MAEDICA – a Journal of Clinical Medicine
2022; 17(1): 215-225
https://doi.org/10.26574/maedica.2022.17.1.215

MÆDICA - a Journal of Clinical Medicine

CASE REPORT

Histopathologically Confirmed Pulmonary Mucormycosis as a Complication of COVID-19: a Case Report from Romania and Insight into Pathology

George Sebastian GHERLAN[a, b] Maria Cristina HOARA[a], Sebastian George SMADU[a], Corneliu Petru POPESCU[a, b], Petronela IONESCU[a, c], Simin-Aysel FLORESCU[a, b]

[a]Department of Infectious Diseases, "Dr. Victor Babes" Clinical Hospital of Infectious and Tropical Diseases, Bucharest, Romania

[b]Infectious Disease Clinic, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[c]Anatomopathology Department, "Dr. Victor Babes" Clinical Hospital of Infectious and Tropical Diseases, Bucharest, Romania

ABSTRACT

COVID-19 has proven to be an independent risk factor for secondary infectious complications. Amongst them, mucormycosis has recently been noticed more frequently than in the past. Caused by molds belonging to the Mucorales order, this is a rare, but potentially fatal infection unless adequately treated. Ear, nose and throat involvement is prevalent with often expansion to the orbit, sinuses or brain. Pulmonary, cutaneous and gastrointestinal infections are also recognized. Classical risk factors for progression to angioinvasive disease include poorly controlled diabetes mellitus, defects in phagocytic function (prolonged neutropenia, glucocorticoid treatment), immunosuppressive therapy associated with transplantation, malignancy, elevated levels of free iron as well as iron chelators (deferoxamine). In addition, immune dysregulation rendered by COVID-19 itself may contribute or solely lead to invasive mold disease. The largest experience comes from India, which has dealt with a challenging epidemic of COVID-19-associated mucormycosis (CAM). To our knowledge, no previous studies have reported CAM in Romania. We therefore present a case of severe COVID-19 pneumonia initially complicated by bacterial superinfection and secondary sepsis at admission in an unvaccinated 61-year-old male who presented in our clinic with respiratory failure and digestive symptoms. Although improvement occurred rapidly following antiviral, empiric large spectrum

Address for correspondence:
Maria Cristina Hoara
Postal address: Spitalul Clinic de Boli Infectioase si Tropicale Dr. Victor Babes, Sos. Mihai Bravu 281, sector 3, Bucuresti, Romania
Email: cristina_hoara@yahoo.com

Article received on the 20th of January 2022 and accepted for publication on the 10th of March 2022

antibiotics and pathogenic medication, unfavorable clinical course ensued later on. Biological and imaging investigations were consistent with pulmonary superinfection in the form of multiple different-sized upper right field opacities, which eventually evolved to form cavities. Differential diagnosis was thoroughly performed. Since unable to sterilize the lung by means of medication alone, the patient underwent major thoracic surgery with removal of the entire right lung. Microscopic study of the damaged tissue was able to determine the presence of broad, aseptate hyphae which morphologically belong to Mucorales. *A diagnosis of pulmonary mucormycosis was established and proper antifungal treatment was initiated, with full recovery of the patient.*

Keywords: SARS-CoV-2, COVID-19, mucormycosis, invasive fungal disease, pulmonary abscess.

INTRODUCTION

Apart from its well-known potential severe clinical course, COVID-19 has also raised concern upon a particular opportunistic fungal disease – COVID-19-associated mucormycosis (CAM). By far, India has been most affected, reporting more than 28,000 cases by June 2021 (3).

Mucorales are ubiquitous fungi to which humans are constantly exposed. Risk factors for developing mucormycosis include poorly controlled diabetes mellitus and diabetic ketoacidosis, glucocorticoid treatment which interferes with innate immunity mechanisms, prolonged neutropenia (as in hematologic malignancy), immunosuppressive therapy associated with transplantation, cancer, elevated levels of free iron as well as iron chelators (deferoxamine) which act as siderophores (1, 2). COVID-19-associated mucormycosis seems to result particularly from the association between COVID-19's immune dysregulation and corticoid therapy used for its treatment, all combined with a high prevalence of often poorly controlled diabetes in low- and middle-income countries (3).

The disease is often fatal and diagnosed lately because of its previous low incidence which renders low degree of suspicion. Recently, new guidelines for the diagnosis and management of mucormycosis have become available. Confirmatory diagnosis implies microscopic analysis or culture of normally sterile material or tissue nucleic acid amplification (3-5).

We herein report the case of a 61-year-old male with COVID-19 pneumonia and mixed pulmonary superinfection manifested as multiple excavating abscesses, which eventually led to right pneumonectomy. Histopathological assessment of biopsy specimen established the diagnosis of pulmonary mucormycosis. ❑

CASE PRESENTATION

On April 7th 2021, a 61-year-old man history of hypertension, smoking and occasional alcohol abuse, who had not been vaccinated against COVID-19, was admitted to "Dr. Victor Babes" Clinical Hospital for Infectious and Tropical Diseases in Bucharest, Romania. He presented with asthenia, myalgia and diarrhea of recent onset. He had been tested positive for SARS-CoV-2 infection by RT-PCR on April 2nd. Clinical examination indicated dyspnea, hypoxemia (oxygen saturation was 88 percent on room air), crackles in both inferior halves of lung fields and low blood pressure (81/57 mm Hg). Chest X-ray demonstrated typical COVID-19 pneumonia with bilateral basal ground-glass opacities and consolidations. Thoracic CT scan was not performed at that moment.

Initial blood tests (Table 1) revealed markedly raised C-reactive protein (CRP) and procalcitonin, strongly suggesting bacterial coinfection. Moreover, a diagnosis of sepsis was established based on the SOFA criteria, with renal, respiratory and circulatory failures. Hyperglycemia led to diagnosis of new onset diabetes mellitus. Extensive microbiological workup was performed, with blood, urine and stool cultures, nasal and pharyngeal swabs as well as immunochromatography testing for Clostridioides difficile and serological testing for HIV and hepatitis B and C viruses. All came back negative.

Antiviral treatment with Remdesivir was administered as a classical five-day course. Empiric antibiotic treatment was initiated by associating Meropenem and Linezolid for seven days. Intra-

TABLE 1. Dynamics of laboratory data

Test	Unit	Lab normal range	April 7th – admission	April 11th	April 19th – opacities on CT	April 22nd – *C. krusei* in BC	May 7th after *A. baumannii* in sputum	June 16th – second admission	July 26th - discharge
WBC	cells/mm^3	4000-10000	30900	12000	28900	25500	15000	15700	9700
Ne	cells/mm^3	2000-7000	28800	9400	25800	22700	13800	11800	6800
Hb	g/dL	11.7-16.1	14.9	15.9	12.2	11.2	9.1	7.3[a]	8.9
PLT	*10^3/mm^3	150-400	180	188	263	278	246	496	354
Cr	mg/dL	0.6-1	5.3	1.4	-	0.9	0.8	2.2	1.8
Gly	mg/dL	70-110	205	-	-	314	360	119	165
Na	mmol/L	136-145	136	139	131	128	135	139	131
K	mmol/L	3.5-5.1	3.8	5.2	4.5	4	4	2.3	3.3
ALT	IU/L	6-59	320	150	-	47	81	18	34
AST	IU/L	0-37	203	-	-	-	-	32	-
LDH	IU/L	135-214	401	-	-	-	193	307	-
Fib	mg/dL	200-400	993	564	-	892	583	644	649
D-D	mcg/mL	0-0.5	5.88	>20	1.26	1.87	1.2	2	-
CRP	mg/dL	0-0.3	43	3.8	27.5	34	5.6	17	7.1
PCT	ng/mL	0-0.5	101	-	1.28	3.8	0.15	0.4	0.19
Fer	ng/mL	13-150	4582	-	-	3250	1191	1951	2592

[a]Patient received blood transfusion

Abbreviations: WBC=white blood cells, Ne=neutrophils, Hb=hemoglobin, PLT=platelets, Cr=creatinine, Gly=glycemia, Na=sodium, K=potassium, ALT=alanine aminotransferase, AST=aspartate aminotransferase, LDH=lactate dehydrogenase, Fib=fibrinogen, D-D=D-dimer, CRP=C- reactive protein, PCT=procalcitonin, Fer=ferritin.

venous Dexamethasone was administered in order to reduce the hyperinflammatory reaction (24 mg/day at first, then gradually tapered until cessation 14 days later). Supplementary oxygen was also provided, with decreasing volumes until removal six days later. Subsequently, initial symptoms disappeared with great improvement in biological values and follow-up X-ray from April 13th, which showed almost complete resorption of previously described lung infiltrates (Table 1).

Nevertheless, clinical and paraclinical worsening occurred soon after. Starting April 19th, the patient began experiencing productive cough with high volume grey sputum, which rapidly became hemoptoic (dark red coloration) for a brief period. This event triggered performance of imaging check-up on the same day. Chest X-ray showed new right upper lung field consolidation, whilst native thoracic CT scan revealed multiple nodular opacities projected within the same area. Amongst these, the largest was described as macronodular, measuring 6/7 cm, corresponding to the X-ray lesion (Figure 1). Growing levels of CRP, but not as much of procalcitonin, were noted. RT-PCR for SARS-CoV-2 detection became negative on April 20th (Figure 1).

In light of this unfavorable turn of events, after previous de-escalation, same broad-spectrum antibiotic as well as antifungal treatment with Voriconazole for invasive aspergillosis coverage were initiated, since we have had frequently encountered this fungal superinfection in our COVID-19 patients. Also, sputum, urine and blood cultures were performed, together with a multiplex PCR assay from sputum specimen. Results came positive for two *Candida* species, *C. albicans* (recovered from sputum and urine) and *C. krusei*, respectively. The latter was recovered from sputum and, more significantly, from blood culture (a single set drawn in absence of fever). By this time, oral Voriconazole had already been initiated four days previously. Anidulafungin was also introduced only later (lack of availability) for a nine-day course, since echinocandins are fungicidal against *Candida* spp., unlike

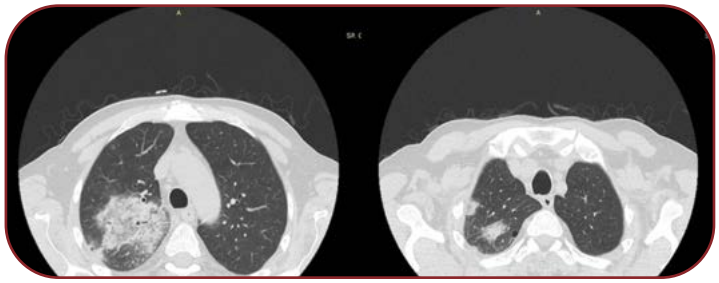

FIGURE 1. First CT scan – four nodular opacities were described

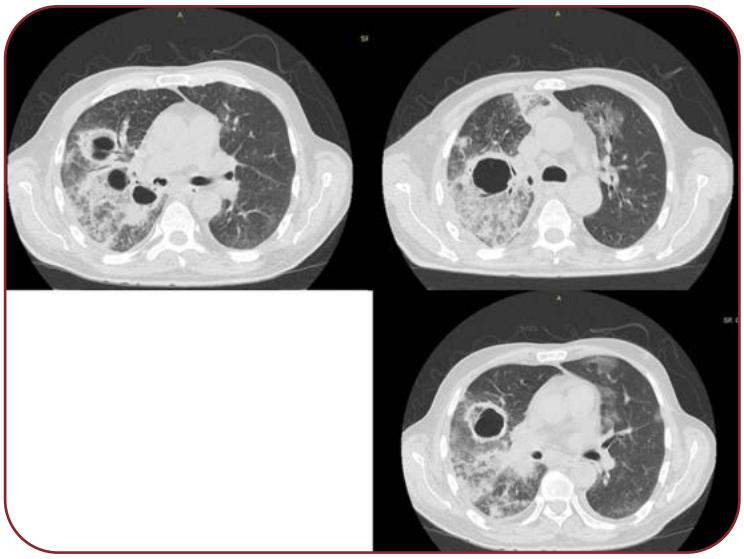

FIGURE 2. Increase in both number and dimension of lesions, with cavity formation within the right upper lobe were described, largest cavity measuring 39/39 mm

azoles. Furthermore, PCR assay detected the gene of *Klebsiella pneumoniae* in sputum. Tuberculosis was not overlooked – Ziehl-Neelsen stain did not reveal acid-fast bacilli and PCR for *Mycobacterium tuberculosis* was negative.

Mild shortness of breath reemerged on April 26th, which triggered the reintroduction of smaller doses of Dexamethasone (8 mg/day, tapered until cessation 14 days later) and low volumes of supplementary oxygen (intermittently used throughout hospital stay) as well as performance of another CT scan, which demonstrated disease progression towards cavitation (Figure 2). Invasive pulmonary aspergillosis was suspected as the patient was already receiving specific antifungal coverage.

Since increasingly volumes of purulent sputum were still eliminated under broad-spectrum antibiotic and antifungal treatment, further series of sputum cultures were performed. Therefore, as of May 6th, repetitive isolation of *Acinetobacter baumannii* (carbapenemase-producing strain) and different *Candida* species (*C. krusei*, *C. lipolytica*) was obtained. Moreover, a second multiplex PCR assay identified *A. baumannii*'s specific gene, without tracing *K. pneumoniae*'s this time. Also, a single set of blood cultures was drawn during a sub-febrile episode on May 10th (otherwise afebrile patient during the entire hospital stay), which returned negative (clearance of *C. krusei*). At the time of *A. baumannii* recovery from sputum, empiric coverage with Colistin (to which the organism proved to be susceptible) had already been initiated based on clinical and biological grounds (persistently high levels of inflammatory markers).

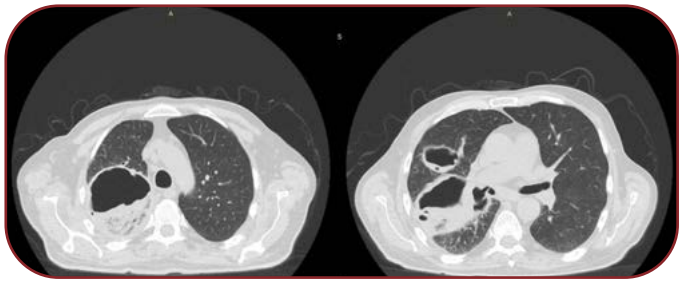

FIGURE 3. Six thick-walled cavitary images were noticed within the right upper lobe; the largest measured 10/10/13 cm and communicated directly with the right main bronchus

Antimicrobials have been constantly reviewed in order to cover all etiologies that might have contributed to lung superinfection, since it is well known that recovery of bacteria and fungi from purulent cellular debris is scarce. Existence of alternative microbial agents was suggested by lack of improvement under correct treatment of the identified ones. Therefore, Ampicillin and Vancomycin were introduced for coverage of anaerobic organisms (predilection for right upper lobe) and Staphylococcus aureus (counterargument – lesions are usually bilateral), respectively, both known for pulmonary abscess formation. By this time, Meropenem and Linezolid had been eliminated after correct *K. pneumoniae* treatment and Linezolid-induced anemia (16-day course). In addition, Ceftazidime-Avibactam was prescribed for eight days (became unavailable thereafter) for multidrug-resistant Gram-negative agents' coverage. Tuberculosis has been repeatedly ruled out, including by means of Lowenstein-Jensen culture (that was still in progress) and molecular amplification. Despite all efforts, the third thoracic CT scan (Figure 3) showed disease progression suggestive of multiple excavating abscesses.

The patient was transferred to the thoracic surgery clinic on June 2nd. There, on June 7th he underwent major surgery – although the operative team had planned preserving the right lower lobe, intraoperative inspection revealed massive destruction of the right main bronchus. Therefore, right pneumonectomy was performed followed by slow recovery (Figure 4). A sample of purulent collection was sent to the local lab, with isolation of fungal organisms, besides *A. bauman-*

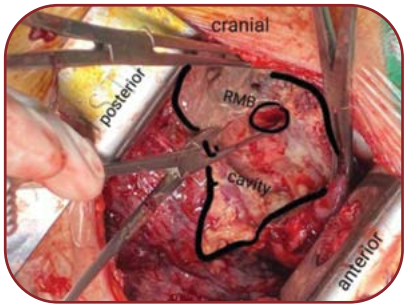

FIGURE 4. Intraoperative aspect – the right main bronchus (RMB) opens directly into the main cavity; its walls are almost entirely destroyed

nii, without possibility of species identification. During the entire stay, the patient received an antimicrobial treatment comprising Colistin, Ampicillin, Vancomycin and Voriconazole, similarly to that administered in our clinic. In addition, a lung biopsy specimen was sent to the local anatomical pathology laboratory. Following H&E staining, optical microscopy revealed an aspect compatible with mucormycosis.

The patient returned to our hospital on June 16th for continuation of antimicrobial therapy, given that surgical sutures had been performed within a contaminated medium with high risk of trachea fistulization. Inflammatory biomarkers were still significantly elevated (Table 1). Persistent cough with production of low volumes of light grey sputum raised worry upon involvement of the remaining lung, which was not confirmed by chest imaging. Culture from sputum samples showed clearance of *A. baumannii*.

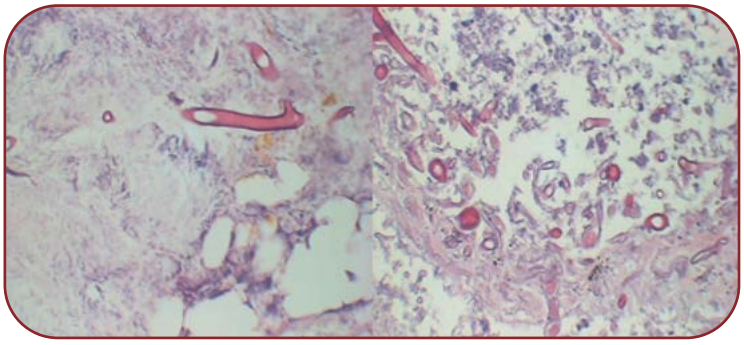

FIGURE 5. Hematoxylin&Eosin staining of lung tissue specimen. Large, aseptate hyphae can be seen in longitudinal and transversal sections amongst necrotic tissue; it seems that two filaments branch at an almost right angle (view circle); this aspect pleads for mucormycosis, since *Mucorales* exhibits non-dichotomous irregular branching at occasional right angles (1, 2).

Considering the pulmonary mucormycosis suspicion, we kindly requested the microscopic slide and the paraffin embedded tissue block for a second opinion provided by our clinic's anatomopathologist. Careful microscopic analysis of the H&E-stained slide supported the presence of broad, aseptate hyphae belonging to *Mucorales* (Figure 5). Therefore, according to diagnosis guidelines (3-5), a confirmed diagnosis of pulmonary mucormycosis was established.

Until second opinion results and proof of *A. baumannii* clearance from sputum were available, the patient received continuous antibacterial coverage as well as antifungal treatment with Voriconazole for possible pulmonary aspergillosis (for a total of 18 days after surgery). Aspergillosis has always been kept in mind, since to the authors' knowledge it had been more often associated to COVID-19 pneumonia rather than mucormycosis. It is also known that recovery of invasive fungal agents from culture is scarce, so repeatedly negative sputum cultures for *Aspergillus* spp. could not rule out this infection. PCR testing and detection of galactomannan were not available. In addition, differentiating filamentous fungi by optical microscopy is a difficult task, which was the final reason for not switching antifungal agents until our specialist agreed upon the diagnosis. Moreover, we did not benefit from an alternative way of diagnosing mucormycosis, since neither intraoperative tissue specimen had been sent for cultivation (*Mucorales* grows from minced, not homogenized tissue, due to friability of non-septated hyphae), nor tissue nucleic acid amplification was available.

Starting June 24th, all other antimicrobial agents were withdrawn and first-line treatment against mucormycosis with Liposomal Amphotericin B was initiated. Although discouraged (5), we used smaller doses (3-4 mg/kg/day). Recommended dosage is of 5-10 mg/kg/day. This decision was individualized in the context of previous surgical removal of lesions. In addition, even if liposomal preparation of Amphotericin B is less nephrotoxic and does not warrant renal adjustment, we considered it prudent to lower daily dosage since the patient's renal function had been damaged by prolonged use of Colistin.

But why treat at all after surgery? We decided beginning specific antifungals given that bilateral involvement is common and damage could have been yet subclinical. Moreover, the remaining

thoracic cavity was left filled with mixed gas-fluid collections (blood, exudate), according to a follow-up CT scan (Figure 6). These fluids could have represented proper culture medium for fungal growth. The thoracic surgeon stated that tube thoracostomy was not indicated, since high fluid consistency would not allow cavity evacuation. Instead, he agreed on performing diagnostic thoracentesis, with drainage of 80 mL of brown liquid – defibrinated blood, which was sent to our microbiology lab for bacterial (aerobic and anaerobic) and fungal cultures – all negative.

As soon as second-line specific treatment was available, after a six-day course of liposomal Amphotericin B, intravenous Isavuconazonium sulfate was initiated, carrying the advantages of lack of nephrotoxicity and side effects associated with infusion. We used appropriate loading doses for 48 hours, then a daily dose of 372 mg (prodrug, equivalent to 200 mg of active Isavuconazole) for a total of 27 days. Therefore, a 33-day course of antifungal treatment was completed. Meanwhile, renal function improved considerably. Before discharge on July 26th, we performed a last thoracic CT scan that showed disappearance of gas bullae and normal aspect of left lung (Figure 7). The patient left the hospital in good shape, with spontaneous oxygen saturation of 98% and no indication for secondary antifungal prophylaxis. Moderately elevated levels of CRP were still noted.

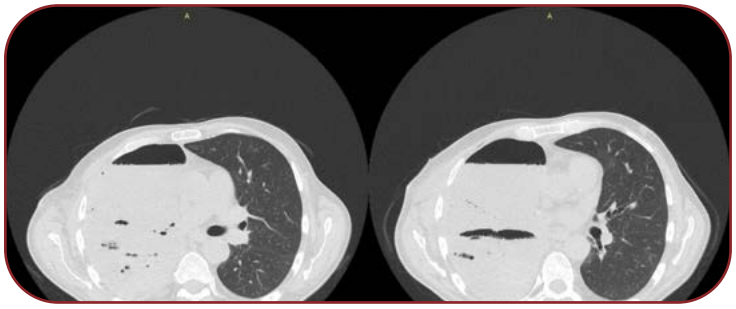

FIGURE 6. A mixed gas-fluid collection occupies almost the entire right thoracic cavity

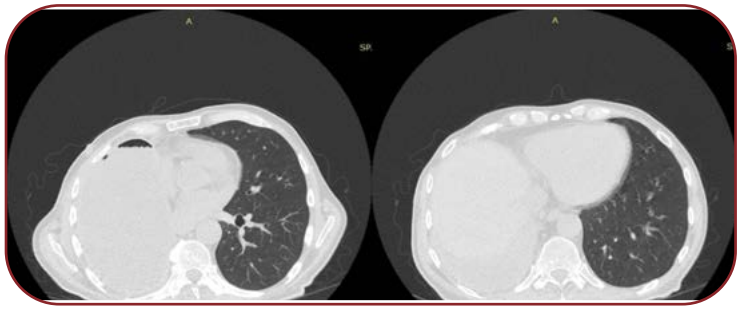

FIGURE 7. Classical postpneumonectomy aspect – rib retraction and different collection densities suggestive of decomposing blood

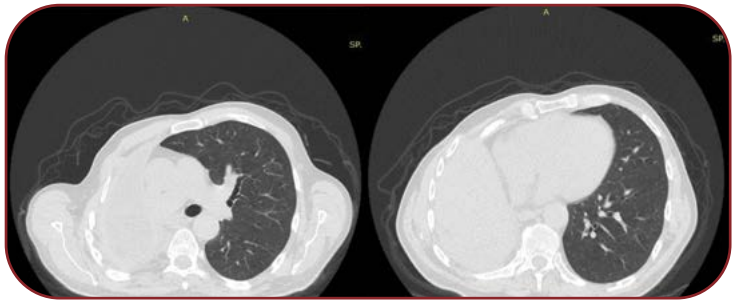

FIGURE 8. Right fibrothorax and normal aspect of left lung

A follow-up checkup in November 2021 revealed a man in good physical health, with possibility of medium and intense effort practice without shortness of breath, a slightly elevated level of CRP (lower than at discharge) and complete fibrosis of right hemithorax on CT scan. The left lung had normal aspect (Figure 8).

Insight into pathology

Since the onset of COVID-19 pandemic, many aspects of the disease's pathophysiology have been clarified, but new observations are constantly emerging and require further research. Amongst them, as noted in other viral illnesses such as influenza or AIDS, rising numbers of opportunistic fungal infections linked to SARS-CoV-2 infection have been registered in the past year. Although not entirely, this is likely a consequence of the immune dysregulation rendered by the virus-host interaction, which will therefore be thoroughly addressed.

COVID-19 involves profound alterations in immune response, which characterize rather severe forms of disease with poor prognosis. There is still debate over the nature of these abnormal reactions, since both immunosuppression as well as hyperinflammation have been documented. A markedly decreased level of lymphocytes is often noticed, whereas other patients may develop a cytokine storm that requires immunosuppressive therapy. Sometimes, both occur in the same individual. Earlier studies have focused on the first hypothesis, one postulating that an initial immunosuppression would allow increased viral burden which could eventually lead to excessive inflammatory responses (6), whilst a second one

found lower levels of IFN-ϒ and TFN-α in COVID-19 compared to septic and non-septic critically ill patients as a result of both innate and adaptive immunity suppression (7).

Nevertheless, increase in IL-6 cytokine titers have also been noticed. Even though one paper showed that levels are lower comparing to sepsis or acute respiratory distress syndrome (ARDS) of other causes (8), eventually IL-6 became the best laboratory predictor of respiratory failure and death in COVID-19 (9), supporting the concept of a cytokine storm as cause of clinical deterioration. Moreover, benefit from inhibiting hyperinflammation – non-specifically, by means of corticosteroids, or specifically, with modulators of Janus kinase (JAK) and IL-6 pathways has been proven in several studies (10, 11). One study also explains coexistence of lymphopenia and cytokine storm – it seems that high IL-6 levels induce exhaustion of lymphocytes (12). The downside of immunosuppressive therapy consists of greater incidence of superinfections. Corticosteroid use is known as a classical risk factor for opportunistic fungal infections. Invasive candidiasis, aspergillosis and mucormycosis are all triggered by low counts or dysfunction of neutrophils and although corticosteroids cause neutrophilia, they inhibit cell migration into the tissue rendering them unavailable for phagocytosis and therefore impairing innate immunity. To sum up, both the disease and its treatment are to blame for the new challenge ahead – COVID-19-associated fungal opportunistic infections.

The literature reports various opportunistic infections of bacterial, fungal, viral and protozoal etiology associated with COVID-19 (13). Amongst them, invasive mold and yeast superinfections are of particular interest for this paper. As shown above, these pathogens thrive in proper conditions for multiplication, especially when high doses of corticosteroids are used, but patient's comorbidities and ICU setting also contribute. Many cases of invasive candidiasis (mostly with highly resistant *C. auris*, which is worrisome) (14) and aspergillosis (15) have been linked to SARS-CoV-2 infection. While clinicians had been more accustomed to these occurrences, the pandemic has drawn attention to mucormycosis as well, which has been largely reported across India (not exclusively) and represents a major burden for patients and health systems alike, since it requires specific investigations and complex therapeutical approaches.

Suspecting such complications is key to timely diagnosis and proper treatment coverage, but discriminating between fungal etiologies is not an easy task. Usually, the setting that triggers specific IFD (invasive fungal disease) workup in COVID-19 consists of deteriorating clinical status in a patient with a rather lengthy hospital stay experiencing a typically moderate-severe form of disease and (re)appearance or worsening of respiratory failure while already receiving maximal therapy (antiviral, empiric large spectrum antibiotics and pathogenic medication); increased levels of inflammatory markers and especially new suggestive pulmonary lesions on chest X-ray or CT also point towards a possible development of IFD. Dynamics of C-reactive protein and procalcitonin levels are somewhat debated, as it seems they do not have the same diagnostic value as in bacterial infections. Some studies postulate that combination of raised CRP and low procalcitonin is highly associated with fungal infections in immunocompromised patients with leukemia or undergoing chemotherapy (16, 17).

Specific lung lesions associated with IFD are classically described when HRCT (high-resolution computed tomography) is performed, but spotting new consolidations, nodules or cavities on chest X-ray is also significant and should be followed by CT scan for a more detailed description. Referring to invasive aspergillosis and mucormycosis, typical abnormalities include segmental or lobar consolidations with or without a halo sign (surrounding ground-glass), air crescent sign, reverse halo sign, multiple nodules, cavities and pleural effusion. These represent clinical criteria that support probable invasive pulmonary mold disease according to two current guidelines for diagnosis of IFD and mucormycosis alone, respectively (4, 5). However, some of them rather pledge for mucormycosis: reverse halo sign, presence of more than 10 nodules, pleural effusions and accompanying sinusitis (2, 4). One study that reported three cases of COVID-19-associated pulmonary mucormycosis along with another 13 reviewed from the literature showed that the most frequent CT findings were consolidations and thin to thick-walled cavities, some with an air-fluid level (69% each), followed by pleural effusion (47%), pneumothorax and nodules (18.8% each) and reverse halo sign (13%) (18).

Whenever IFD is suspected, and COVID-19 setting has been no exception, invasive pulmonary aspergillosis is usually blamed and empirically covered until results of laboratory tests are obtained (when available). That is because at least until the CAM epidemic in India, this particular mold infection had been more frequently described and better acknowledged by clinicians than mucormycosis. In addition, given that the two mold diseases may share same CT findings, the diagnosis task becomes even harder. Typically, Voriconazole is initiated since it is fungicidal against *Aspergillus* spp. and largely accessible. Unfortunately, when etiology assumption is mistaken, this antifungal not only is inefficient, but its usage may lead to breakthrough disseminated mucormycosis, too (1, 2).

Other prominent risk factors for developing mucormycosis are poorly controlled diabetes mellitus and ketoacidosis. Patients with such comorbidities are prone to experience the rhino-cerebral form of illness. Sometimes, first clinical recognition of hyperglycemia occurs as a result of investigating mucormycosis. Also, elevated levels of free iron support fungal growth in serum and tissues, predisposing to rapidly fatal disseminated disease in iron-overloaded patients and ones treated with a chelator such as deferoxamine. This acts like a siderophore, directly delivering iron to the *Mucorales*. Moreover, it seems that hyperglycation of iron-sequestering proteins disrupts this linkage and increases free iron level. Cancer, solid organ and hematopoietic stem cell transplantation and their respective immunosuppressive therapies are also ideal settings for progressing to angioinvasive fungal disease. Impairment of phagocytic function by means of prolonged neutropenia (hematologic malignancies) or use of glucocorticoids is as important, especially since the latter represent the main pathogenic medication for COVID-19's cytokine storm. In plus, corticosteroids are known to alter carbohydrate metabolism with secondary hyperglycemia, therefore posing an even higher threat (1, 2). Few studies have even signaled cases with no other risk factors for CAM than COVID-19 and its treatment, which suggests the need for judicious use of glucocorticoids and immunomodulators such as tocilizumab and anakinra. Avoidance in the absence of a clear benefit is advisable (18-20).

There are several clinical presentations of mucormycosis based on involvement of a particular anatomic site: rhino-sinusal (with different degrees of extension to the orbit or brain), pulmonary, cutaneous, gastrointestinal and disseminated. One systematic review reporting 100 cases of CAM showed that the rhino-orbital form of disease was most prevalent (50% of patients), while rhino-sinusal and rhino-orbito-cerebral followed (17% and 15%, respectively). Only 8% of individuals had pulmonary lesions (21). Another paper concerning 47 CAM subjects stated that the mean duration between the diagnosis of COVID-19 and appearance of first signs of CAM was 12.1 ± 4.6 days, but reminded that rhino-orbital involvement may appear even after recovery from the viral infection (22).

Diagnosis of mucormycosis is divided in proven, probable and possible, based on evidence supporting it. In order to prove disease, either microscopic examination of an otherwise sterile material obtained by needle aspiration or biopsy with visualization of broad aseptate or paucisseptate hyphae accompanied by tissue damage or recovery of mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site must be performed. In addition, amplification of fungal DNA by PCR from tissue is also confirmatory. Probable diagnosis combines three criteria: a host factor – various causes of immunosuppression as discussed above, a clinical feature – at least one CT pattern suggestive of mucormycosis as shown previously or signs indicating sino-nasal disease (acute localized pain, including pain radiating to the eye, nasal ulcer with black eschar and extension from the paranasal sinus across bony barriers towards the orbit or brain) and a mycological evidence – recovery by culture or microscopic detection of fungal elements using non-sterile specimens (sputum, bronchial brush, bronchial aspirate, bronchoalveolar lavage or sinus aspirate). There are no specific *Mucorales* biomarkers to help within the diagnostic process (3-5).

Once discovered, mucormycosis dictates rapid onset of targeted treatment in order to limit destruction and consequent patient death. Unfortunately, effective antifungals are not widely available and carry great costs. Moreover, extensive surgical debridement is usually necessary for cure. Liposomal Amphotericin B is the first-line

choice, replacing the less tolerated deoxycholate formulation. Second-line antifungals are Isavuconazole and Posaconazole. Duration of treatment is not well defined, but it is advisable to continue until permanent reversal of immunosuppressant factors and complete response on imaging, which could mean weeks to months (4).

To sum up, COVID-19-associated mucormycosis poses a new threat to health systems worldwide but especially in low- and middle-income countries, where accessibility to health care is limited and resources are scarce. The CAM epidemic within the pandemic in India has brought international attention to this previously little-known illness and has definitely been a tough lesson, yet again, about how challenging COVID-19 is and how important it is to prevent it (22). ❑

DISCUSSION

Mucormycosis is a rapidly progressive and often fatal invasive illness in the absence of antifungal treatment. This does not seem to be our patient's case. Therefore, few questions are left to be answered: when did he acquire this fungal infection? What are patient's risk factors for developing invasive fungal disease? Which etiology was at first responsible for the right upper lobe consolidations? To what extent has each of the microbial agents contributed to such a tissue damage?

First agents to be isolated from sputum just a few days after CT scan proved formation of multiple lung opacities were two species of *Candida*, *C. albicans* and *C. krusei*. It is common knowledge that recovery of *Candida* from sputum is almost never indicative of underlying pulmonary candidiasis and usually represents colonization, but, more importantly, we also found *C. krusei* in blood culture, which is clinically significant since this species is not a usual skin contaminant. Hence, we can fairly assume that the patient developed invasive candidiasis at the moment of his deterioration, based on few risk factors: use of parenteral glucocorticoids, antibacterial agents and establishment of new diagnosis of diabetes mellitus. However, no trace of typical target-like abscesses in liver or spleen was noticed on abdominal CT, nor patient accused vision impairment or neurological symptoms, in order to suspect dissemination within the eye or brain. Besides candidemia, the fungus could have triggered pulmonary affliction with cavity formation, as also described in literature (23).

At the same time, *K. pneumoniae* was also identified from sputum by means of multiplex PCR, which was a significant finding, due to the organism's predilection for upper lobes abscesses formation in alcoholic and diabetic individuals (24), similarly to our case. Its contribution is also suggested by elimination of dark red sputum for a few days, resembling hemoptysis witnessed in pneumonia caused by this Gram-negative bacillus.

Hemoptysis is also an important marker for mucormycosis, which is an angioinvasive disease. Together with the first CT scan description of multiple nodular opacities which soon after excavated to form cavities, lesions that are both suggestive for this fungal infection (3-5), it may support diagnosis of pulmonary mucormycosis from the beginning of clinical deterioration. Procalcitonin levels may not rise with localized infections such as abscesses (25), as noted in our case (although it usually pleads for bacterial over fungal etiology), whilst panfungal β-D-glucan test has no use in indicating *Mucorales* presence, since this genus wall does not contain the antigen. Our patient tested repeatedly negative for β-D-glucan, which instead might sustain mucormycosis by excluding other fungal etiologies. This is also a counterargument for presence of invasive candidiasis, which should have triggered an important raise in levels of this marker, but exceptions may occur. Notably, first determination of this marker was made under effective treatment covering *Candida krusei,* four days prior to negative control blood culture.

Then, starting May 6th until surgical removal of the right lung, *A. baumannii* has been persistently recovered from sputum and also identified from multiplex PCR assay. By the time of its first isolation, multiple pulmonary cavities had already been formed. Therefore, it is fair to assume that it did not contribute to initial consolidation, but instead aggravated lesions' progression. Literature confirms that *A. baumannii* may cause multiple lung abscesses (26).

We cannot exactly determine when mucormycosis developed, but identification of risk factors that may have led to it is warranted. First, our patient has never experienced neutropenia, which is more frequently associated to pulmonary involvement. Instead, he had impaired neu-

trophils function due to glucocorticoid usage. Also, he was a diabetic patient with only partially insulin-controlled glycemic values, since glucocorticoids impair this balance as well. Poorly controlled diabetes mellitus is more commonly linked to the rhino-sinusal and rhino-orbito-cerebral manifestations, as predominantly noticed in India's post-COVID-19 mucormycosis cases (3), but exceptions do exist.

Another particular role belongs to hyperferritinemia. High levels of ferritin and IL-6 have been largely noticed in hyperinflammatory states of COVID-19. IL-6 stimulates production of ferritin and hepcidin, which in turn determines sequestration of iron, with secondary anemia (which our patient had). Increased cellular iron load eventually causes tissular damage and free iron release into the circulatory system where it becomes available for *Mucorales* (3). To continue, prolonged usage of Voriconazole may lead to breakthrough mucormycosis, since it does not cover *Mucorales* but inhibits growth of most other fungi. Lastly, as shown within the review, COVID-19 has proven to be a risk factor in itself. Anyways, given the fact that our patient's pulmonary damage was yet less severe than that of literature descriptions, it is reasonable to affirm that his case was a more slowly progressing and atypical one, possibly explained by lack of causes for severe immunosuppression such as hematologic malignancy or transplantation.

In the end, we chose not to provide secondary prophylaxis for our patient since damaged tissue had been surgically removed with no new lesions formation and predisposing factors had been slowly eliminated – no use of glucocorticoids at discharge and better controlled glycemic values. Ferritin was still high, but it is usually expected to decrease slowly. The patient was referred to a pulmonologist for periodic check-ups. ❑

CONCLUSION

Undoubtedly, COVID-19 represents a real challenge for both medical practitioners and researchers. Posing a threat not only by means of its unique pathophysiology, which leads to extensive lung damage and multiorgan impairment, it also represents a new predisposing factor for severe secondary infective complications. Amongst them, mucormycosis with different localizations is to be sought with a high index of suspicion, since early diagnosis and targeted treatment greatly improve prognosis. Our clinical case is proof that daily practice encounters with CAM are not uncommon and that complex management is efficient, therefore effort should be invested in establishing the correct diagnosis whenever possible. ❑

Conflicts of interest: none declared.

Financial support: none declared.

Acknowledgements: We thank our collaborator Dr. Andrei Dobrea, Thoracic Surgery Clinic, Bucharest University Emergency Hospital, for providing surgical treatment and intraoperative images (Figure 4). We also thank Dr. George Simion, Anatomopathology Clinic, Bucharest University Emergency Hospital, for facilitating microscopic diagnosis in our case.

References

1. **Kontoyiannis DP, Lewis RE.** Agents of Mucormycosis and Entomophthoramycosis. In: Bennett JE, Dolin R, Blaser MJ, ed. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 9th ed. Philadelphia: Elsevier, 2020, pp 3117-3130.
2. **Spellberg B, Ibrahim AS.** Mucormycosis. In: *Kasper DL, Fauci AS, ed. Harrison's Infectious Diseases*. 3rd ed. New York: McGraw-Hill Education Medical, 2017, pp 1020-1031.
3. **Rudramurthy SM, Hoenigl M, Meis JF, et al.** ECMM/ISHAM recommendations for clinical management of COVID-19 associated mucormycosis in low- and middle- income countries. *Mycoses* 2021;64:1028-1037.
4. **Cornely OA, Alastruey-Izquierdo A, Arenz D, et al.** Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *Lancet Infect Dis* 2019;19:e405-e421.
5. **Donnelly JP, Chen SC, Kauffman CA, et al.** Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis* 2020;71:1367-1376.
6. **Ritchie AI, Singanayagam A.** Immunosuppression for hyperinflammation in COVID-19: a double-edged sword? *Lancet* 2020;395:1111.
7. **Remy KE, Mazer M, Striker DA, et al.** Severe immunosuppression and not a cytokine storm characterizes COVID-19 infections. *JCI Insight* 2020;5:e140329.
8. **Leisman DE, Ronner L, Pinotti R, et al.** Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes.

Lancet Respir Med 2020;8:1233-1244.
9. **Chen LYC, Hoiland RL, Stukas S, et al.** Confronting the controversy: interleukin-6 and the COVID-19 cytokine storm syndrome. *Eur Respir J* 2020;56:2003006.
10. **The REMAP-CAP Investigators.** Interleukin-6 Receptor Antagonists in Critically Ill Patients with COVID-19. *N Engl J Med* 2021;384:1491-1502.
11. **The RECOVERY Collaborative Group.** Dexamethasone in Hospitalized Patients with COVID-19. *N Engl J Med* 2021;384:693-704.
12. **Tang Y, Liu J, Zhang D, et al.** Cytokine Storm in COVID-19: The Current Evidence and Treatment Strategies. *Front Immunol* 2020;11:1708.
13. **Abdoli A, Falahi S, Kenarkoohi A.** COVID-19-associated opportunistic infections: a snapshot on the current reports. *Clin Exp Med* 2021 (ahead of print: cited 2021 Oct 15); (20 p.). doi:10.1007/s10238-021-00751-7
14. **Arastehfar A, Carvalho A, Nguyen H, et al.** COVID-19- associated Candidiasis (CAC): an Underestimated Complication in the Absence of Immunological Predisposition? *J Fungi* (Basel) 2020;6:211.
15. **Lai CC, Yu WL.** COVID-19 associated with pulmonary aspergillosis: a literature review. *J Microbiol Immunol Infect* 2021;54:46-53.
16. **Markova M, Brodska H, Malickova K, et al.** Substantially elevated C-reactive protein (CRP), together with low levels of procalcitonin (PCT), contributes to diagnosis of fungal infection in immunocompromised patients. *Support Care Cancer* 2013;21:2733-2742.
17. **Roques M, Chretien ML, Favennec C, et al.** Evolution of procalcitonin, C-reactive protein and fibrinogen levels in neutropenic leukemia patients with invasive pulmonary aspergillosis or mucormycosis. *Mycosis* 2016;59:383-390.
18. **Garg M, Prabhakar N, Muthu V, et al.** CT Findings of COVID-19-associated Pulmonary Mucormycosis: A Case Series and Literature Review. *Radiology* 2022;302:214-217.
19. **Garg D, Muthu V, Seghal IS, et al.** Coronavirus Disease (Covid-19) Associated Mucormycosis (CAM): Case Report and Systematic Review of Literature. *Mycopathologia* 2021;186:289-298.
20. **Mohammadi F, Badri M, Safari S, et al.** A case report of rhino-facial mucormycosis in a non-diabetic patient with COVID-19: a systematic review of literature and current update. *BMC Infect Dis* 2021;21:906.
21. **Dilek A, Ozaras R, Ozkaya S, et al.** COVID-19-associated mucormycosis: Case report and systematic review. *Travel Med Infect Dis* 2021;44:102148.
22. **Selarka L, Sharma S, Saini D, et al.** Mucormycosis and COVID-19: An epidemic within a pandemic in India. *Mycoses* 2021;64:1253-1260.
23. **Yasuda Y, Tobino K, Asaji M, et al.** Invasive candidiasis presenting multiple pulmonary cavitary lesions on chest computed tomography. *Multidiscip Respir Med* 2015;10:11.
24. **Baudrand H, Mbatchou Ngahane BH, Marcu M, et al.** Pneumopathie communautaire abcédée à Klebsiella pneumoniae (Lung abscess due to community acquired Klebsiella pneumoniae). *Rev Mal Respir* 2009;26:773-778 (French).
25. **El-Azeem AA, Hamdy G, Saraya M, et al.** The role of procalcitonin as a guide for the diagnosis, prognosis, and decision of antibiotic therapy for lower respiratory tract infections. *Egypt J Chest Dis Tuberc* 2013;62:687-695.
26. **Kokkonouzis I, Christou I, Athanasopoulos I, et al.** Multiple lung abscesses due to Acinetobacter infection: a case report. *Cases J* 2009;2:9347.