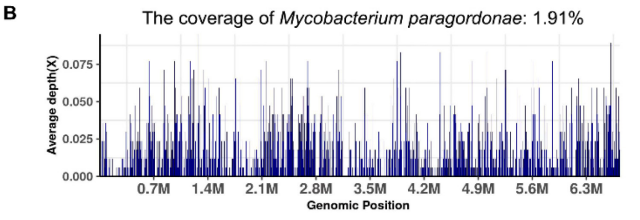
in Korea in 2014 [1]. The pathogenesis, imaging features, and prognosis of MPG infection are poorly characterized. The few limited research findings on MPG seem contradictory. For example, Kim et al. found that MPG grows well at 25 °C but poorly at 37 °C, implying that the optimum MPG growth temperature is 25-30 °C [1]. However, Li et al. [12] found that MPG could grow between 25 °C and 37°C, with 37 °C being the optimum growth temperature [1]. Furthermore, previous research has shown that MPG grows slowly, and Li et al. found that lesions in MPGPD patients remained essentially stable after a year of follow-up without treatment. However, in the present case, lung lesions grew rapidly in less than half a year. The above studies indicate the need for further research to better understand the characteristics and progression of MPG infection.

MPG, like most NTMs, should be regarded as an opportunistic pathogen, which is most commonly acquired in individuals with susceptible factors or specific environments [13,14]. HIV infection, renal failure requiring long-term hemodialysis, diabetes, various diseases receiving immunosuppressive therapy, including glucocorticoids, and malignant tumors receiving radiotherapy and/or chemotherapy are known to enhance susceptibility to NTM infection [15-18]. Previous case reports of infection by MPG are summarized in Table 2 [12,19-22]. Cheung *et al*. reported a 55-year-old man who developed MPG-infected peritonitis after receiving peritoneal dialysis for two years [20]. Tan *et al*. reported a 53-year-old patient with a lumbar abscess, in whom MPG was identified as the causative pathogen via biopsy mNGS [22]. Many recent reports have shown that the occurrence of NTMPD and NTM positivity after surgical resection of lung cancer is not uncommon [23,24]. This is likely attributable to the decreased local immunity due to the destruction of local lung tissue structure during the operation and NTM inhalation in specific circumstances, increasing the risk of NTM infection. Our patient was an elderly, overweight (BMI 27.22 kg/m^2) woman with a history of lung cancer resection. In addition, she had a habit of a closed sweat-steaming environment (once a week, each session lasting approximately one hour). We speculated that the

Figure 3. Results of metagenomics next-generation sequencing (mNGS) of lung biopsy. A: non-tuberculosis mycobacterium (NTM) was detected in the lung tissue biopsy, the sequence number was 6951, and the identified species were Mycobacterium paragordonae (MPG), with a sequence number of 1617 and a confidence of 99%. B: The MPG genome coverage was 1.91%. The abscissa denotes genome size, while the ordinate denotes average sequencing depth. The uniform distribution and the large sequencing depth of the detected sequences on the whole genome indicate the high credibility of the pathogen in the sample species. C: the species composition of the lung tissue microbes, with 1,617 species-specific reads mapped to the genome of MPG.

A

Genus				Species		
Type	Name	Relative abundance	Sequence number	Name	Confidence	Sequence number
G+	*Mycobacterium*	4.7 %	6,951	*Mycobacterium paragordonae*	99%	1,617



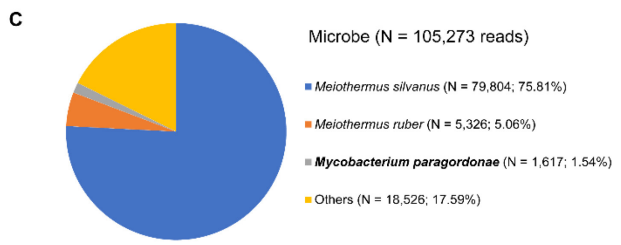


Figure 4. Flowchart showing the timeline for diagnosis, treatment, and follow-up.

Table 2. Previously reported cases of infection by MPG.

Authors	Year	Age (yrs.)	Sex	Primary disease	Infection site	Diagnostic test
Cheung *et al*. [20]	2017	55	M	CAPD for 2 years	Peritoneum	Mycobacterial culture of peritoneal dialysate effluent
Tan *et al*. [22]	2021	53	M	None	Lumbar vertebra	mNGS of lumbar necrotic tissue specimens
Li *et al*. [12]	2022	42.38 ± 9.92	M (3), F (5)	Hepatitis B (2), diabetes mellitus (1), pulmonary maculopathy (1), gout (1)	Lumbar vertebra	MALDI-TOF MS and WGS of BALF (5) or sputum (3)
Uchiyama *et al*. [19]	2023	55	F	NR	Pulmonary	Mycobacterial culture of bronchial secretions
Jinah *et al*. [21]	2023	60	F	Hypothyroidism, dyslipidemia	Pulmonary	Mycobacterial culture of pericardial fluid

CAPD: Continuous ambulatory peritoneal dialysis; mNGS: metagenomic next-generation sequencing; MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass; WGS: whole-genome sequencing; BALF: bronchoalveolar lavage fluid; NR: not reported.

patient repeatedly inhaled the aerosol containing MPG in a closed sweat-steaming environment, which coupled with local ischemia and poor drainage of the stump of the resected lung led to the occurrence of MPGPD.

The imaging features of NTM lung disease are similar to those of pulmonary tuberculosis, including nodular changes distributed along the bronchi, centrilobular nodules, bronchiectasis, patchy shadows, fibrous cavitation changes in the upper lobe, and other special types such as "hot tub lung" manifestation and generally no pleural effusion [25-27]. In a cohort study of 388 patients with NTM lung disease, 14 patients (3.6%) had solitary nodule/mass or mass-like consolidation on imaging [28]. A solitary nodule/mass or mass-like consolidation with obvious calcification at the local margin, as described in this MPGPD case, is extremely rare. These cases suggest that NTM lung disease can also present with solitary nodules/masses or mass-like consolidations, easily misdiagnosed as malignant tumors. To avoid inappropriate treatment, clinicians should be aware of this clinical presentation and take concerted steps to confirm the diagnosis.

The diagnosis of NTM lung disease, including MPGPD, has always been challenging in clinical practice. On the one hand, samples from the respiratory tract, such as sputum, bronchial lavage fluid, and bronchoalveolar lavage fluid, are prone to NTM contamination or colonization. It is easy to isolate contaminating bacteria such as MG, *Mycobacterium haemophilum*, and *Mycobacterium mucogenicum*, which may cause misdiagnosis [2,3,6,29,30]. NTM isolated from sterile lung tissue samples, such as lung biopsy, on the other hand, frequently indicates pathogenic bacteria. However, because of the limitations of the materials obtained, the positivity rate is low, which may lead to misdiagnosis. The traditional NTM culture method relies on bacterial growth and biochemical reactions, which usually take several weeks, and the results can be affected by many factors, posing a significant challenge to clinical timeliness [2,3,31].

Next-generation sequencing, which can sequence thousands to billions of DNA fragments independently and simultaneously, has become a popular technique for microbial detection in recent years. mNGS is particularly useful for diagnosing infections that are difficult to detect [32,33]. For respiratory system infection, the Infectious Diseases Society of China recommends mNGS of respiratory specimens if the pathogen cannot be identified within three days by traditional laboratory tests and empirical anti-infection treatment is ineffective [34] (A, II). The mNGS is gaining popularity as a viable alternative to routine bacterial culture for NTM [35,36]. According to the Tuberculosis Branch of the Chinese Medical Association, histological demonstration of granulomatous inflammation or acid-fast bacilli, plus positive NTM culture or molecular detection in biopsy specimens is confirmative of NTM disease [11]. The lung biopsy results of our patient qualified the above criteria, and the ddPCR results using the designed MPG-specific primers were positive, confirming that the patient had MPGPD. Of note, the American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) diagnostic criteria for NTM do not include molecular biology positivity. Many hospitals in China do not perform NTM culture because it is time-consuming and the positive rate is low. To reduce the rate of missed diagnosis, the Tuberculosis Branch of the Chinese Medical Association has added positive molecular biology as one of the diagnostic criteria for NTM.

The ATS/IDSA guidelines currently have not recommended any specific treatment plan for MPG

infection, but the most effective antibacterial drugs for MG infection *in vitro* include ethambutol, rifabutin, clarithromycin, linezolid, and fluoroquinolones [2]. In a recent study by Li *et al*., clinical isolates of MPG showed good sensitivity to drugs used to treat common NTM diseases [12]. Only two MPG strains were rifampicin-resistant, and all isolates were clarithromycin-sensitive [12].

We administered the quadruple oral regimen recommended in the guidelines for MG infection. The chest CT showed a significant reduction in the lesion after one month of treatment and almost complete resolution four months later, indicating that the ATS/IDSA protocol for MG infection also applies to the treatment for MPGPD.

Conclusions

MPGPD is a rare NTM infection. Individuals without immunodeficiency can also be infected under certain conditions. The imaging manifestations of MPGPD are diverse, and the lesions may show rapid development. Biopsy mNGS can enable quick diagnosis of MPG infection and is a good alternative to routine NTM microbial testing. The ATS/IDSA protocol for MG infection also appears to be an effective treatment for MPG infection.

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Consent for publication

Written informed consent for publication of the clinical details and the clinical images was obtained from the patient. A copy of the consent form is available for review by the editor of this journal.

Availability of data and materials

Please contact the author for data requests.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Conception and design: WWH and HMS; Administrative support: HMS; Provision of study materials or patients: WWH, WJW, ZXH, YLL, YS, BY, and HMS; Histomorphological description: QYX; mNGS detection and MPG-ddPCR primers design and detection: BY; Collection and assembly of data: WWH and HMS; Data analysis and interpretation: All authors; Manuscript writing: All authors; Final approval of manuscript: All authors.

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