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Case report

Rhizopus microsporus and Mucor racemosus coinfection following COVID-19 detected by metagenomics next-generation sequencing: A case of disseminated mucormycosis

Lihan Hai [a], Peihong Li [b], Zheng Xiao [c], Jinxia Zhou [b], Bo Xiao [b], Luo Zhou [b,d,*]

[a] Department of Neurology, Xing'an League People's Hospital, Ulanhot, Inner Mongolia, China
[b] Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, China
[c] Department of Pathology, First Hospital of Changsha, Changsha, Hunan, China
[d] National Clinical Medical Research Center for Geriatric Diseases (Xiangya Hospital), Central South University, Changsha, Hunan, China

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ABSTRACT

Mucormycosis is an invasive opportunistic fungal infection, which may be lethal and mostly affects patients with immunodeficiency or diabetes mellitus. Among Mucorales fungi, Rhizopus spp. is the most common cause of mucormycosis, followed by genera such as Mucor and Lichtheimia. Here we report a patient with severe COVID-19 infection who developed nasal pain, facial swelling, prominent black eschar on the nasal root. CT scan revealed pansinusitis along the maxillary, ethmoidal, and sphenoid sinuses. Mixed mold infection with Rhizopus microsporus and Mucor racemosus was detected by blood metagenomics next-generation sequencing (mNGS) and later nasal mucosa histological investigation confirmed mucormycosis. Severe COVID-19 infection led to the patient's thrombocytopenia and leukopenia. Later disseminated mucormycosis aggravated the infection and sepsis eventually resulted in death.

It is the first case report of mucormycosis in which R. microsporus and M. racemosus as the etiologic agents were found simultaneously in one patient. COVID-19 infection combined with disseminated mucormycosisis can be fatal and mNGS is a fast, sensitive and accurate diagnostic method for fungi detection.

1. Introduction

The global epidemic of coronavirus disease 2019 (COVID-19) continues to be a major health issue worldwide. The most common symptoms are fever, dry cough, fatigue, and shortness of breath and sometimes in severe cases, the disease leads to acute respiratory distress syndrome. According to the World Health Organization, disease severity is determined as mild, moderate, severe, or critical. COVID-19 can lead to severe fungal co-infections in patients with underlying health conditions, and previous reports have documented the occurrence of associated mucormycosis in severe cases.

Mucormycosis is an invasive opportunistic fungal infection, mostly affecting immunocompromised patients, such as those with uncontrolled diabetes and long-term use of corticosteroids. There is an approximately 46% mortality rate in the patients infected [1]. Among Mucorales fungi, Rhizopus spp. is the most common cause of mucormycosis, followed by genera such as Mucor and

* Corresponding author. Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, China.
E-mail addresses: zhouluo33@163.com, zhouluo@csu.edu.cn (L. Zhou).

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Lichtheimia, accounting for 70–80% of all mucormycosis cases [2]. It is reported that in all those cases only 50% of fungal cultures were positive [3]. Moreover, in many clinical reports the pathogens of mucormycosis cases were not identified to the genus or species level.

Compared to fungal culture, mNGS is a relatively precise and fast method to obtain pathogenic results. Here we report a severe COVID-19 patient diagnosed with mucormycosis confirmed by the metagenomics next-generation sequencing (mNGS) and histopathological findings. Based on the mNGS results, our case was positive for the presence of Rhizopus microsporus and Mucor racemosus.

2. Case report

A 67-year-old male patient with uncontrolled diabetes who experienced COVID-19 infection at the end of December 2022 was admitted to our hospital on January 1st , 2023, with shortness of breath and hypoxia.

On admission, he had a temperature of 36.5 °C, heart rate of 116, respiratory rate of 26, blood pressure of 150/88 mmHg, and O_2 saturation of 90% in room air. The laboratory examinations showed: white blood cell count (10^9/L): 2, creatinine (umol/L): 178, blood sugar (mmol/L): 11.6, hemoglobin A1C (%): 10.1, platelet count (10^9/L): 17, albumin (g/L):20.5, ferritin (ng/ml): > 2000, indicating inflammatory state with severe thrombocytopenia and leukopenia.

After admission, the patient was placed on Paxlovid against COVID-19. He wasn't treated with steroids because of high blood sugar. Other therapies also included granulocyte-macrophage colony-stimulating factor (GM-CSF) injection, use of gamma-globulin and Albutein, etc. The patient gradually improved with platelets increasing, and without hypoxia and shortness of breath. However, the patient suddenly progressed on January 11th when he complained the new symptom of nasal ache, along with dysnea and platelets decreasing again (Table 1). Physical examination showed facial and nasal swelling. Uncontrolled diabetes combined with COVID-19 infection made it easier for the patient to develop secondary fungal infection, so when the condition had worsened abruptly, we had a lot of microbiological workups done, including blood, lower respiratory tract sputum, nasal secretion and urine cultures. All came back negative. The mNGS of blood was underwent On January 11th. Since highly suspected of fungi infection, though β-D-Glucan (G) and Galactomannan (GM) tests of blood sample remained negative, the patient was treated with liposomal amphotericin B at a daily dosage of 250mg on January 13th, later on which day the result of mNGS came back, indicating a total of 22 sequence reads of Rhizopus microsporum and 19 reads of Mucor racemosus that accounted for 0.16% and 0.13% of all reads respectively (Supplementary Material).

The next day black eschar on the patient's nasal root was observed (Fig. 1, a). A noncontrast Computed tomography (CT) scan of the head was then conducted which revealed pansinusitis along the maxillary, ethmoidal, and sphenoid sinuses (Fig. 1b and c). CT scan of the lungs showed bilateral lung infiltrations consistent with pulmonary mucormycosis (Fig. 1, d). Subsequently the diagnosis of mucormycosis was further reconfirmed by histopathological evaluation of tissue samples from nasal mucosa (Fig. 1e and f). Platelet, cryoprecipitate and plasma transfusion were administered given his thrombocytopenia with hemorrhagic tendency. Continuous renal replacement therapy (CRRT) was performed in view of acute kidney dysfunction accompanied by severe metabolic acidosis and sepsis. Adequate management of the hyperglycemia and metabolic acidosis with intravenous fluids and continuous insulin infusion was initiated. Unfortunately, the patient rapidly aggravated with respiratory and circulatory failure. Norepinephrine, terlipressin and

Table 1
Laboratory results of the patient from admission (01.01.23), condition improved (09.01.23), suspected mucor diagnosis (11.01.23), and after treatment (16.01.23).

Date	01.01.23	09.01.23	11.01.23	16.01.23	Normal range
Blood cell count					
Red cell count	3.69	3.56	3.37	3.32	4.3–5.8 × 10^12/L
Hemoglobin	100	98	91	90	130–175g/L
White cell count	2.0	1.8	1.3	33.0	3.5–9.5 × 10^9/L
Platelets	17	81	28	9.0	125–350 × 10^9 / L
inflammatory markers					
CRP	106	89.8	106	123.0	0–8mg / L
Ferritin	> 2000	–	1543.0	4144.0	30–400ng / mL
Procalcitonin	3.97	4.827	15.14	34.07	0–0.1ng / ml
Kidney function					
Creatinine	178	336.0	355.9	410	41.0–111.0 μmol / L
BUN	10.5	24.33	29.48	38.22	3.60–9.50mmol / L
Arterial blood gas					
PH	–	7.34	7.32	7.12	7.350–7.450
PCO_2	–	25	18	30	35–45 mmHg
PaO_2	–	124	149	121	80–100 mmHg
Glucose	–	16.2	14	22.3	3.9–5.8mmol/L
hemoglobin A1C	10.1	–	–	–	4–6%
BS	11.6	–	–	–	3.9–6.1mmol/L
albumin	20.5g	22.1	16.6	19.1	40.0–55.0g/L

Abbreviations: CRP, c-reactive protein; BUN, blood urea nitrogen; PH, pondus hydrogenii; PCO_2, partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; BS, blood sugar.

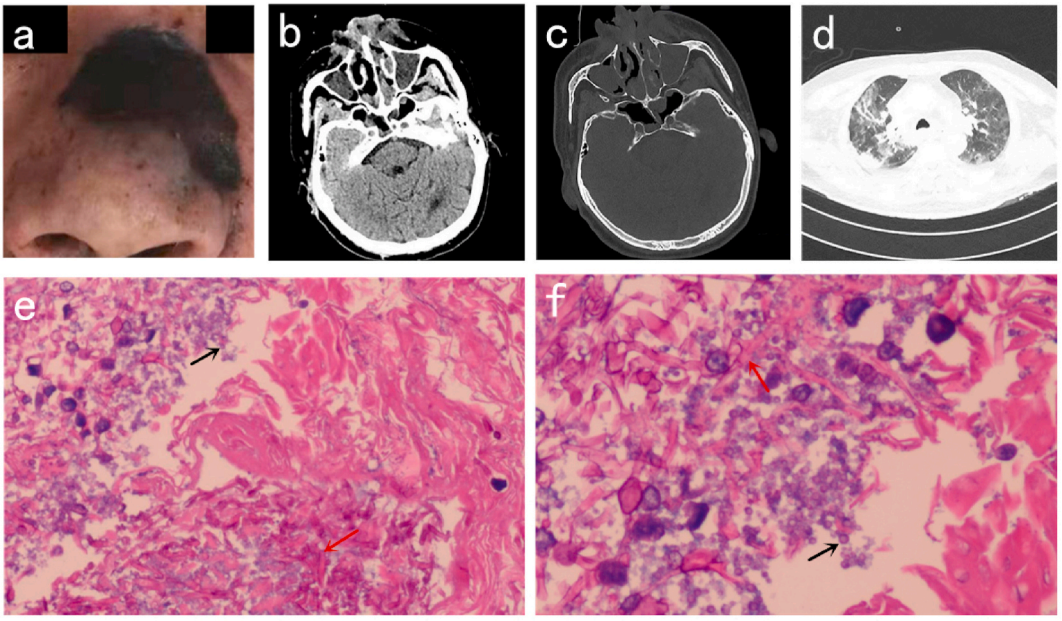

Fig. 1. (a) Black eschar on the nasal root. (b and c) Computed tomography (CT) scan of the head revealing much inflammatory effusion along bilateral maxillary, ethmoidal, and sphenoid sinuses. (d) CT of the lungs showing bilateral lung infiltrations consistent with pulmonary mucormycosis. (e and f) Haematoxylin and eosin staining of nasal mucosa showing presence of a large amount of fungal hyphae (red arrows)and spores (black arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hydrocortisone were used but still unable to maintain stable blood pressure. The patient lost the chance to operation and showed no response to all those treatments above. He was discharged from hospital on January 18th and finally deceased.

3. Discussion

The case emerged when Omicron variant was the primary pathogenic strain. Probably due to weakened virulence of Omicron compared to Delta and the racial differences, in the mainland of China, the literature about COVID-19 associated mucomycosis is scarce.

Our case is consistent with others indicating that diabetes mellitus is the most common underlying risk factor of mucormycosis [1, 2]. COVID-19 would further increase the risk. During the COVID-19 pandemic, patients are on immunosuppressive drugs, glucocorticoids, thus are at a higher risk of mucormycosis [3]. Though our patient wasn't treated with glucocorticoids, his diabetes and hyperglycaemia brought about an inflammatory state that could be potentiated by the activation of antiviral immunity to SARS-CoV2, which favoured fungal infections.

The patient's new symptom of nasal ache was subtle and may be easily neglected. We strongly suggest nasal ache may be an important initial symptom indicating mucor infection, since it represents nasal cavity affected where mucor spores like to invade and reside firstly.

A study showed that in the diagnosis of invasive fungal rhinosinusitis the sensitivities of the G and GM tests alone were 60.0% and 28.6%, respectively. When the G and GM tests were parallel combined, the sensitivity was 66.7% [4]. So negative results definitely cannot rule out fungal infection. Therefore, we remained a high suspicion of fungal infection though results of G and GM tests in our patient were negative. Histopathologically, mucorales hyphae typically have a variable width of 6–16 μm, and are nonseptate. Obtaining a diagnosis of mucormycosis on histomorphological basis sometimes is challenging, because it is hard to distinguish untypical hyphae of mucorales from Aspergillus spp. It is strongly recommended to confirm the diagnosis of mucormycosis in tissue by culture or by application of molecular identification technique [5]. In our case the mNGS provided the initial diagnosis accurately, while the gold standard diagnostic histological confirmation of non-septate hyphae in nasal tissue was six days later when the patient was terminally ill. Moreover, the mNGS provided the exact pathogens of Rhizopus microsporus and Mucor racemosus in species. It is the first case that these two pathogens were found simultaneously in a disseminatedly infected patient. The Rhizopus microspores and Mucor racemosus related death rate is reported to be 64% and 52% respectively [6].

Besides bloodstream infection, CT scan confirmed both rhinosinusitis and pulmonary mucormycosis. Therefore disseminated mucormycosis was diagnosed. The characteristic radiological finding of mucormycosis on CT of the lungs is the halo sign, a ring of ground glass opacity surrounding a nodular infiltrate. The noncontrast CT scan revealed mucosal thickening of the sinuses, indicating an ongoing sinonasal inflammatory process.

The concurrent infection with severe COVID-19 and mucor made timely diagnosis challenging. A large study showed that among

patients with severe COVID-19, 17% had second concurrent active condition, including second infections, and they were highly prone to a delayed second diagnosis [7]. In our case, because of the concurrent infection situation, we actually didn't know the exact time of mucor infection. Nasal symptom may be a sign. We suggest it is vital to maintain a close surveillance of patients post severe COVID-19 infection.

Simultaneous infection is rare and fatal even when treated with antifungal drugs. Surgical debridement plays a vital role as an adjunctive treatment in mucormycosis if the patient's condition permits [5]. Early diagnosis and treatment are critical in order to improve clinical outcomes. In our case, anti-fungal treatment was not initiated until two days after nasal symptom appeared. The delayed treatment may be partially responsible for the poor outcome. Based on a study by Son HJ et al., neutropenia, thrombocytopenia, positivity of non-sterile culture, use of steroid and treatment without surgery contribute to a poor outcome in patients with pulmonary mucornycosis [8]. Our case is consistent with the above conclusions. We hold that thrombocytopenia, neutropenia and treatment without surgery in our case contributed to the poor prognosis. The worst complication of thrombocytopenia in a patient with mucormycosis is intracranial hemorrhage [9], as a case report has indicated. Treatment is determined by the underlying cause of the thrombocytopenia. In our case, getting the infection under control was the top priority. Another vital management was platelet transfusion. However, we failed to elevate the level of platelet count. As shown in Table 1, the patient's kidney function was getting worse and worse. Moreover, inflammatory markers, including serum levels of ferritin, procalcitonin, and C-reactive protein were elevated in relation to high levels of circulating pro-inflammatory cytokines and chemokines. Performing CRRT was essential to improve the kidney function, get rid of the harmful circulating cytokines and correct acid-base imbalance. Mechanical ventilation support and vasoactive drugs were needed to sustain patient's vital signs at the late stage of the disease. In general, comprehensive managements are needed for mucormycosis patients with complicated conditions.

Overall, the mNGS may be a fast, sensitive and accurate diagnostic method for early pathogen detection and point the way toward treatment earlier. We highlight the need for high index of suspicion of mucormycosis after severe COVID-19 infection, performing imaging earlier and initiating empiric anti-fungal treatment from the onset of presenting symptoms given the worse outcomes of this disease.

4. Conclusion

COVID-19 associated mucormycosis is life-threatening especially for those with mixed mold infection. In view of the severity of this disease, the promptly empiric anti-fungal treatment is suggested once mucormycosis is suspected. The mNGS may provide a fast, sensitive and accurate diagnosis. Our case highlights the diagnostic value of the mNGS in fungal infection.

Consent

Patient consent for publication was obtained from the relative for the publication of all images, clinical data and other data included in the manuscript.

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Data availability statement

Data associated with our study has not been deposited into a publicly available repository. Since our paper is a case report. All data supporting the findings of this study are available within the paper and its Supplementary information.

Ethics declarations

All patient's legal guardians provided informed consent for the publication of patient's anonymised case details and images.

Approval by an ethics committee was not needed for this study because it is a case report that does not involve experiments.

CRediT authorship contribution statement

Lihan Hai: Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Investigation. **Peihong Li:** Formal analysis, Writing – original draft. **Zheng Xiao:** Formal analysis, Writing – original draft. **Jinxia Zhou:** Data curation, Formal analysis, Writing – review & editing. **Bo Xiao:** Conceptualization, Funding acquisition, Writing – review & editing. **Luo Zhou:** Conceptualization, Funding acquisition, Writing – review & editing, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25840.

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