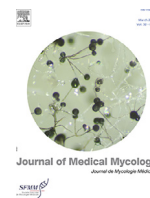




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Case report

Pulmonary mucormycosis in the aftermath of critical COVID-19 in an immunocompromised patient: Mind the diagnostic gap

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ABSTRACT

Mucormycosis has recently been recognized as a severe complication of COVID-19 with high fatality rates. We report a fatal case of COVID-19 associated mucormycosis (CAM) in a non-diabetic immunocompromised patient, who was first misdiagnosed and treated for COVID-19 associated aspergillosis (CAPA). The risk factors and initial clinical presentation of CAPA and CAM are similar, but CAM has a more aggressive course and CAPA and CAM are treated differently. Dedicated diagnostic workup is essential to ensure early treatment of CAM with surgical debridement and targeted antifungal therapy.

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Introduction

Critical COVID-19 is associated with increased risk of invasive fungal infections. Reported rates of COVID-19 associated pulmonary aspergillosis (CAPA) varies from 0 to 33% [1]. Recently there has been a rise in cases of COVID-19 associated mucormycosis (CAM), most extensively reported in India [2,3], with an associated mortality of 14–70% depending on site of infection [2–4]. Mucormycosis is a rapidly growing angioinvasive infection caused by moulds of the Mucorales species. It most often affects the rhino-, orbito-, cerebral- or pulmonary regions [4]. Type 2 diabetes, corticosteroids and immunosuppression are major risk factors for CAM [4]. The immunomodulatory effects and pulmonary endothelial damage caused by SARS-CoV-2, in combination with iatrogenic immunosuppression and hyperglycemia induced by corticosteroids, are believed to create “the perfect storm” for mucormycosis. CAM and CAPA may share radiographic and clinical features, but the treatment of CAM differs from that of CAPA, and due to the aggressive course of CAM, prompt specific diagnosis and treatment is required to avoid fatal outcome.

We report a case of CAM in a non-diabetic immunocompromised patient first misdiagnosed as CAPA and discuss the pathogenesis, risk factors, diagnostic measures and treatment of CAM.

Case report

A Caucasian male in his fifties, who had a renal transplantation 20 years previously, presented with septic shock and was admitted directly to the intensive care unit (ICU) (day 0), where he tested positive for SARS-CoV-2.

Two years previously, he was diagnosed with polymorph post transplantation lymphoproliferative disease (PTLD). He was treated with rituximab, chemotherapy and radiotherapy and had complete remission. Secondary to the radiotherapy the patient developed ileus, resulting in ileostomy and short bowel syndrome (SBS). The patient had received his second dose of the Pfizer-BioNTech COVID-19 vaccine 50 days prior to admission, 9 months after the last dose of rituximab.

Before ICU admission, the patient had 3 days of general malaise without other symptoms. At presentation, he was afebrile, the blood pressure was 65/40 mmHg and he had oliguric renal failure with a creatinine of 719 $\mu\text{mol/L}$, a markedly elevated C-reactive protein of

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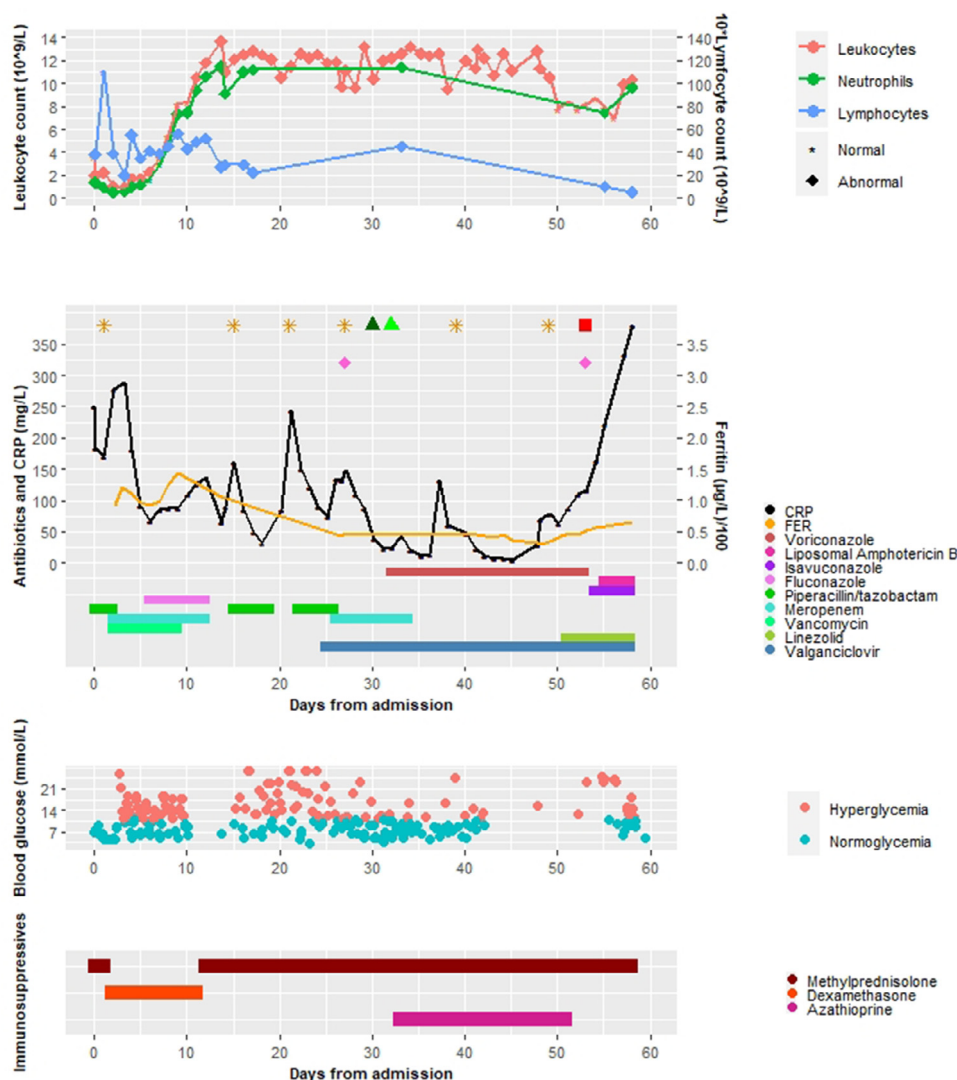


Fig. 1. Timeline of inflammatory markers, blood glucose and medication in a patient with COVID-19 associated mucormycosis.

Microbiological findings: orange asterisk = positive SARS-CoV-2 by PCR (cycle threshold values available: day 18 = 24, day 20 = 24, day 26 = 28), dark green triangle = galactomannan index blood 1.5; light green triangle = galactomannan index BAL-F 0.6; red square = *Rhizopus microsporum* in culture of sputum.

pink diamonds = Time of computed tomography (CT)

CRP = C-reactive protein, FER = ferritin

Hyperglycemia ≥ 11.0 mmol/L.

252 mg/L and ferritin of 9270 ug/L (Fig. 1). At day 1, polymerase chain reaction (PCR) and next-generation sequencing was performed, as described in detail in supplementary material, with the result of the SARS-CoV-2 B.1.1.7. variant with the N501Y mutation. Maintenance immunosuppressive therapy was paused (azathioprine 50 mg/day and solumedrol 30 mg/day) and dexamethasone 6 mg was prescribed. Remdesivir was omitted due to renal insufficiency. During the next 4 days, progressive hypoxemic respiratory failure required high flow oxygen therapy up to FiO₂ 80–100%, flow 30 L/min at day 4. A chest computed tomography (CT) scan revealed a central lung embolus. Throughout the ICU admission the patient was treated with empiric antibacterial therapy, fluconazole (Fig. 1), therapeutic tinzaparin and high flow oxygen therapy but did not need mechanical ventilation. Cultures of blood, tracheal secretions and urine were without growth of relevant pathogens.

On day 10 he was sufficiently stabilized for ICU discharge but continued to have general malaise, renal insufficiency and elevated inflammation markers. During the following weeks, fever, chest pain and elevated inflammatory markers persisted, despite treatment

with broad spectrum antibiotics, and blood glucose levels remained difficult to regulate (Fig. 1).

On day 24, CMV was detected by PCR in blood (1400 copies/ml) and treated with valganciclovir.

Because of ongoing signs of infection without a focus, a high-resolution CT scan was done showing a large infiltrate with central necrosis compatible with invasive fungal infection or necrotic sequelae of the central embolus (Fig. 2A). Bronchoscopy was performed on day 31. Cultures of bronchoalveolar lavage fluid (BAL-F) were without growth. BAL-F was not examined for moulds by PCR. *Aspergillus* galactomannan antigen (GM) was detected with Platelia™ *Aspergillus* Ag (Bio-Rad, Marnes-la-Coquette, France) resulting in optical density index of 0.6 in BAL-F, and 1.5 in serum.

Invasive pulmonary aspergillosis was suspected and voriconazole was started. The patient was discharged with oral voriconazole on day 42 but was readmitted after five days due to fever, cough, general malaise and thoracic pain. Upon readmission, sputum samples were sent for culturing and on day 53 mold isolates, that grew in two of two consecutive sputum samples taken four days apart, were identified as *Rhizopus microsporum* by morphology, MALDI-ToF mass

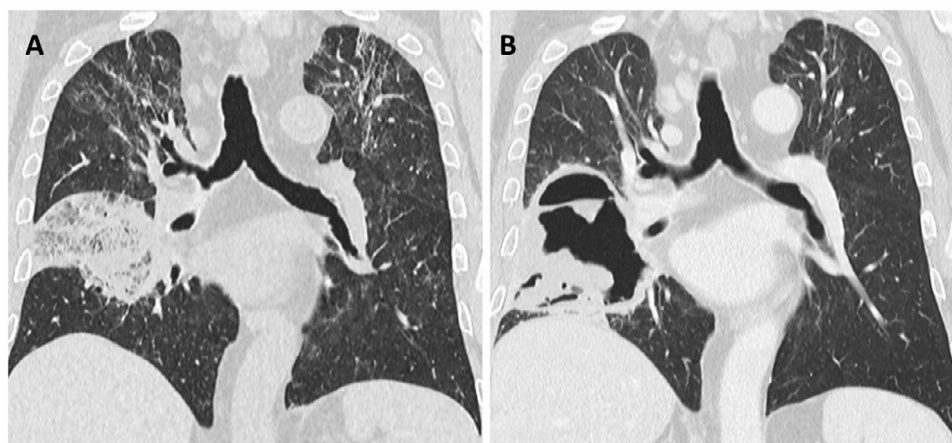


Fig. 2. Radiological findings of pulmonary COVID-19 associated mucormycosis.

A: High resolution computed tomography (CT) scan day 27 after first admission. B: CT scan day 53 after first admission.

spectrometry and molecularly as previously described [5]. Antifungal susceptibility testing was performed using the reference method EUCAST E.Def 9.3 [6] resulting in MICs for voriconazole, isavuconazole and amphotericin B of 4 mg/L, 0.5 mg/L and 0.06 mg/L, respectively. CT scan demonstrated severe progression of the lung infiltrate (Fig. 2B). Voriconazole was switched to intravenous treatment with high-dose liposomal amphotericin B (5 mg/kg/day) and isavuconazole (200 mg three times a day for two days followed by 200 mg once daily).

At the time of diagnosis of *R. microsporum*, the infection involved all lobes of the right lung and the pericardium with close relation to the major vessel. Radical surgical debridement was discussed but rejected because of high risk of fatal bleeding due to the localization and progressed state of the infection.

The patient quickly deteriorated with hemoptysis, respiratory distress and renal failure. Embolization of lung arteries by coiling was unsuccessful. The patient passed away on day 60.

Discussion

In 2021 there has been a surge in cases of CAM, with the vast majority occurring in India [2,3]. In a review by Pal et al., data from 99 CAM patients were pooled [4]: The mortality rate was 34%. The median time interval from diagnosis of COVID-19 to mucormycosis diagnosis was 15 days. A total of 85% of the patients had diabetes mellitus, 85% were treated with corticosteroids for COVID-19, the majority had rhino-orbital-(cerebral) mucormycosis, and 10% had pulmonary mucormycosis, of whom 70% died [4]. A large scale multi-center study of CAM has recently been published by Sen et al. reporting characteristics of 2826 Indian patients with COVID-19-associated rhino-orbital-cerebral mucormycosis (ROCM) [2]. Garg et al. confirms findings of the previous review including high proportions of diabetic and corticoid treated patients amongst the ROCM cases. However, Garg et al. report a lower mortality rate of 14%, which likely reflects that the CAM patients with highest mortality, such as pulmonary mucormycosis [7], were not included in their report.

Several risk factors for CAM have been identified [4,8] and a number of these were present in the case we present. Prior to COVID-19 infection the patient was immunocompromised due to treatment with azathioprine and methylprednisolone. During the COVID-19 infection he was treated with dexamethasone and had persistent lymphopenia. SARS-CoV-2 was repeatedly detected by PCR over several weeks with cycle threshold values <30 indicating ongoing viral replication. Mucorales require iron to grow and increased iron-availability, including elevated ferritin levels, is a risk factor for CAM [8].

Our patient had high levels of ferritin throughout the course of infection. Due to low plasma-levels of zinc, he received zinc-supplements, which has been found to improve growth of *Rhizopus* [9]. He had SBS, received parenteral nutrition and had corticoid-induced hyperglycemia. Diabetes and hyperglycemia are also known to predispose to mucormycosis and to severe COVID-19 [10,11]. Elevated glucose levels impair the immune function in several ways, particularly by reducing the innate immune systems' response towards invading pathogens [10]. Both SARS-CoV-2 and Mucorales species enter the endothelial cells through glucose regulated protein 78 (GRP78) [8,12], which is presented on the cell surface during cell stress, including glucose dysregulation and COVID-19 infection [8]. Dexamethasone has also been shown to upregulate GRP78 [13]. A recent study suggests that the new SARS-CoV-2 variants bind more tightly to GRP78 than the original strains [12], which might partly explain the increasing incidence of CAM.

Diagnosis of invasive mold infection is challenging, especially in patients with COVID-19. Bronchoscopy is not always possible due to severe respiratory distress or to the risk of transmission of SARS-CoV-2 associated with aerosol generating procedures. Culture of BAL-F has a low sensitivity ($\approx 50\%$) and the radiographic changes of COVID-associated pulmonary fungal infection are often unspecific [7,14]. Our case was first diagnosed with probable invasive pulmonary aspergillosis on the basis of the ECMM/ISHAM consensus criteria for CAPA [14], whereof the patient fulfilled 1) the host factor criteria due to need of intensive care, 2) the clinical criteria in terms of symptoms and chest CT findings and 3) the mycological criteria with evidence of aspergillosis with a GM index ≥ 0.5 in serum. However, a positive GM in serum with negative BAL GM is an atypical finding for CAPA [1]. Treatment with voriconazole for invasive aspergillosis was started and no further diagnostic testing was sought initially. Galactomannan is a fungal cell wall component mainly produced by the *Aspergillus* species and Mucorales species are not known to produce this polysaccharide. Detection of GM in serum and BAL-F from the present case could either represent a mixed mold infection, with *Aspergillus* sp. being outgrown by *R. microsporum*, or false positive results. False positive results of GM in serum have previously been seen in patients receiving beta-lactam treatment [15], however our patient had finished the course of piperacillin/tazobactam 3 days before blood sampling for GM was done. Reports of mucormycosis cases with positive GM have been published previously [16,17], as well as reports of CAM cases with concomitant aspergillosis [18,19].

There is no validated biomarker for Mucorales species yet, which makes diagnosis even more difficult. Molecular test for detection of circulating Mucorales DNA are available for clinical use, however

standardization of this method is ongoing [11]. This direct detection with a short turnaround time can facilitate early diagnosis, which is essential to ensure timely treatment to stop the rapid growth of Mucorales and progression of the infection.

Our case of *R. microsporium*, as the causative agent of pulmonary mucormycosis, confirms previous reports of high frequency of pulmonary manifestations due to this specific organism [20]. This might be associated to the smaller size of the *R. microsporium* spores, enabling inoculation in the lower respiratory tract.

Mucorales' angioinvasive growth causes vessel thrombosis and necrosis in the infected tissue, leading to impaired blood circulation and poor penetration of systemic antifungal therapy. Surgical treatment with radical removal of infected tissue is key for improving outcomes. In addition to surgery, timely initiation of antifungal therapy is important. The drug of choice for treatment of Mucorales spp. is high-dose liposomal Amphotericin B (5–10 mg/kg) [11]. Additionally, isavuconazole has been approved as first line treatment by the FDA. Voriconazole is not recommended due to the low susceptibility of Mucorales to this drug [11]. Liposomal Amphotericin B is nephrotoxic and isavuconazole and posaconazole are alternatives in patients with preexisting renal compromise.

In the present case *R. microsporium* was first detected in a sputum culture 22 days after the positive GM test and 33 days after the first chest X-ray with an infiltrate suggestive of invasive fungal infection. Only at this point was the right antifungal therapy for mucormycosis started, and the infection was too widespread to be removed surgically.

Our case underlies the importance of rapid and accurate detection and identification of the causative pathogen by culture or molecular based methods, when CAM is a possible diagnosis, in order to secure timely and targeted treatment, including surgical debridement. Repeated sampling is recommendable if initial tests are negative or inconsistent. CAM should be recognized as a differential diagnosis in patients with suspected invasive fungal infections and therapeutic coverage of Mucorales species could be considered until the causative pathogen has been identified, allowing for targeted therapy.

Conclusion

Critical COVID-19 is associated with risk of invasive fungal infections including mucormycosis. We demonstrate the rapid angioinvasive and fatal course of CAM in a case first misdiagnosed and treated as CAPA. When invasive fungal infection is suspected, diagnostic measures for detection of the causing pathogen should be performed insistently.

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Declaration of Competing Interest

The authors CGC and JHL have no conflicts of interest.

The author MS has the following conflict of interest: Outside the current work, MS has received speaker honoraria (personal fees) from Gilead and MSD.

The author MCA has the following conflict of interest: MCA has, over the past 5 years, received research grants/contract work (paid to the SSI) from Amplyx, Basilea, Cidara, F2G, Gilead, Novabiotics, Scynexis and T2Biosystems and speaker honoraria (personal fees) from Astellas, Gilead, Novartis, MSD, and SEGES. She is the current chairwoman of the EUCAST-AFST.

The author MH has the following conflict of interest: MH has received travel grants from Gilead and GSK, speaker's honorarium from GSK, MSD and Gilead, research grant from Takeda, and has

participated in advisory boards for GSK, Gilead, MSD, AstraZenica and Sobi.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mycmed.2021.101228.

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