Pneumonia Caused by Lichtheimia Ramosa During the COVID-19 Pandemic in Hubei Province, China: A Case Report and Literature Review

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Case report

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Abstract

Background

Mucor infection cannot be ignored in patients with pulmonary shadowing with cavitation. This paper reports a case of mucormycosis during the COVID-19 pandemic in Hubei Province, China.

Case Presentation

A anesthesiology doctor was initially diagnosed as COVID-19 due to changes in lung imaging. Later Lichtheimia ramose was found by Metagenomic next generation sequencing (mNGS) in the Bronchoalveolar lavage uid (BALF). After adjusting amphotericin B for anti-infective treatment, the patient's infection lesions were shranked and the symptoms were significantly relieved.

Conclusion

The diagnosis of invasive fungal infections is very difficult, mNGS can make an accurate pathogenic diagnosis of invasive fungal diseases for the clinic and provide a basis for clinical treatment.

Background

Recently, COVID-19 is still pandemic in worldwide. India has reported multiple cases of COVID-19 combined with mucormycosis (MM) (1). Mucormycosis is an uncommon opportunistic infection caused by filamentous fungus, with a high degree of morbidity & mortality (2, 3).

Lichtheimia ramosa is a fungal organism belonging to the genus Lichtheimia, Mucor and Zygomycetes. People with low immune function are susceptible (4), which can lead to mucormycosis, which is characterized by mycelia invading blood vessels, causing thrombosis and necrosis, and can lead to lesions in the nose, brain, digestive tract and respiratory tract (5). Mucor fungi rarely infect hosts with normal immune function (6). Because of the rapid onset and rapid progress of mycosis, the positive rate of traditional detection methods is low, the general antibacterial treatment is ineffective, the prognosis is poor and the mortality rate is high, and the diagnosis of the disease is usually made clear by autopsy after death, so it is of great significance to determine the pathogen as early as possible to improve the prognosis and reduce the mortality rate of patients.

In recent years, mNGS screening technology for pathogen has developed rapidly, with the high throughput and speed. Theoretically, it can theoretically detect the characteristics of all known pathogens, and identify the pathogens of critical and intractable infectious diseases in an early, rapid and accurate manner, providing a basis for clinical treatment (7). A case of Mucormycosis assisted by mNGS was reported, in order to provide some experience for clinical diagnosis and treatment of invasive fungal diseases.

Case Presentation
The patient, male, 51 years old, medical worker in the Department of Anesthesiology, was admitted to the Department of Respiratory Medicine of the First People's Hospital of Tianmen City for treatment on September 21, 2020 due to "intermittent chest pain and cough for more than 5 months".

The patient was admitted to Department of Infectious Diseases of our hospital for treatment due to 'chest pain for 3 days' on March 26, 2020. (On March 20, 2020. At this moment, All COVID-19 patients in Tianmen City, Hubei Province were cleared.)

Physical examination: T: 36.5 °C, P: 87 times/min, R: 18 times/min, BP: 129/85 mmHg, SpO2: 98%, the pharynx was not red, bilateral tonsils were not large, superficial lymph nodes were not large, respiratory sounds in both lungs were rough, and no rales of dry or wet were heard. Past history: history of acute renal insuciency in 2016; No history of hypertension, coronary heart disease, diabetes, tuberculosis, hepatitis; Has a history of allergy to ginseng wheat. Since the onset of the disease, appetite loss, slightly poor spirit.

The patient had been tracheally intubated in a patient with COVID-19. The clinical diagnosis was "viral pneumonia (COVID-19 cannot be ruled out)", based on medical history, physical examination and ancillary tests. The patient was treated with anti-infective (moxifloxacin), anti-viral (Abidol) and symptomatic supportive treatment. 12 days in hospital, chest pain, chest tightness, shortness of breath, cough and other symptoms relieved and discharged.

Due to 'chest pain and discomfort', accompanied by chest sulking and short breath after activities", the patient was hospitalized for many times, with poor results. Later, the patient was transferred to the First Affiliated Hospital Of Guangzhou Medical University.

Auxiliary examination: The patient was admitted to the hospital on March 26 for routine blood tests with a slightly high neutrophil ratio (78.5%, normal range: 40%-75%) and a slightly low lymphocyte ratio (14.70%, normal range: 40%-75%). Interleukin-6 (IL-6) was 28.68 pg/ml (reference range: <7 pg/ml); liver and kidney function, coagulation function, ESR, respiratory viruses were not significantly abnormal, and SARS-CoV-2 was negative. The patient’s blood count began to rise in April (Table 1). During the course of the disease, the changes in the patient's blood count are detailed in Table 1.

Blood T-SPOT negative, Fiberoptic bronchoscopy: old bloodstains can be seen at the opening of the upper right lobe of the lung. Cryptococcus antigen negative, Aspergillus antigen detection was 5.6ug/L (normal range < 0.5 ug/L), X-pert TB-DNA negative, Nine respiratory tract infection pathogens IgM antibody negative, 1-3-β-D-glucan test(G test) and Galactomannan test(GM test) was negative. Patient’s BALF was tested by mNGS ( 

\[ \text{Lichtheimia ramose}: 260 \text{ reads, Relative abundance: 52.95\%} \] 

on September 3 (Fig. 3). For further confirmation, Patient’s BALF was tested by PCR (Mucorales DNA: positive, cycle threshold (ct) = 24.61, normal range: <45)
Table 1
Changes in blood routine, C-reactive protein, and ESR during hospitalization

| Test items       | Reference range | Mar-26 | Mar-27 | Apr-3 | Apr-24 | Aug-18 | Aug-29 | Sep-23 |
|------------------|-----------------|--------|--------|-------|--------|--------|--------|--------|
| WBC(×10^9/L)     | 3.5–9.5         | 8.02   | 6.76   | 5.57  | 5.16   | 10.54  | 7.54   | 6.02   |
| NEU# (×10^9/L)   | 1.8–6.3         | 6.29   | 5.36   | 3.98  | 3.61   | 9.42   | 5.87   | 4.45   |
| NEU%             | 40–75           | 78.50  | 79.20  | 71.40 | 69.90  | 89.30  | 77.90  | 74.00  |
| LYM# (×10^9/L)   | 1.1–3.2         | 1.18   | 0.83   | 0.84  | 0.68   | 0.67   | 1.13   | 0.83   |
| LYM%             | 20–50           | 14.70  | 12.30  | 16.90 | 13.20  | 6.40   | 15.00  | 13.80  |
| CRP(mg/L)        | ≤ 6             | 9.99   | 11.32  | 5.15  | 8.96   | 35.08  | 5.77   | 35.94  |
| ESR(mm/h)        | 0–20            | -      | 11     | 68.2  | -      | 56     | 30     | 45     |

On March 26, the first computed tomography (CT) at the first admission (Fig. 1.A) The chest CT suggested infectious lesions in both lungs, with multiple patchy ground glass shadows with blurred margins in both lungs, with more obvious lesions in the right lower lung and bilateral pleural thickening and adhesions. The patient was readmitted on April 23, a repeat chest CT (Fig. 1.B) suggested that multiple patchy and striated high-density foci were seen in both lungs, with blurred margins of some of the foci, and the foci in the lower lobe of the right lung were slightly larger than before, with new small patchy ground-glass density foci in the anterior segment of the upper lobe of the right lung, and the foci in the lower lobe of the left lung were slightly reduced in size. A repeat chest CT (Fig. 1.C) on May 29 suggested that multiple patchy and striated high-density foci were seen in both lungs. On August 29, a repeat chest CT showed multiple patchy and striated high-density foci in both lungs, with a slight reduction in the extent of the foci in the lower lobe of both lungs, new ground glass-like and patchy solid high-density foci in the upper lobe of the right lung (Fig. 1E) and the lower lobe of the left lung (Fig. 1D), with poorly defined borders. On October 1, the chest CT (Fig. 1F) showed partial cavity formation in the right upper lung and a small amount of fluid in the right pleural cavity. On November 30, the chest CT (Fig. 1G) showed the cavity formation in the right upper lung was slightly smaller than before, and the right pleural effusion had basically disappeared. H, On May 27, 2021, the chest CT (Fig. 1H) showed showed a partially reduced lesion in the upper lobe of the right lung and the cavity in the lesion was reduced.

Pathology and etiological examination: CT-guided percutaneous lung biopsy was performed on April 24, lung biopsy tissue submitted for pathological examination and pathogenic examination. Tissue biopsy revealed: coagulative necrosis of some alveolar tissue (Fig. 2A), fibrin-like exudates in tissue cells in the alveolar cavity, and fibers and blood vessels at the edges of necrotic alveolar tissue Hyperplasia (Fig. 2B), Fungal fluorescence staining (negative) (Fig. 2). The tissue was stained to find bacteria and fungi:
bacteria, fungal hyphae and spores were not found; tissue culture (common bacteria, anaerobic bacteria, fungi): no bacterial growth.

On September 21, the patient was transferred to the First People's Hospital of Tianmen City (Respiratory and Critical Care Medicine Department) for continued treatment. Based on the patient's medical history, clinical presentation, mNGS, and Based on the patient's history, clinical manifestations, mNGS, CT and other examination results, the patient was considered to have a pulmonary fungal infection with Lichtheimia ramosa as the pathogenic organism, and was treated with amphotericin B antifungal therapy at a dose of 30 mg/10h intravenously pumped. During the hospitalization, the blood potassium was low, and the urea and creatinine were increased (Table 2), and symptomatic treatments such as potassium supplementation and kidney protection were performed. On October 12, Examination of chest CT showed that most of the lesions were reduced in size, and the upper right lung cavity was reduced (Fig. 1F). The patient's cough and chest tightness were relieved, he was discharged and amphotericin B was continue oral at outpatient service.

Table 2
Changes in serum potassium, urea, and creatinine during hospitalization

| Test items  | Reference range | Aug-29 | Sep-23 | Sep-25 | Sep-27 | Sep-29 | Oct-1 | Oct-4 |
|------------|-----------------|--------|--------|--------|--------|--------|-------|-------|
| K (mmol/L) | 3.5–5.3         | 4.61   | 3.22   | 3.02   | 2.72   | 2.91   | 3.34  | 3.56  |
| Urea (mmol/L) | 3.1-8          | 4.55   | 8.63   | 7.73   | 7.25   | 6.35   | 8.11  | 8.75  |
| CREA (mmol/L) | 57–97           | 90.1   | 140.1  | 132.7  | 136.4  | 127.1  | 163.4 | 138.3 |

Discussion

Mucor fungi are widely in the environment including soil, manure, grasses and air (8). Autopsy studies have shown that Mucor is the third most common fungus in invasive fungal infections (9, 10). In Europe, the incidence of Mucor mycosis is increasing, among which Rhizopus is the most common (11). Mucormycosis is a kind of systemic conditional pathogenic fungal infection with rapid onset, rapid progression and high mortality. People with low immune function are susceptible to infection, especially those with diabetes, leukemia, immunodeficiency diseases and malignant tumors. Large doses of antibiotics, corticosteroids are the most vulnerable to infection (12). At first, the disease mainly occurs in nasal mucosa or sinuses, and then extends to orbital soft tissue, facial palate and brain. It can also spread throughout the body, with a poor prognosis. Lung infection by Mucor fungi is a rare case (13). In this case, the patient was in good health and had no history of hypertension, coronary heart disease, diabetes, tuberculosis and other chronic medical history, and did not use large doses of antibiotics and hormones, so he was not a group susceptible to Mucor fungi. There was no obvious abnormality in the orbital and central nervous system. Routine blood examination showed a slightly higher ratio of neutrophils and a slightly lower ratio of lymphocytes, and there was no significant change in the whole course of the disease. C-reactive protein and erythrocyte sedimentation rate did not change significantly.
in the early morning, but increased significantly in the late stage (see Table 1); chest CT (Fig. 1.A) indicated infective lesions in both lungs; The patient's pathologic features and clinical manifestations are atypical.

The diagnosis of invasive fungal disease usually relies on fungal culture and biopsy, but its low positivity rate often causes patients with mycosis to miss the optimal treatment time (14). Autopsy is too late for clinical treatment (15, 16). Galactomannan(GM) test is a routine method for clinical detection of fungal antigens(17), but Mucor does not produce antigenic substances, so GM test is not helpful for the diagnosis of Mucor. Compared with the traditional genetic testing methods, NGS testing has obvious technical advantages and extensive clinical applications. NGS can generate high-throughput sequencing data covering specific regions of the genome (from several genes to hundreds of genes to whole exome or whole genome) at one time (18), and can simultaneously detect the DNA/RNA sequences of various bacteria, viruses, fungi or parasites with high accuracy (19). The cost of mNGS testing is relatively high, but compared with traditional testing methods, the average cost of single gene and single locus testing is lower, and most patients can afford it. mNGS has high sensitivity and is helpful for the detection of unknown pathogens, which is superior to the traditional clinical testing methods. mNGS can provide direct clues for accurate diagnosis of complex infections. For the diagnosis of invasive fungal infection, the detection cycle of fungal culture (the traditional "gold standard") is long and the positive rate is low. The results of G test and GM test lack specificity and poor sensitivity. In this case, tissue fungal fluorescent staining, tissue culture, and tissue Gram staining were all negative. The pathogen of infection could not be identified. Although the patient's SARS-CoV-2 test was negative, we administered antiviral and bacterial therapy based on the patient's history, clinical manifestations, and examination results, with poor results. In this case, the patient was from Tianmen, Hubei Province, who developed right chest pain in March 2020. The pain worsened after exercise, shortness of breath was obvious, and there was no headache, fever, cough and sputum. At the moment of COVID-19 epidemic, the patient was a medical worker in Hubei Province, whose chest CT showed infectious lesions (Fig. 1A). According to the history, clinical manifestations and CT examination results, antiviral and anti-infective treatment was performed, with poor efficacy. Later, the patient was hospitalized for "chest pain" for several times. According to the patient's condition, he was given a variety of anti-infection treatment regimens. The patient's condition showed no significant improvement, but worsened in August, with ground glass and pleural effusion (Fig. 1D), chest pain and discomfort worsened, accompanied by chest sulkiness and shortness of breath after activities (20). Considering the high mortality rate of trichinosis, mNGS can be used as a routine test(21). In this case, mNGS was used to detect its BALF, and the results suggested Lichtheimia ramosa infection with undetected drug-resistant genes, and the patient was treated with the choice of amphotericin B. The patient's bilateral lung lesions were reduced (Fig. 1F,1G), the chest pain symptoms were alleviated, and the cough was completely relieved with significant efficacy. Amphotericin B can cause pharmacological hypokalemia and renal damage, and the patient's blood potassium was low and urea and creatinine were increased several times during hospitalization (Table 2), suggesting that the clinic should regularly test the patient's electrolytes and renal function changes, and pay attention to potassium supplementation and renal protection. mNGS results were negative when the patient
underwent percutaneous puncture lung tissue biopsy in April 2020, suggesting that the selection of BALF specimens for mNGS testing can improve the positive rate.

**Conclusion**

Due to the rapid onset, rapid progression and high mortality of mycosis, early diagnosis and selection of sensitive antifungal agents are the keys to a good prognosis (22). This paper reports a case of Lichtheimia ramosa, a pathogen of unknown pulmonary infection, which was identified by mNGS test, and its treatment regimen was adjusted according to the mNGS test report, and a good curative effect was obtained. Because of the difficulty in diagnosing invasive fungal infections, mNGS should be a routine test for patients with pneumonia of unknown cause, especially those with high suspicion of mycosis. Based on the mNGS results, the actual infectious pathogens can be determined, and sensitive drugs can be selected to improve the cure rate of mycosis. In conclusion, mNGS can provide accurate and rapid etiological diagnosis for invasive mycosis in clinical practice, and provide therapeutic basis for clinical treatment.

**Abbreviations**

mNGS: Metagenomics next-generation sequencing; BALF :Bronchoalveolar lavage fluid ;WBC: White blood cell;

NEU#: neutrophil count; LYM# :lymphocyte count; NEU%: neutrophil ratio; LYM%: lymphocyte ratio; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; CREA: creatinine; CT: Computed tomography.

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Written informed consent was obtained from the patient for publication of this Case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

**Availability of data and materials**

Data and materials are available.

**Competing interests**

The authors declare that they have no competing interests.

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Authors’ contributions

QJS, SHL, JBZ, JYT contribute to thesis selection and design, data collection; YFH participate in data analysis and interpretation; PYC and FWZ contributes to critical review of the intellectual content of an article; QJS and SHL contribute to the manuscript writing. HW Provide funding support. QJS draft the manuscript and HL provide language modification service.

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**Figures**
CT examination results of the patient's chest:

A, On March 26, the chest CT suggested infectious lesions in both lungs, with multiple patchy ground glass shadows with blurred margins in both lungs, with more obvious lesions in the right lower lung and bilateral pleural thickening and adhesions.

B, The patient was readmitted on April 23, suggested that multiple patchy and striated high-density foci were seen in both lungs, with blurred margins of some of the foci, and the foci in the lower lobe of the right lung were
slightly larger than before, with new small patchy ground-glass density foci in the anterior segment of the upper lobe of the right lung, and the foci in the lower lobe of the left lung were slightly reduced in size. C, On May 29, the chest CT suggested that multiple patchy and striated high-density foci were seen in both lungs. D, On August 29, the chest CT suggested infectious lesions in both lungs, with multiple patchy ground glass shadows with blurred margins in both lungs, with more obvious lesions in the right lower lung and bilateral pleural thickening and adhesions. showed multiple patchy and striated high-density foci in both lungs, new ground glass-like and patchy solid high-density foci in the lower lobe of the left lung. E, On August 29, new ground glass-like and patchy solid high-density foci in the upper lobe of the right lung with poorly defined borders. F, On October 1, showed partial cavity formation in the right upper lung and a small amount of fluid in the right pleural cavity. G, On November 30, the chest CT showed the cavity formation in the right upper lung was slightly smaller than before, and the right pleural effusion had basically disappeared. H, On May 27, 2021, the chest CT showed a partially reduced lesion in the upper lobe of the right lung and the cavity in the lesion was reduced.

![Figure 2](image)

**Figure 2**

Pathology of lung biopsy tissue: A, Coagulative necrosis of some alveolar tissue; B, fibrin-like exudates in tissue cells in the alveolar cavity, and fibers and blood vessels at the edges of necrotic alveolar tissue Hyperplasia.
Figure 3

mNGS result of Patient's BALF: A, Coverage and Sequencing depth. B, Sequencing quality

Supplementary Files

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