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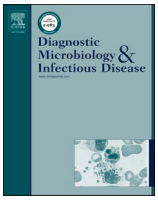

Diagnosis of *Histoplasma capsulatum* pulmonary infection by tNGS: a case report

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ABSTRACT

We report a case of a Chinese male patient with a history of riverside sampling work who presented with a cough and sputum production. Chest computed tomography revealed multiple lesions in both lungs, and bronchial examination indicated inflammatory changes. Histoplasmosis was identified through targeted next-generation sequencing (tNGS)detection of bronchoalveolar lavage fluid and diagnosed as *Histoplasma capsulatum* pneumonia. Lung biopsy and mNGS test further confirmed that the pathogen causing the pulmonary infection was *Histoplasma capsulatum*. Finally, the lesions were reduced and improved after three months of oral itraconazole and fluconazole (Diflucan) treatment. This case report highlights the advantages of tNGS technology, including rapid detection, broad coverage, high accuracy, and low cost. Therefore, tNGS can be a valuable supplement for diagnosing histoplasmosis and other pathogens.

1. Introduction

Histoplasma capsulatum is an environmental biphasic fungus with mold or mycelium and yeast morphology. It exists in the form of hyphae or microspores in the natural environment. After being inhaled into the lungs, it can reach the terminal bronchioles and alveoli and transform into yeast in neutrophils or alveolar macrophages [1]. The majority (approximately 90 %) of exposed patients remain asymptomatic or experience self-limited symptoms. The incubation period of acute onset is usually 1-3 weeks. The severity of the disease is closely linked to exposure to histoplasmosis, host immunity, pulmonary function, and extreme age. Histoplasmosis can be classified based on the frequency of onset (acute, subacute, and chronic), duration of onset (primary or recurrent disease), distribution (intrapulmonary, mediastinal, disseminated, and isolated extrapulmonary), and severity (asymptomatic, mild, and moderate to severe) [2]. Due to the diverse manifestations of histoplasmosis, diagnosis is often delayed.

Histoplasma capsulatumis can be divided into *Histoplasma capsulatum var.capsulatum* and *H.capsulatum var.duboisii*. The capsulatumis variety is found in all continents in the world, mainly in the Mississippi River and Ohio River Valley [3]. The duboisii variety exhibits an apparent regional epidemic, primarily found in Uganda, Nigeria, Zaire, Senegal, and other African countries, with infected patients mainly experiencing skin and bone involvement [4]. In China, histoplasmosis has not received sufficient attention due to the challenges of *Histoplasma capsulatumis* culture, limited diagnostic methods, and relatively few local cases. Researchers reviewed 525 cases of histoplasmosis in China between 1990 and 2011 and from 2012 to 2022 and found an increasing number of cases, with the epidemic spreading from the Yangtze River region to the southern and southwestern areas [5,6].

Histoplasmosis is a significant public health concern that requires control through appropriate preventive measures and timely treatment. It is recommended that multiple diagnostic approaches be employed for histoplasmosis, including laboratory, imaging, histopathological, microbiological, and serological evaluations [2]. The gold standard for the diagnosis of *Histoplasma capsulatum* is culture and pathological examination [7]. However, atypical radiological manifestations and low culture detection rates make accurate diagnosis challenging. Recently, metagenomic next-generation sequencing (mNGS) has been widely used in the diagnosis of pathogens of numerous infectious diseases, including respiratory infections. The mNGS offers the advantage of unbiased sequencing of all genetic material in samples, making it useful for

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detecting rare pathogens. However, it has the disadvantages of high cost and susceptibility to interference from the host genome. Pathogen-targeted next-generation sequencing (tNGS), which combines ultra-multiplex polymerase chain reaction (PCR) with high-throughput sequencing, is a cost-effective and cost-effective sequencing technique that enriches specific pathogens and is unaffected by the host genome. It can detect 206 pathogens (Supplementary Table 1) and covers >95 % of respiratory infections. The detection performance of tNGS and mNGS in bronchoalveolar lavage fluid samples is similar [8]. We present a case report of pulmonary infection caused by *Histoplasma capsulatum* using tNGS.

2. Case description

A 38-year-old male patient from Yunnan Province was hospitalized at a local hospital due to uninduced paroxysmal cough, production of yellow-green thick sputum in the morning, chest tightness, and shortness of breath after exercise. After eight days of anti-infective treatment with amoxicillin-clavulanate potassium, the symptoms of chest tightness and shortness of breath improved. However, the patient continued to experience a moderate amount of white, sticky sputum with coughing. Chest computed tomography (CT) indicated an increase in the severity of the lesions.

On August 17, 2023, the patient was transferred to Dehong Prefecture People's Hospital and admitted for further examination of bilateral lung lesions. The patient experienced elevated uric acid levels, occasionally consumed alcohol, and did not smoke. Chest CT revealed multiple lesions in the dorsal segment of the lower lobe of the right lung, the superior lingual segment of the upper lobe of the left lung, the dorsal segments of the lower lobe of the left lung, and the posterior and outer basal segment of the lower lobe of the left lung (Fig. 1A and 1B). Infection was suspected. Laboratory examination on August 18, 2023, revealed elevated levels of white blood cells (WBC) at 9.68 × 10^9/L, neutrophil percentage at 70.2 %, erythrocyte sedimentation rate at 59 mm/h, C-reactive protein (CRP) at 12.69 mg/L, total cholesterol at 5.26 mmol/L, low-density lipoprotein cholesterol at 3.39 mmol/L, fibrinogen at 4.66 g/L, and d-dimer at 0.71 mg/L. Bilirubin, liver enzymes, prothrombin time, and renal function were within normal ranges. Serological tests for HIV, hepatitis A, hepatitis B, hepatitis C, and venereal diseases were negative. Urine and stool tests were normal. Serological tests for common respiratory pathogens, including respiratory *syncytial virus, adenovirus, influenza virus A, influenza virus B, parainfluenza virus, Chlamydia pneumoniae, Legionella pneumophila, and Mycoplasma pneumoniae*, were also negative. Sputum culture results were negative. Tuberculosis-related tests, including γ-interferon and Xpert MTB/RIF were negative. Following admission, the patient was empirically treated with moxifloxacin due to elevated white blood cell, neutrophil, and CRP levels (Fig. 2). A bronchoscopic examination on August 21, 2023, revealed bronchial mucosal inflammation (Fig. 1C and 1D). Bronchoalveolar lavage fluid (BALF) was collected and sent for tNGS. On August 22, 139 reads of *Histoplasma capsulatum* were detected by tNGS (Supplementary Table 2). Upon inquiry into his life history, the patient revealed that he worked as a staff member of the Water Conservancy Bureau and conducted investigations and sampling at the riverside more than a month before the onset of the disease. Based on the tNGS results and the patient's occupational history, *Histoplasma capsulatum* was identified as the causative microorganism of the lung disease, and oral itraconazole treatment was initiated. From August 26, 2023, to September 14, 2023, the patient took itraconazole orally, 200 mg three times daily for the first three days, followed by 200 mg twice daily for the next three days.

The chest CT scan of the patient on September 17, 2023, was compared with that from August 17, 2023. The results indicated that the lesions in the anterior segment of the upper lobe of the left lung were slightly enlarged, with increased solid components. The number of lesions in the dorsal segment also increased, and the left pleura thickened, accompanied by a small amount of pleural effusion (Fig. 1E and 1F). Bronchial inflammatory changes were observed via bronchoscopy (Fig. 1G and 1H). BALF was collected for mNGS, which did not detect *Histoplasma capsulatum*, revealing only respiratory colonization bacteria (Supplementary Table 3). Laboratory examination displayed an elevated WBC count of 11.58 × 10^9/L, an erythrocyte sedimentation rate of 30 mm/h, CRP at 9.63 mg/L, interleukin at 616.07 pg/mL, fibrinogen at 4.66 g/L, and a galactomannan (GM) test result of 0.88. Since there was

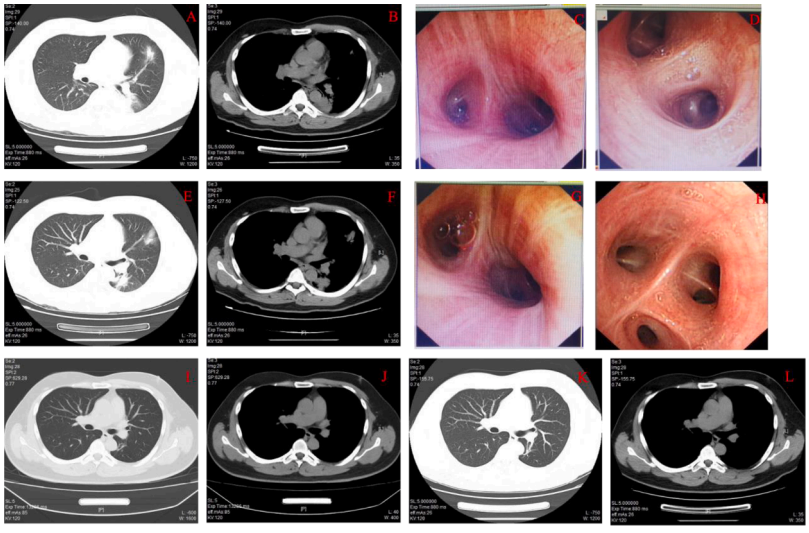

Fig. 1. Chest CT scan and bronchoscopy examination of the patient. (A-B) On August 17, 2023, the pulmonary window and mediastinal window display multiple lesions in the dorsal segment of the right lower lung lobe, the superior lingual segment of the left upper lung lobe, the dorsal segment of the left lower lung lobe, and the posterior and lateral basal segments of the left lower lung lobe. (C-D) On August 21, 2023,inflammatory changes in the left upper lobe bronchitis. (E-F) About half a month, the pulmonary and mediastinal windows depict a slightly enlarged lesion area in the anterior segment of the left upper lobe, with an increase in solid components. (G-H) Inflammatory changes in the left upper lobe bronchitis. (I-J) Three month later, the pulmonary window indicates that the lesions in the lingual segment and lower lobe of the left lung have decreased less than before, with a small amount of fibrous foci in the apicoposterior segment of the left upper lung lobe. The mediastinal window appears normal. (K-L) On May 2, 2023,the pulmonary window indicates that the lesions in the left lung have been absorbed, and the mediastinal window appears normal.

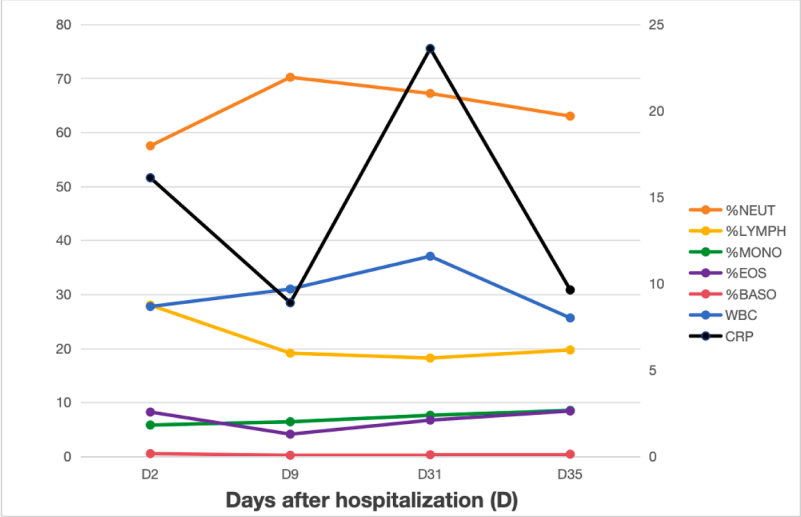

Fig. 2. Changes in blood routine and CRP. Changes in blood routine tests based on the episode of treatment: curves of neutrophils percentage, lymphocytes percentage, monocytes percentage, eosinophils percentage, basophils percentage, WBC, and CRP.

no improvement after the treatment for *Histoplasma capsulatum*, a lung biopsy was recommended for the patient. Lung tissue samples were obtained via percutaneous puncture and examined through pathology and mNGS. Pathological results revealed that chronic inflammation with granulomatous inflammation and the presence of fungi (Fig. 3A-F). The mNGS analysis of the lung puncture tissue detected 116 reads of *Histoplasma capsulatum*(Supplementary Table 4 and Supplementary Fig. 5). Based on the combined findings from tNGS, mNGS, and histopathological examination, the patient was diagnosed with *Histoplasma capsulatum* pneumonia. Due to the poor response to itraconazole, the patient was switched to oral fluconazole (Diflucan) capsules at 0.45 g once daily for eight weeks, starting from September 30, 2023.

After three months of antifungal treatment for histoplasmosis, the chest CT scan on November 24, 2023, displayed a reduction in lesions in the lingual segment and lower lobe of the left lung, with the cord shadow in the lower lobe of the right lung being absorbed. A few fibrous foci were also observed in the posterior segment of the upper lobe of the left lung (Fig. 1I and 1J). During follow-up until May 2, 2024, the patient continued to receive fluconazole (Diflucan) treatment at 450 mg once daily, with relief from the cough symptoms and no other discomfort. Further absorption of lesions was noted in the subsequent chest CT (Fig. 1K and 1L).

3. Discussion

To our knowledge, this is the first reported case of *Histoplasma capsulatum* pneumonia diagnosed using tNGS. The patient's clinical symptoms included coughing with sputum, chest tightness, and shortness of breath after exercise, with chest CT revealing multiple lung lesions consistent with the characteristics of *Histoplasma capsulatum* infection. However, traditional diagnostic methods made achieving a timely and accurate diagnosis challenging. Five days after admission, *Histoplasma capsulatum* was detected in BALF through tNGS, which enabled the rapid identification of the pathogen and timely treatment initiation. The use of tNGS played a crucial role in this case.

Clinically, the most common symptoms of pulmonary histoplasmosis include coughing, difficulty breathing, discomfort, fever, and chills. It is often misdiagnosed as bacterial pneumonia or viral respiratory disease [9]. Chronic pulmonary histoplasmosis is characterized by shortness of breath and frequent coughing, accompanied by fever, weight loss, and night sweats. Chest CT typically reveals interstitial fibrosis and pleural thickening, developing from focal or diffuse infiltration, which must be

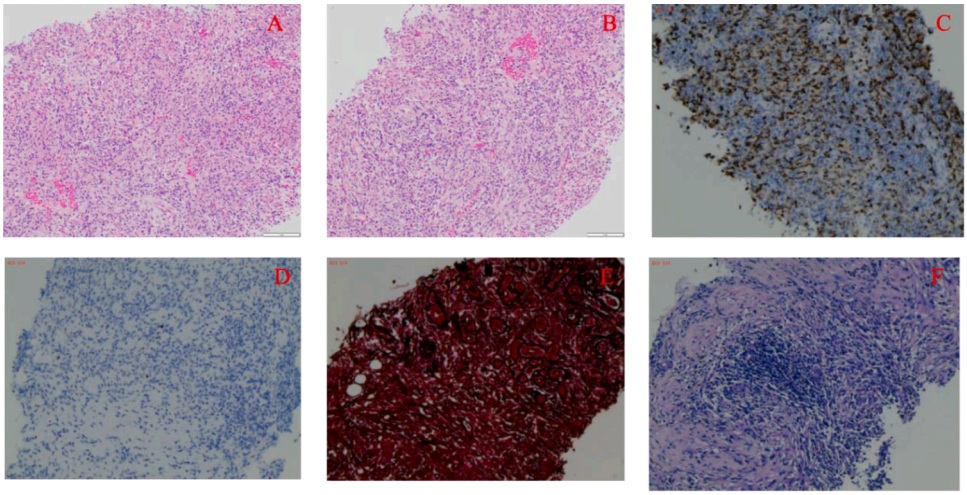

Fig. 3. Histopathological evaluation. (A-B) *He*matoxylin-eosin stain. (C) CD68. (D) S-100 stain. (E) Periodic Acid-Schiff stain (PAS). (F) Gomori-methenamine silver stain (GMS). A, B and C suggests acute and chronic granulomatous inflammation of the lung tissue in the lower lobe of the left lung. D indicates that s-100 negative excludes tumor-related lesions. E and F show the presence of fungi.

differentiated from pulmonary tuberculosis [10]. In disseminated histoplasmosis, the reticuloendothelial system (liver, spleen, bone marrow, and lymph nodes), gastrointestinal tract (particularly the oral mucosa), central nervous system (causing focal lesions or diffuse meningitis), and adrenal glands (leading to adrenocortical insufficiency) are the most common sites of infection [2,11,12]. Clinical manifestations include fever, hepatosplenomegaly, and lymph node enlargement. Unlike other fungal infections, the skin is a rare site of extrapulmonary infection in histoplasmosis, except in patients with advanced HIV.

Histoplasma capsulatum mainly exists in damp soil containing large bird droppings or bat guano, especially in areas with moderate temperatures and rivers flowing through. Therefore, climate, humidity, and soil characteristics such as high nitrogen content favor the growth of Histoplasma capsulatum [10,13]. In specific environments, such as chicken coops, construction sites, or during activities such as excavation, demolition, and cave exploration, the risk of infection increases [10]. This case occurred in the Dehong region of Yunnan, which has a subtropical climate. The patient had visited the riverside for sampling and investigation more than a month before the onset of the disease, during which time they were exposed to a damp soil environment. This aligns with the typical epidemiological history of histoplasmosis.

Pathological examination is the gold standard for diagnosing Histoplasma capsulatum and can reveal the characteristic pathological changes of histoplasmosis. Tissue responses vary depending on the duration of infection and the degree of lesion. In older lesions, Histoplasma balls or calcified nodules containing a small number of pathogens are typically surrounded by fibrosis. In non-acute disseminated cases, epithelioid cell granulomas form. Phagocytes and neutrophils may contain spores, though their number and size vary. In patients with acute disseminated infection, there is extensive infiltration of histiocytes in the lungs, liver, spleen, bone marrow, and lymph nodes. Disseminated infections often reveal yeast-form Histoplasma capsulatum in bone marrow and liver tissue. The identification of 2-4 μm oval micro-yeasts in tissue biopsy can aid in rapid diagnosis. Periodic acid-Schiff (PAS) stain, Gomori methenamine silver (GMS) stain, and Wright-Giemsa stain of pathological sections can all detect Histoplasma capsulatum [14,15]. In this case, hematoxylin and eosin staining and CD68 staining of the lung biopsy tissue from the patient's left lower lung lobe indicated acute and chronic granulomatous inflammation. Positive PAS and GMS staining revealed the presence of fungi. Combined with the results of BALF tNGS and lung tissue mNGS, the pulmonary infection caused by Histoplasma capsulatum was confirmed.

There are two kinds of antifungal agents in the treatment of histoplasmosis [16]. One is azole drugs, which inhibit the biosynthesis of necessary ergosterols in fungal cell membranes. Itraconazole, voriconazole, and posaconazole are considered to be tissue cytoplasmic fungicides, but fluconazole (Diflucan) is considered to have only fungal inhibitory activity. The second type is the polyene compound, amphotericin B (AmB), which forms a complex with ergosterol in the fungal plasma membrane, causing membrane rupture and eventually leading to cell death. In this case, the patient initially received 200 mg of itraconazole three times a day for the first three days, followed by 200 mg twice a day for 20 days, but indicated no signs of improvement or worsening. To confirm the diagnosis of histoplasmosis detected tNGS, the pathogen was re-confirmed through lung biopsy pathology and mNGS. The treatment plan was then adjusted to oral fluconazole (Diflucan) capsules 0.45 g once daily for eight weeks, which led to improvement in the pulmonary lesions. While itraconazole is the first-line treatment for mild to moderate histoplasmosis, it was ineffective in this case, which warrants further consideration. In China, patients with histoplasmosis are commonly treated with fluconazole (Diflucan) or itraconazole; however, statistics indicate that fluconazole (Diflucan) is less effective than itraconazole, with a higher recurrence rate in patients treated with fluconazole (Diflucan) compared with those treated with itraconazole.

4. Conclusion

In summary, China is emerging as a new epidemic area for histoplasmosis, with the number of cases increasing annually and the geographic range of the epidemic expanding. For patients with a history of fieldwork and pulmonary lesions in tropical and subtropical climates, histoplasmosis should be considered and investigated. Furthermore, tNGS offers advantages such as rapid detection, broad coverage, high accuracy, and cost-effectiveness. This technique has proven to be highly effective in detecting difficult-to-culture organisms, atypical manifestations, and uncommon pathogens and holds significant potential for histoplasmosis diagnosis.

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Institutional review board statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Dehong Prefecture People's Hospital (protocol code DYLL-KY2024004 and 1 April 2024).

Informed consent statement

Written informed consent has been obtained from the patient to publish this paper.

Data availability statement

Data are contained within the article.

Author statement

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

CRediT authorship contribution statement

Yong-min Yang: Writing – original draft, Investigation, Methodology, Writing – review & editing. **Lian-jing Chai:** Writing – review & editing, Investigation, Methodology. **Rui-feng Zhang:** Investigation, Methodology. **Yu-juan Fan:** Investigation, Methodology. **Chen-Xue Mao:** Project administration. **Ai-lian Ding:** Project administration.

Declaration of competing interest

Chen-Xue Mao, Yong-min Yang,Rui-feng Zhang and Yu-juan Fan are employed by Kunming Kingmed Institute for Clinical Laboratory Co., Ltd.The authors declare that they have no potential conflicts of interest that might be relevant to the content of this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2025.116892.

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