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Review Article

COVID-19 associated mold infections: Review of COVID-19 associated pulmonary aspergillosis and mucormycosis

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Abbreviations: ACE2, Angiotensin-converting enzyme 2; BALF, Broncheoalveolar lavage fluid; CAM, COVID-19 associated mucormycosis; CAPA, COVID-19 associated pulmonary aspergillosis; GM, Galactomannan; non-BALF, Fluid obtained from lower respiratory tract by non-BAL procedure; non-CAPA, Patients with SARS-CoV-2 virus infection who did not develop CAPA.

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Abstract COVID-19-associated mold infection (CAMI) is defined as development of mold infections in COVID-19 patients. Co-pathogenesis of viral and fungal infections include the disruption of tissue barrier following SARS CoV-2 infection with the damage in the alveolar space, respiratory epithelium and endothelium injury and overwhelming inflammation and immune dysregulation during severe COVID-19. Other predisposing risk factors permissive to fungal infections during COVID-19 include the administration of immune modulators such as corticosteroids and IL-6 antagonist. COVID-19-associated pulmonary aspergillosis (CAPA) and COVID-19-associated mucormycosis (CAM) is increasingly reported during the COVID-19 pandemic. CAPA usually developed within the first month of COVID infection, and CAM frequently arose 10—15 days post diagnosis of COVID-19. Diagnosis is challenging and often indistinguishable during the cytokine storm in COVID-19, and several diagnostic criteria have been proposed. Development of CAPA and CAM is associated with a high mortality despiteappropriate anti-mold therapy. Both isavuconazole and amphotericin B can be used for treatment of CAPA and CAM; voriconazole is the primary agent for CAPA and posaconazole is an alternative for CAM. Aggressive surgery is recommended for CAM to improve patient survival. A high index of suspicion and timely and appropriate treatment is crucial to improve patient outcome.

Introduction

Invasive mold infections (IMI) associated with COVID-19 are emerging as severe complications with high mortality. Diagnosis and treatment of IMIs due to two common filamentous fungi, *Aspergillus* and *Mucorales*, respectively, are challenging. Typically, IMI develops in immunosuppressed patients, such as hematopoietic stem cell or organ transplant recipients, those who have prolonged agranulocytosis, in patients with severe lymphopenia during acute illness such as influenza associated respiratory distress, or in those with severe sepsis requiring intensive care unit (ICU) support.

COVID-19 is characterized by a profound inflammation initiated by damaged alveolar cells, and injury to respiratory epithelium and vascular endothelium, in which monocytes and neutrophils play a key role.[1,2] In severe COVID-19 disease, delayed or poor induction of interferon (IFN) response or the presence of auto-IFN antibodies was associated with increased viral replication and induction of hyperinflammation.[2] Immunosuppressive therapy with corticosteroids or anti-interleukin-6 (IL-6) blockers can help to control the inflammation in COVID-19,[3,4] but predisposes patients to fungal superinfection.[5,6] The pathogenesis of fungal super-infection in COVID-19 is facilitated by the loss of alveolar epithelium barrier and dysregulated innate and cellular immunity.[7] Distinguishing COVID-19-associated pulmonary aspergillosis (CAPA) or COVID-19-associated mucormycosis (CAM) from a concurrent proinflammatory cytokine storm in COVID-19 is difficult. Timely identification and treatment of CAPA or CAM is important for improving patient outcome, especially in areas with a high prevalence of aspergillosis and mucormycosis.

To date, several diagnostic criteria for CAPA and CAM have been proposed by several groups.[8—11] However, there is a lack of consensus on which criteria is more appropriate or applicable. In addition, in the setting of COVID-19, invasive diagnostic procedures are limited due to concerns with transmission of SARS-CoV-2. Herein, we reviewed studies of CAPA and CAM in COVID-19 patients regarding the epidemiological data, diagnostic laboratory indicators, clinical characteristics, risk factors, prognostic factors and treatment strategy from the published articles in PUBMED, MEDLINE, and Google Scholar databases during the period of January 2020 to March 2022. We also introduce the pathogenesis and role of SARS-CoV-2 in CAMI by a brief review.

Covid-19 associated pulmonary aspergillosis (CAPA)

Pathogenesis

A brief summary of the pathogenesis of CAPA is shown in Fig. 1.The first impact of COVID-19 virus is interruption of the respiratory ciliary[12] and epithelium system.[1] The damaged pneumocytes and disrupted lung epithelial barrier attract neutrophils and monocytes released extracellular trap, resulting in disjunction between endothelial cells.[2] SARS-CoV-2 infection also leads to endothelial cells activation, swelling, and destruction, then thrombus formation in endovascular systems.[13] After SARS-CoV-2 infection, the disrupted lung epithelial barrier creates an environment conducive for fungus hyphae invasion into lung parenchyma.[14] The link between the two pathogens is the sharing surface markers that recognize both the virus and *Aspergillus*. SARS-CoV-2 infection can activate Toll-like receptor 4 (TLR4) expression that further induces ACE2 expression.[15] The *Aspergillus* molecules such as galactosaminogalactan (GAG), melanin, galactomannan (GM), and chitins[16] have an affinity to bind or modify the function of lung epithelial cells, macrophages, T and B cells, C-C chemokine receptor type 2 presenting (CCR2+) monocytes, and endothelial cells[17] through pattern recognition receptors (PRRs) such as macrophage inducible Ca-dependent lectin receptor (Mincle), dectin-1, dectin-2, NOD-like receptors (NLRs), TLR2 and TLR4.[18] The galactomannan and beta-glucan of *Aspergillus* further diminish the TLR4-mediated immunity, such as phagocytosis.[19] *Aspergillus* is also known as a pathogen capable of both endothelium invasion and angiogenesis.[20,21] Proteomic study suggests that proteins expressed by *Aspergillus*-infected endothelial model are involved in apoptosis and affects adaptive and innate immunity.[21] In patients complicated with CAPA, the ACE2, hypoxia-inducible factor 1α (HIF-1α), cytokines including interleukin 1-beta (IL-1B), tumor necrosis factor-α (TNF-α) were also significantly more expressed compared to those in non-CAPA patients.[14,19] As a result, a vicious cycle between COVID-19 and *Aspergillus* increased the risk of mutual infection and aggravates the severity of disease.

The immunomodulatory effect of the SARS-CoV-2 associated with fungal infection was by both innate and adaptive immunity. Once entering the host cells, the virus can modulate the expression of type I and III interferon,[22] decrease production of IFN-γ,[23,24] and affect the activation of antigen presenting cells who presented with PRRs,

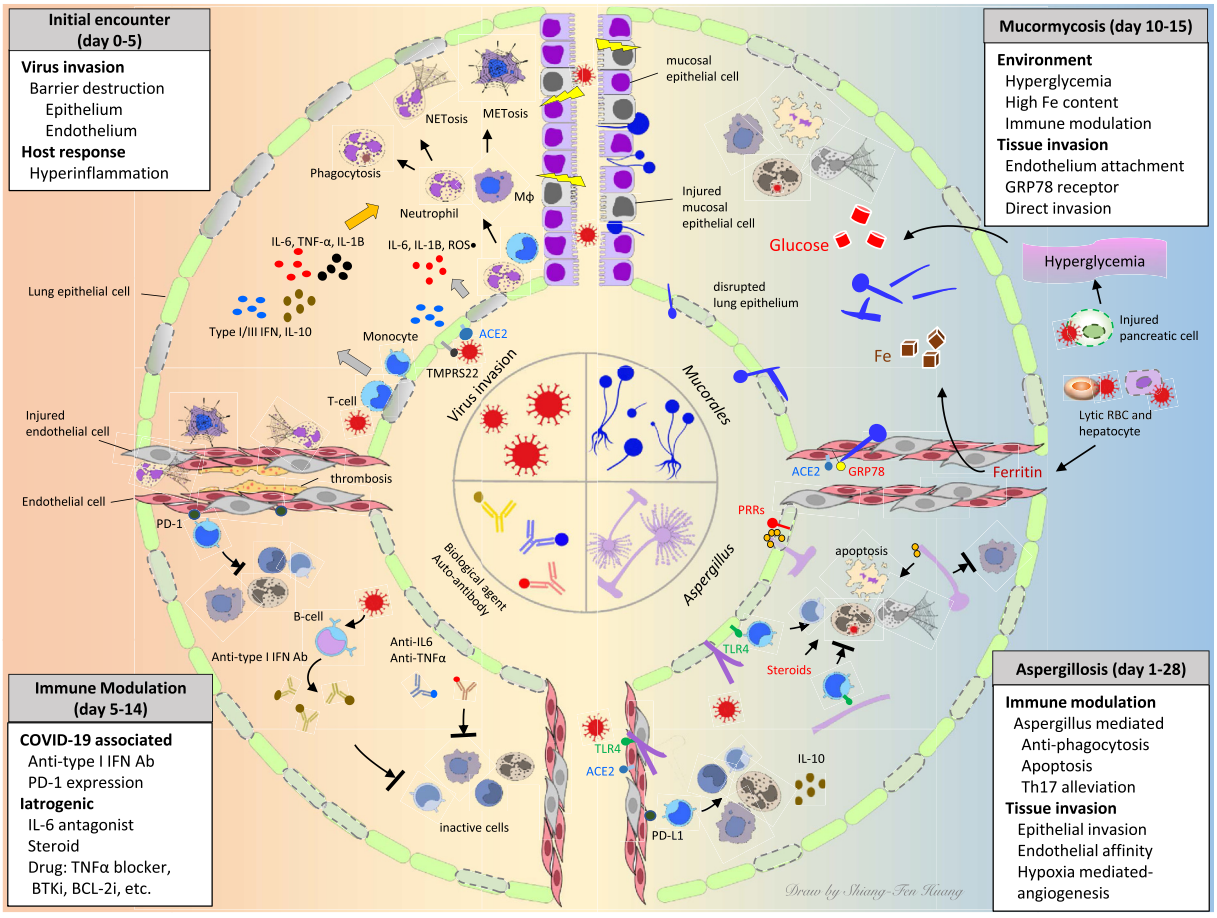

Figure 1. Pathogenesis of CAPA and CAM **Abbreviations:** ACE2: Angiotensin-converting enzyme 2, BCL-2i: B-Cell Lymphoma 2 inhibitors, BTKi: Bruton's Tyrosine Kinase inhibitors, GRP78: glucose-regulated protein 78, IFN: interferon, IL: interleukin, METosis: macrophage extracellular trap generation, Mφ: macrophage, NETosis: neutrophil extracellular trap generation, PD-1: program cell death protein 1; PD-L1: program cell death protein-ligand 1, TMPRS22: transmembrane protease serine 22, ROS•: reactive oxygen species, TLR4: Toll-like receptor 4, Th17: T-helper 17 cell immune cascade, TNFα: tumor necrosis factor *alpha*.

such as TLR4, TLR7, and TLR8[25]. The SARS-CoV-2 resulted in increased NADPH cascades and ROS oxidative stress system[26,27] related to overwhelmed inflammatory reaction. However, SARS-CoV-2 infection led to decreased protective cellular immunity through increased expression of programmed cell death protein 1 (PD-1) expression[28—30] and IL-1B cascades.[25] The *Aspergillus* fungal wall alpha-(1,3)-Glucan also increases expression of ligand of PD-1 (PD-L1) on human endothelial cells.[31] The use of PD-L1 blockade could improve anti-SARS-CoV immune abnormalities,[30] and showed beneficial effect when co-administered with antifungal agent in an invasive pulmonary aspergillosis model.[32] Meanwhile, the B cells may produce neutralizing autoantibody against type I IFN (IFN-α2, IFN-ω, IFN-β)[33] especially in patients aged >70 and with higher mortality. Interestingly, the type I IFN (IFN-β)-mediated dendritic cell can stimulate protective T-cells with anti-*Aspergillus* ability[34]; that type I IFN may be considered an adjuvant therapy for severe COVID-19 in the future.[35]

Before the era of COVID-19, increased *Aspergillus* infections has been observed during treatment with target therapy, such as Bruton's Tyrosine Kinase inhibitors (BTKi),[36,37] B-Cell Lymphoma 2 (BCL-2) inhibitors,[38,39] medication those used for hematological malignancy,[40] and immune modulators for rheumatoid arthritis (RA).[41,42] The Tocilizumab aimed to suppress inflammatory response in RA, sepsis, and COVID-19 by inhibiting overwhelmed inflammation activities,[43] phagocytosis,[44] restore endothelial dysfunction and abridged neutrophil extracellular trap (NET) generation,[45] which were essential for anti-*Aspergillus* infection.[46.47] Theoretically, tocilizumab may increase the incidence of *Aspergillus* infection;[42,48] however, large scale epidemiological report for increased aspergillosis in patients with RA who received Tocilizumab therapy was lacking. Methylprednisolone resulted in broad-spectrum effect on cellular and adaptive immunity, such as increasing ROS system with tissue destruction in IA.[49] Steroids also inhibit phagocytosis by increasing apoptosis of neutrophil,[50] alleviate CD4+, Th1+, Th17+ lineage T cells immunities, which are essential against *Aspergillus* infection.[51] Beeke Tappe et al. demonstrated the recent corticosteroid exposure might predispose common COVID-19 and *Aspergillus* co-infection through diminished cellular immunity and exhausted T cells.[52]

Epidemiology

The incidence of CAPA varied by countries, and most studies reported an observation period for 1 month to 6 months during the year 2020. In a meta-analysis including 28 studies with 3184 COVID-19 patients admitted to the ICU,[53] the cumulative incidence for CAPA was 12.3% (294/3184), and were the highest in central Europe and Asia countries (21—31.4%), followed by UK, Spain, south Europe,[54] and north American countries (<10%). Jon Salmanton-García et al. reported the incidence of CAPA in ICU patients to be 6.8% (131/1902) in 19 facilities across Europe, Mexico, Pakistan, Argentina, and the United Kingdom. Patients included those with suspected IA by literature review (160 patients) and reported in the FungiScope registry databases (129 patients), from March—August 2020.[55] The incidence of CAPA in those with ICU admission was 0.1% (2/163) in South America and 20% (3/15) in Ireland. Notably, seven cases of CAPA registered in FungiScope databases were from South America, mid Asia, and Australia.[55]

Risk factors for developing CAPA

Older age is the most common host factor for developing CAPA.[56—58] In a multicenter study, requirement for respiratory support, anti-IL-6 monoclonal antibody (Tocilizumab),[56,57] initial disease severity and respiratory distress were risk factors for developing CAPA.[56—58]

In the subgroup analysis among patients with ICU stay, host factors for CAPA included underlying COPD, bronchiectasis, HIV infection, liver cirrhosis, cardiovascular disease, active malignancy status, older age, and solid organ transplant recipient[57,58,]. Iatrogenic risk factors included use of T or B cell immunosuppressants, dexamethasone plus anti-IL-6 therapy,[59] long-term corticosteroids[60] and renal replacement therapy (RRT) during ICU stay.[57]

Clinical characteristics

The onset of CAPA ranged from the first day to the 28th day after ICU admission, with a mean of 7.3 days.[53,61] Patients with CAPA had higher SOFA scores, shorter duration from illness to ICU admission (mean 11 $\pm$ 2.5 days),[60] a higher probability for ventilation support requirement, ECMO support,[56] and renal replacement therapy.[57,60] Overall, patients with CAPA had a higher mortality than those without CAPA.[56,57,62,63] The utility of laboratory biomarkers (such as C-reactive protein, ferritin, ESR, or creatinine) in the diagnosis of CAPA have not been evaluated. Current literature suggests that every patient with respiratory distress and without treatment response or deterioration are potentially at risk for CAPA.[11]

Mycological evidence of *Aspergillus spp.*

Culture

The culture positivity rate of *Aspergillus* species in respiratory specimens varied widely across different studies and populations. The lowest rate was 42% in bronchoalveolar lavage fluid (BALF) or innon-BALF in a multicenter study,[57] and a higher positive rate of 90% was found in cases with a previous history of airway diseases[64] (Table 1). In a review of 28 published studies with 134 CAPA cases reported during Jan to Oct, 2020 [63], 101 cases had cultures positive for *Aspergillus*. The most frequently identified species were *Aspergillus fumigatus* (86%), followed by *Aspergillus flavus* (5.9%), *Aspergillus niger* (4.9%), and other (3.9%). Less than 5% of patients with CAPA were confirmed by histology. Another review article summarized 85 patients with CAPA reported from Jan to April, 2020; 66% of them were culture positive with *A. fumigatus*, followed by *A. flavus* (9%), and 21% were un-identified *Aspergillus*.[65] There is a regional variation in the most commonly identified *Aspergillus* species.[58] In European countries, *A. fumigatus* was the most frequently isolated species. In Pakistan, a higher proportion of cultures isolated was *A. flavus* (60%). *A. flavus* accounted for 10—16% of identified species in Belgium, Netherlands, and 0—10% in other European countries such as in France, Germany, and Austria.[66] Non-*fumigatus* and non-*flavus*

Table 1 The positive rates of mycological examinations in the cases with and without COVID-19-associated pulmonary aspergillosis after SARS-CoV-2 infection.[57,63,64,67,82]

Examination	Positive rate (%)	
	CAPA[a]	non-CAPA
Histopathology[b]	4.1	0
Growth of *Aspergillus* spp.[c]	42—90	0—67
Serum		
BDG (>80)	40.9—58	0
Galactomannan index (>0.5)[d]	11—33	0—19
PCR	30.7—56	0—15.8
Respiratory samples [e]		
Galactomannan index (>1.0)	38—100	53.3
PCR	66.6	42
BALF		
Galactomannan index (>1.0)	46—100	50—68
PCR	17—89	2—83
Other respiratory sample[f]		
BDG (+)	42	66
Galactomannan index (>1.0)	92.5	66
PCR	15—100	—

[a] CAPA included proven, probable, possible, and putative CAPA according to different diagnostic algorithm.
[b] Proven pulmonary aspergillosis with presence of filaments hyphae in lung tissue through bronchoscopy, concurrent with positive Galactomannan index or culture positive for *Aspergillus spp*. in BALF or bronchial aspiration.
[d] With significant difference in the mortality rate, if serum galactomannan index is > 0.5 in the cases of CAPA.[63]
[c] Please see reference [57,64,67,82] and the description in section Mycological evidence of *Aspergillus spp*..
[e] Respiratory samples by any type of specimens, including BALF, non-BALF, trachea aspiration, and others.
[f] Respiratory samples collected by method other than BAL, including tracheal aspiration and lower respiratory tract fluid by non-BAL procedure.
Abbreviations: BDG: Beta-D-glucan, BALF: bronchioalveolar lavage fluid; CAPA: Covid-19-associated pulmonary aspergillosis; PCR: polymerase chain reaction for *Aspergillus*. non-CAPA: Patients with SARS-CoV-2 virus infection who did not develop CAPA.

Aspergillus were frequently found in America and Spain, such as *Aspergillus citrinoterreus*, *Aspergillus lentulus*, and *Aspergillus nidulans*, which had been reported with >10% frequency in isolated *Aspergillus* in Spain.[67]

The rate of azole resistance by detecting mutations in the TR34/L98H gene *cyp51A* in *A. fumigatus* was 3.4% (3/87) in a systemic review.[63] Another longitudinal survey of 27 *Aspergillus* strains from CAPA in a German ICU reference center reported 3.7% azole resistance with a TR34/L98H mutation.[68] Recently, a single center study of 329 ICU patients during March—April, 2020 in Austria,[69] found that all 22 *A. fumigatus* colonies from respiratory samples of CAPA patients were susceptible to caspofungin (CAS), isavuconazole (ISZ), voriconazole (VOR), posaconazole (POS), micafungin (MIC), and amphotericin B (AmphB) by E-test.

The source of *Aspergillus* has not been comprehensively investigated; however, a comparison of 35 *Aspergillus* isolates by genotyping for *cyp51A* gene from respiratory samples of CAPA patients and 8 strains from ICU environment found no correlation. This suggested that CAPA was unlikely to be hospital acquired but rather may be due to pre-colonization in the host or may be community-acquired infection.[70]

Current consensus for diagnosis of CAPA includes a positive culture for *Aspergillus* species, with compatible radiological imaging and clinical manifestations. Histological findings are not mandatory for diagnosis, considering the possible transmission risks of SARS-CoV-2 when performing invasive diagnostic procedures. Nearly 60—90% of patients with CAPA reported in the literature had a positive culture of *Aspergillus* from their respiratory tract specimens.[63,64] Despite a low rate of antifungal resistance (<5%), treatment response was poor, and mortality remained high. In a small case series, a high mortality of 50%—71% was reported, even though appropriate antifungal therapy with documented susceptibility was administered.[69] A case report of an HIV-infected patient with CAPA due to infection with a multi-drug resistance *A.* spp. died despite use of combination anti-fungal regimen.[71] In a meta-analysis enrolling 134 cases of CAPA,[63] none of the mycological factors were associated with mortality.

Biomarkers

Beta-D-glucan (BDG) is a biomarker for pan-fungal infection used for early diagnosis of invasive fungal disease (IFD). Although it is not specific for *Aspergillus* infection, several meta-analyses and reviews showed a higher positivity rate of BDG than serum galactomannan (GM) antigen in CAPA patients.[63]

GM antigen test is used to detect invasive aspergillosis; however, it has cross reactivity with other fungal infections, such as *Penicillium* and *Fusarium* spp.[72] GM index (>0.5) in serum may represent invasive aspergillosis and is associated with a higher mortality in CAPA.[63] GM index (>1.0) in BALF[73—75] or non-BAL aspiration[11] were considered a biomarker for diagnosis of invasive pulmonary aspergillosis (IPA). The level of GM index in BALF decreases in response to voriconazole - based therapy in CAPA.[76]

Rapid molecular detection methods using PCR to target internal transcribed spacer region (ITS1), 28S rRNA, and 18S rRNA genes[77] had been used for detection of *Aspergillus*[78] and diagnosis of IA.[79] AsperGenius® can identify *cyp51A* gene of *A. fumigatus* with TR34/L98H and TR46/Y121F/T289A[80] mutations; MycoGENIE® can detect TR34/L98H,[81] and FungiPlex® can detect TR34/TR46[81] mutation for azole resistance. Other commercial kits have additional benefits in identification of non-*fumigatus Aspergillus* species; for example, *Aspergillus* spp. ELITe MGB® Kit uses a quantitative qPCR method for 18S rRNA and had been used for diagnosis of CAPA.[54] Although an added benefit in PCR detection of *Aspergillus* directly from serum and BALF was described,[68] a comparison evaluating the accuracy of different kits to detect CAPA has not been published. There is currently no consensus on which commercial method is better for detection of CAPA or for non-COVID-associated invasive aspergillosis.

Currently, multi-model detection of CAPA by fungus culture, biomarkers in respiratory samples (PCR, GM, or BDG), and in serum (PCR, GM or BDG) is suggested to improve diagnostic sensitivity. A higher mortality has been associated with the number of positive mycological criteria in patients with CAPA. In patients with CAPA fulfilling four mycological criteria, a lower survival was found compared

to those with less than two mycological criteria.[64] Significant predictors of mortality in CAPA include positive biomarkers in blood, such as PCR (odds ratio (OR): 11.67, 95% confidence interval (CI): 3.06—55.5) or GM index(OR: 5.79, 95% CI: 1.98—16.9)[63], and a concordant positive culture and biomarker-based mycological evidence.[69] Giacobbe et al. compared different methods and found that a positive GM index concurrent with a positive culture of *Aspergillus* in BALF was a prognostic factor for 90-day mortality in CAPA.[76,82]

Among various mycological examinations, the highest proportion with positive findings for CAPA was the fungus culture, followed by BDG, GM index in BALF and then in serum (Table 1). PCR methods provided an accurate and rapid identification for specific *Aspergillus*, and were included in the recent diagnostic criteria for CAPA.[11,75] However, the sensitivity of the PCR method varies with the PCR kit and DNA extraction procedure used.

Diagnosis

A surveillance for CAPA is suggested in individuals with clinical deterioration and abnormal chest images.[11] To maximize the sensitivity and specificity, mycological evidence by fungus culture and biomarkers in serum or lower respiratory tract using BAL or non-BAL procedure were suggested in several guidelines.[11,73—75,83] The BAL procedure was often unfeasible in patients with COVID-19, and alternative methods such as tracheal aspiration and non-BAL fluid (non-BALF) acquired from the lower respiratory tract were acceptable substitutes to aid in the diagnosis of CAPA.

In published articles regarding CAPA, commonly used criteria include revised EORTC/MSG criteria,[75] influenza-associated PA criteria (IAPA),[74] AspICU algorithm,[83] BM-AspICU algorithm,[10] BM-AspICU with severe influenza infection[73] and ECMM/ISHAM consensus criteria.[11] The host population constitute the major differences among them. For EORTC/MSG criteria, the invasive pulmonary aspergillosis (IPA) is defined in population with prolonged neutropenic fever or immunocompromised status. AspICU is the earliest diagnostic algorithm to define patients in ICU and with host factors for developing IPA, and only consider *Aspergillus* culture from lower respiratory tract (LRT) as mycological evidence. The BM-AspICU employed GM test as biomarker to increase the diagnostic sensitivities, and IAPA criteria is specific for patients with influenza co-infection. Diagnostic criteria for CAPA by ISHAM/ECMM[11] is specific for COVID-19 complication, which is similar to IAPA criteria[74] in the characteristics described on chest imaging. Due to the high accuracy and rapid detection by PCR method, this was introduced into the modified EORTC algorithm[75] and ISHAM/ECMM criteria for CAPA.[11] A summary and comparison for different algorithms to define IPA and CAPA is summarized in Table 2. Both BM-AspICU and ISHAM/ECMM criteria consider the SARS-CoV-2 infection as host factor in the development of IPA and CAPA. Only the AspICU and BM-AspICU defined the condition of *Aspergillus* colonization, that were without indication for anti-fungal therapy; and the ECMM/ISHAM, EORTC/MSG and the IAPA criteria defined a new category *Aspergillus* tracheobronchitis, which also contributed to morbidity during COVID-19 infection.

Current antifungal therapy and predictors of clinical outcome

The overall mortality of patients with CAPA were from 43% to 71.4% and was higher than in patients without CAPA ($p < 0.05$).[53,62] The mortality in CAPA patients without anti-mold therapy ranged from 59% to 90%, compared to 38%—66.7% among CAPA patients who had received anti-mold treatment.[67,84] However, one meta-analysis including 20 studies demonstrated that none of the mycological factors affected mortality in CAPA.[62]

Primary anti-mold regimens used in the treatment of CAPA were diverse. Currently, there are no RCTs regarding which regimen is the most appropriate treatment for CAPA. One prospective, observational study described 14 patients with CAPA who received voriconazole, resulting in a lower mortality compared with those who received other anti-mold agents, though not reaching statistical significance (46% vs 59%, $p = 0.30$).[76] According to recent publications, the most common regimens used for CAPA included voriconazole, isavuconazole, and amphotericin B. One meta-analysis demonstrated that case fatality rates of CAPA were not significantly different between different triazoles, but a marginally inferior survival was found for anidulafungin therapy compared to other triazoles.[63] A phase 3 clinical trial for isavuconazole-based therapy for CAPA was initiated since 2021 (*ClinicalTrials.gov*: NCT04707703).

There is no consensus whether anti-mold prophylaxis is necessary in COVID-19 infection. Two small observational studies found that the administration of posaconazole or inhaled liposomal amphotericin-B could decrease the incidence of CAPA,[85,86] but the antifungal prophylaxis did not reduce the mortality.

COVID-19 associated mucormycosis (CAM)

Introduction

A rise in the incidence of mucormycosis was seen recently with the advent of the COVID-19 pandemic, and COVID-19 is increasingly regarded as a possible risk factor of mucormycosis. COVID-associated mucormycosis (CAM) cases are found worldwide, after a surge of cases reported in India with the second wave of COVID-19 epidemic after April 2021.

Mucormycosis is an uncommon but life-threatening invasive fungal infection caused by the mold in the order of Mucorales, which are rapidly growing saprophytic fungus that is ubiquitous in the environment. The most common genera causing human infections is *Rhizopus*, but also includes *Apophysomyces*, *Cunninghamella*, *Lichtheimia*, *Mucor*, and *Rhizomucor*.[8] Mucormycosis results from the inhalation of sporangiospores, which colonize or infect the nasal turbinates, then spread into the sinuses and palate, and subsequently invade into the orbital or cerebral areas. Ingestion of fungi may cause gastrointestinal mucormycosis, while fungal contamination of wounds can cause cutaneous mucormycosis. In immunocompetent individuals, the host neutrophils can inhibit and kill fungal hyphae.[87] The host respiratory cilia clear inhaled fungus into the gastrointestinal tract. These defenses are impaired in

Table 2 Summary of diagnostic criteria for invasive pulmonary aspergillosis (IPA).

	Revised EORTC/MSG (2020)	ISHAM/ECMM for CAPA	IAPA	AspICU	BM-Asp ICU
Published year [Ref.]	2020[75]	2021[11]	2020[74]	2012[83]	2021[10]
Category	proven, probable, possible	TBs: proven, probable. PA: proven, probable, possible	TBs and IAPA: proven, probable	Proven, putative, colonization	Proven, Probable, possible, colonization
Host factors	immuno-compromised	COVID with ICU, temporal entering criteria	ICU with influenza	ICU with immuno-compromised	ICU, immuno-compromised
Particular pattern on chest image	Wedge shaped, consolidation, tracheobronchitis	Cavity, tracheo bronchitis, non-specific	Cavity, airway plaques, and others	Non-specific	Any infiltrations
Aspergillus culture				[g]	
Lower respiratory tract, trachea	✓	✓	✓	Entry criteria	
BALF	✓	✓	✓	a	✓
Hyphae (in LRT)	✓	✓	✓	b	
Tissue[f]					[h]
Presence of hyphae					c
Culture +					d
Serum/plasma/blood					
Galactomannan index	≥0.5	>0.5	>0.5		≥0.5
PCR	Positive[e]	plus PCR+ in BALF			
BALF/non-BALF					
Galactomannan index	≥1.0, or serum ≥0.7 and BALF ≥0.8	≥1.0, or non-BALF >4.5 once. or non-BALF >1.2 for ≥2 times; or ≥1.0 plus GM index> 0.5 in blood	≥1.0		≥1.0
PCR	Positive^	Ct < 36			

[e] : Two or more duplicate positive tests.
[f] : Proven a[f]pergillosis in modified EORTC, ISHAMM/ECMM, and IAPA algorithm if the histopathology showed hyphae compatible with *Aspergillus* or with culture positive for *Aspergillus* spp. from tissue specimens.
[g] : Mycological evidence for putative IPA: *Aspergillus* culture positive from LRT (entry criteria), and (a) semiquantitive culture from BALF without bacterial growth plus (b) cytological smear showing branching hyphae.
[h] : Proven IPA by BM-AspICU criteria if (c) dichromats septate hyphae in histology concurrent with (d) culture positive in *Aspergillus* from tissue.
Abbreviations: BALF: bronchoalveolar lavage fluid; Ct: Cycle threshold of *Aspergillus* qPCR; TBs: tracheobronchitis; LRT: lower respiratory tract; PA: pulmonary aspergillosis.

immunocompromised hosts. Traditional predisposing conditions for mucormycosis include diabetes mellitus, immunosuppression, hematologic malignancies, allogeneic hematopoietic transplantation, and trauma.

Pathogenesis

A number of factors have been hypothesized to predispose COVID-19 patients to mucormycosis. Firstly, uncontrolled diabetes and corticosteroid therapy were found to be closely linked with the development of CAM. However, the exact causal relationship between COVID-19 and mucormycosis in relation to diabetes and corticosteroid use remains unclear.[88] Hyperglycemia in COVID-19 may be caused by direct infection of the pancreatic islets cells, which express ACE2 entry receptors, by SARS-CoV-2 virus, leading to beta cell failure, and by systemic inflammation resulting in increased insulin resistance.[89] Moreover, corticosteroids used to treat severe COVID-19 infection requiring oxygen or invasive mechanical ventilation may exacerbate hyperglycemia by inducing insulin resistance and beta cell dysfunction. Under acidic and glucose-rich conditions[90] such as that during uncontrolled diabetes mellitus, diabetic ketoacidosis, COVID-19 infection and

with use of corticosteroids, *Rhizopus* hyphae can proliferate and thrive.[91—99]

Secondly, COVID-19 results in dysregulation of the immune system and lymphopenia with decreased CD4+ and CD8+ T cells,[100] which lowers the innate immune defenses against mucormycosis. Use of corticosteroids and immunomodulatory agents, such as anti-IL-6 antagonists to counter COVID-19-induced hyperinflammation also results in immunosuppression. Finally, virus-induced impairment in ciliary function[101] facilitate fungal spread.[102]

Thirdly, COVID-19 may induce an inflammatory state with elevated ferritin levels which predisposes to mucormycosis by increasing serum iron level. Iron is required for the growth of the fungus. In a cross-sectional study involving 63 patients in India, significantly higher levels of ferritin were seen in patients with more severe extent of mucormycosis.[103] COVID-19-induced inflammatory reactions may result in apoptosis of hepatocytes and release of stored labile iron and serum ferritin. The virus may also attack hemoglobin causing dissociation of porphyrins from iron, and induce dysregulation of hepcidin, which is an iron regulatory hormone. All this results in elevated free iron levels in patients with COVID-19.[104] The upregulation of host glucose regulated protein 78 (GRP78), an endothelial co-receptor mediating entry of SARS-CoV-2, may play a role in the endothelial invasion by *Mucorales* in CAM.[105]

Epidemiology and predisposing factors

The prevalence of mucormycosis in patients with COVID-19 admitted to the intensive care units (ICU) was 0.3%—0.8% in a national, multicenter, observational cohort study in France.[102] The predominant co-morbidities associated with CAM included diabetes mellitus (77.1%), hypertension (29.5%), and renal disease (14.3%).[106] Risk factors for CAM in mechanically ventilated COVID-19 patients include older age, treatment with dexamethasone and anti-interleukin-6 (IL-6) antagonists, and long duration of mechanical ventilation (>14 days).[102,106] More males (69.6%) than females (19.6%)[107] were affected, although this was reflective of the male predominance (65.39%) of COVID-19 cases in India.[108] The median time to diagnosis of mucormycosis after COVID-19 diagnosis was 10—15 days.[102,109]

Clinical manifestations

In a recent review of 80 cases of CAM from 18 countries worldwide, rhino-orbital cerebral mucormycosis was the most frequent clinical syndrome (74%), followed by pulmonary mucormycosis (25%), while concomitant disseminated mucormycosis (4%) and gastrointestinal mucormycosis was rare (1%).[102] The most common sites of involvement were the sinuses (79.4%), especially the maxillary sinus (47.4%); the orbits (56.7%) and the lungs (11.3%) were affected in another review of 99 cases.[106]

The clinical manifestations of mucormycosis included fever, nasal passage dryness, periorbital or facial pain, ptosis, proptosis, toothache, loss of tooth, blurry or double vision, and shortness of breath.[110] Neurological symptoms such as headache, confusion, and altered mental status can be found in the later stages.[110]

Diagnosis

Diagnosis of CAM is challenging since the clinical and radiologic findings may overlap with those of COVID-19.[102] Currently, conventional culture and histopathological demonstration of typical *Mucorales* hyphae, which are broad (5—25 μm), aseptate, with ribbon-like, irregular branching, and wide-angle bifurcation up to 90°, remains the mainstay of diagnosis. Histopathology may show an acute suppurative inflammation with focal areas of granulomatous inflammation, although this may be absent in immunosuppressed patients.[8] Invasion into the blood vessels with subsequent thrombosis and inflammation is hallmark for mucormycosis. The organism is very delicate; grinding or homogenization of tissues may destroy fungal hyphae, leading to negative culture results.[111] Blood cultures are rarely positive despite evidence of angioinvasion.

Imaging signs suggestive of pulmonary mucormycosis on computed tomography (CT) or magnetic resonance (MRI) include the halo sign, reversed halo sign, or a ring of ground glass opacity surrounding a nodular infiltrate that is indicative of focal ischemia. The "reverse halo sign" was suggestive of *Mucorales* infection and may be useful for the preemptive initiation of antifungal therapy but is not consistently detected (55%) and can be associated with other mold infections. Thrombosis may be seen on CT angiography.[8] In a study involving 50 patients with rhino-orbital-cerebral CAM, the most common imaging features on MRI were the black turbinate sign/non enhancing sinonasal mucosa (82%), followed by peri-antral soft tissue inflammation (74%) and extra-ocular muscle involvement (76%).[112] MRI has is more sensitive than CT in detecting early angioinvasion of mucormycosis.[113,114] Radiological changes may precede overt clinical symptoms.

Molecular diagnostic methods have a high potential for diagnosing fungal infections in early stages of the disease, though not clinically validated. Polymerase chain reaction (PCR)-based diagnostic methods on BAL fluid, biopsy specimens, and serum has been reported and appear promising. One study evaluated quantitative PCR of the serum in patients with non-COVID-associated mucormycosis with a sensitivity and specificity of 85.2% and 89.8% respectively.[115]

Treatment and prevention

Judicious use of corticosteroids and strict control of glucose in COVID-19 patients may prevent the development of CAM.[116] Once diagnosed, prompt antifungal therapy must be administered. Aggressive surgical treatment has been shown to improve outcomes.[109] Currently, there is a lack of evidence to support the use of iron chelators and hyperbaric oxygen as standard or salvage therapy in the treatment of mucormycosis.

Pharmacologic treatment of COVID-associated mucormycosis is similar to that of mucormycosis in general. Evidence for the treatment of mucormycosis is limited by the rarity of the disease. Global guidelines for the antifungal management of mucormycosis are based on that established by the 2017 European Conference on Infections in Leukemia (ECIL), with an upgrade in 2019 by the European Confederation of Medical Mycology (ECMM).[8] The first line

of treatment recommended is liposomal amphotericin B (L-AMB). A dosage of 5—10 mg/kg of L-AMB is suggested while a higher dose of 10 mg/kg is necessary in patients with central nervous system (CNS) involvement. ECIL guideline suggests that in the absence of CNS involvement, amphotericin B lipid complex (ABLC) may also be used.[117] Amphotericin B deoxycholate has been shown to be effective but is limited by substantial nephrotoxicity, especially given the prolonged duration of treatment at high doses as required in the treatment of mucormycosis.

Isavuconazole was approved by the United States and Europe as an effective oral and intravenous formulation to treat mucormycosis in 2015, following the results of the VITAL study, which showed clinical response rates comparable to that of amphotericin B.[118] Clinical success has been documented in a few patients treated with isavuconazole, either alone or in combination with amphotericin B.[119]

Posaconazole exhibits *in vitro* susceptibility against *Mucorales*. Both the tablet and intravenous forms are recommended by ECMM/ISHAM as an alternative treatment, combination treatment with amphotericin B, salvage therapy, or step-down therapy for mucormycosis. In the recent Indian epidemic of CAM, posaconazole was used as monotherapy in the treatment of a few cases of mucormycosis with varying success.[120]

Although *Rhizopus oryzae* expresses the target enzyme for echinocandins, the echinocandins do not exhibit *in vitro* susceptibility against mucormycosis. The use of echinocandins is not supported in major guidelines including ECMM, ISHAM, ECIL. The impact of combination antifungal therapy with echinocandins in humans are uncertain.

Treatment duration

The optimal treatment duration is unknown.[8] Guidelines from India generally recommend 4—6 weeks of amphotericin B therapy followed by consolidation therapy with posaconazole or isavuconazole for an additional 3—6 months.[121]

Prognosis

Mucormycosis is a grave disease which progresses rapidly and often have poor clinical outcome. Mortality is high for those with extensive involvement and nearly 100% die in cases of disseminated mucormycosis. For CAM, large case series revealed case fatality rates from 14%[94] to 45%.[120] No difference in mortality rates was reported between COVID and non-COVID cases of mucormycosis.[120]

Conclusions

COVID-19 infection is associated with an increased risk of invasive mold infections. During the pandemic of COVID-19 from 2020 to 2021, accumulating evidence demonstrated that CAMI contributed to high morbidity and mortality despite anti-mold therapy; however, the relationship between treatment response and antifungal resistance remains under investigation. Clinicians must maintain high clinical suspicion for these fungal diseases in COVID-19 patients, especially in those who have severe COVID-19 and multiple risk factors. Early diagnosis and aggressive medical and surgical therapy are necessary to improve patient outcome.

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References

1. Parasher A. COVID-19: current understanding of its pathophysiology, clinical presentation and treatment. *Postgrad Med* May 2021;**97**(1147):312—20.
2. Lamers MM, Haagmans BL. SARS-CoV-2 pathogenesis. *Nat Rev Microbiol* May 2022;**20**(5):270—84.
3. Salama C, Han J, Yau L, Reiss WG, Kramer B, Neidhart JD, et al. Tocilizumab in patients hospitalized with covid-19 pneumonia. *N Engl J Med* Jan 7 2021;**384**(1):20—30.
4. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, et al. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. *Cell Host Microbe* Feb 10 2016;**19**(2):181—93.
5. Peng J, Fu M, Mei H, Zheng H, Liang G, She X, et al. Efficacy and secondary infection risk of tocilizumab, sarilumab and anakinra in COVID-19 patients: a systematic review and meta-analysis. *Rev Med Virol* Sep 24 2021:e2295.
6. Kluge S, Strauss R, Kochanek M, Weigand MA, Rohde H, Lahmer T. Aspergillosis: emerging risk groups in critically ill patients. *Med Mycol* Dec 8 2021;**60**(1).
7. Salazar F, Bignell E, Brown GD, Cook PC, Warrisa A. Pathogenesis of respiratory viral and fungal coinfections. Review. *Clinical Microbiology Reveiws* 2022;**35**(1).
8. Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SCA, Dannaoui E, Hochhegger B, 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* Dec 2019;**19**(12):e405—21.
9. Rudramurthy SM, Hoenigl M, Meis JF, Cornely OA, Muthu V, Gangneux JP, et al. ECMM/ISHAM recommendations for clinical management of COVID-19 associated mucormycosis in low- and middle-income countries. *Mycoses* Sep 2021;**64**(9):1028—37.
10. Hamam J, Navellou JC, Bellanger AP, Bretagne S, Winiszewski H, Scherer E, et al. New clinical algorithm including fungal biomarkers to better diagnose probable invasive pulmonary aspergillosis in ICU. *Ann Intensive Care* Mar 8 2021;**11**(1):41.
11. Koehler P, Bassetti M, Chakrabarti A, Chen SCA, Colombo AL, Hoenigl M, et al. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. *Lancet Infect Dis* Jun 2021;**21**(6):e149—62.
12. Robinot R, Hubert M, de Melo GD, Lazarini F, Bruel T, Smith N, et al. SARS-CoV-2 infection induces the dedifferentiation of multiciliated cells and impairs mucociliary clearance. *Nat Commun* Jul 16 2021;**12**(1):4354.
13. Jin Y, Ji W, Yang H, Chen S, Zhang W, Duan G. Endothelial activation and dysfunction in COVID-19: from basic mechanisms to potential therapeutic approaches. *Signal Transduct Targeted Ther* Dec 24 2020;**5**(1):293.
14. S. Feys, S.M. Goncalves, M. Khan, S. Choi, B. Boeckx, D. Chatelain, et al., Lung epithelial and myeloid innate immunity in influenza-associated or COVID-19-associated pulmonary aspergillosis: an observational study, *Lancet Respir Med*, **10**(12), Dec 2022, 1147-1159.

15. Aboudounya MM, Heads RJ. COVID-19 and toll-like receptor 4 (TLR4): SARS-CoV-2 may bind and activate TLR4 to increase ACE2 expression, facilitating entry and causing hyperinflammation. *Mediat Inflamm* 2021;**2021**:8874339.
16. Speth C, Rambach G, Lass-Florl C, Howell PL, Sheppard DC. Galactosaminogalactan (GAG) and its multiple roles in Aspergillus pathogenesis. *Virulence* Dec 2019;**10**(1):976—83.
17. Kumar V, van de Veerdonk FL, Netea MG. Antifungal immune responses: emerging host-pathogen interactions and translational implications. *Genome Med* May 25 2018;**10**(1):39.
18. Patin EC, Thompson A, Orr SJ. Pattern recognition receptors in fungal immunity. *Semin Cell Dev Biol* May 2019;**89**:24—33.
19. Chai LY, Vonk AG, Kullberg BJ, Verweij PE, Verschueren I, van der Meer JW, et al. Aspergillus fumigatus cell wall components differentially modulate host TLR2 and TLR4 responses. *Microb Infect* Feb 2011;**13**(2):151—9.
20. Lopes Bezerra LM, Filler SG. Interactions of Aspergillus fumigatus with endothelial cells: internalization, injury, and stimulation of tissue factor activity. *Blood* Mar 15 2004;**103**(6):2143—9.
21. Curty N, Kubitschek-Barreira PH, Neves GW, Gomes D, Pizzatti L, Abdelhay E, et al. Discovering the infectome of human endothelial cells challenged with Aspergillus fumigatus applying a mass spectrometry label-free approach. *J Proteonomics* Jan 31 2014;**97**:126—40.
22. Kim YM, Shin EC. Type I and III interferon responses in SARS-CoV-2 infection. *Exp Mol Med* May 2021;**53**(5):750—60.
23. Beer J, Crotta S, Breithaupt A, Ohnemus A, Becker J, Sachs B, et al. Impaired immune response drives age-dependent severity of COVID-19. *J Exp Med* Dec 5 2022;(12):219.
24. Alessio G, Imeneo A, Di Lorenzo A, Rossi B, Sorace C, Compagno M, et al. Longitudinal evaluation of the QuantiFERON-TB gold plus assay in hospitalized COVID-19 patients with a first indeterminate result: resolution of inflammation and restoration of T-lymphocyte counts and interferon-gamma production. *Microbiol Spectr* Sep 13 2022:e0185822.
25. Yao Y, Subedi K, Liu T, Khalasawi N, Pretto-Kernahan CD, Wotring JW, et al. Surface translocation of ACE2 and TMPRSS2 upon TLR4/7/8 activation is required for SARS-CoV-2 infection in circulating monocytes. *Cell Discov* Sep 9 2022;**8**(1):89.
26. Alam MS, Czajkowsky DM. SARS-CoV-2 infection and oxidative stress: pathophysiological insight into thrombosis and therapeutic opportunities. *Cytokine Growth Factor Rev* Feb 2022;**63**:44—57.
27. Scioli MG, Storti G, D'Amico F, Rodriguez Guzman R, Centofanti F, Doldo E, et al. Oxidative stress and new pathogenetic mechanisms in endothelial dysfunction: potential diagnostic biomarkers and therapeutic targets. *J Clin Med* Jun 25 2020;**9**(6).
28. Beserra DR, Alberca RW, Branco A, de Mendonca Oliveira L, de Souza Andrade MM, Gozzi-Silva SC, et al. Upregulation of PD-1 expression and high sPD-L1 levels associated with COVID-19 severity. *J Immunol Res* 2022;**2022**:9764002.
29. Sabbatino F, Conti V, Franci G, Sellitto C, Manzo V, Pagliano P, et al. PD-L1 dysregulation in COVID-19 patients. *Front Immunol* 2021;**12**:695242.
30. Loretelli C, Abdelsalam A, D'Addio F, Ben Nasr M, Assi E, Usuelli V, et al. PD-1 blockade counteracts post-COVID-19 immune abnormalities and stimulates the anti-SARS-CoV-2 immune response. *JCI Insight* Dec 22 2021;**6**(24).
31. Stephen-Victor E, Karnam A, Fontaine T, Beauvais A, Das M, Hegde P, et al. Aspergillus fumigatus cell wall alpha-(1,3)-glucan stimulates regulatory T-cell polarization by inducing PD-L1 expression on human dendritic cells. *J Infect Dis* Dec 5 2017;**216**(10):1281—94.
32. Wurster S, Robinson P, Albert ND, Tarrand JJ, Goff M, Swamydas M, et al. Protective activity of programmed cell death protein 1 blockade and synergy with caspofungin in a murine invasive pulmonary aspergillosis model. *J Infect Dis* Aug 17 2020;**222**(6):989—94.
33. Bastard P, Gervais A, Le Voyer T, Rosain J, Philippot Q, Manry J, et al. Autoantibodies neutralizing type I IFNs are present in ~4% of uninfected individuals over 70 years old and account for ~20% of COVID-19 deaths. *Sci Immunol* Aug 19 2021;(62):6.
34. Gafa V, Remoli ME, Giacomini E, Severa M, Grillot R, Coccia EM. Enhancement of anti-Aspergillus T helper type 1 response by interferon-beta-conditioned dendritic cells. *Immunology* Oct 2010;**131**(2):282—8.
35. Zhang Q, Bastard P, Effort CHG, Cobat A, Casanova JL. Human genetic and immunological determinants of critical COVID-19 pneumonia. *Nature* Mar 2022;**603**(7902):587—98.
36. Marchesini G, Nadali G, Facchinelli D, Candoni A, Cattaneo C, Laurenti L, et al. Infections in patients with lymphoproliferative diseases treated with targeted agents: SEIFEM multicentric retrospective study. *Br J Haematol* Apr 2021;**193**(2):316—24.
37. Varughese T, Taur Y, Cohen N, Palomba ML, Seo SK, Hohl TM, et al. Serious infections in patients receiving ibrutinib for treatment of lymphoid cancer. *Clin Infect Dis* Aug 16 2018;**67**(5):687—92.
38. Aldoss I, Dadwal S, Zhang J, Tegtmeier B, Mei M, Arslan S, et al. Invasive fungal infections in acute myeloid leukemia treated with venetoclax and hypomethylating agents. *Blood Adv* Dec 10 2019;**3**(23):4043—9.
39. Davids MS, Hallek M, Wierda W, Roberts AW, Stilgenbauer S, Jones JA, et al. Comprehensive safety analysis of venetoclax monotherapy for patients with relapsed/refractory chronic lymphocytic leukemia. *Clin Cancer Res* Sep 15 2018;**24**(18):4371—9.
40. Little JS, Weiss ZF, Hammond SP. Invasive fungal infections and targeted therapies in hematological malignancies. *J Fungi (Basel)* Dec 10 2021;**7**(12).
41. De Rosa FG, Shaz D, Campagna AC, Dellaripa PE, Khettry U, Craven DE. Invasive pulmonary aspergillosis soon after therapy with infliximab, a tumor necrosis factor-alpha-neutralizing antibody: a possible healthcare-associated case? *Infect Control Hosp Epidemiol* Jul 2003;**24**(7):477—82.
42. Honda H, Kida H, Yoshida M, Tomita T, Fujii M, Ihara S, et al. Recurrent allergic bronchopulmonary aspergillosis in a patient with rheumatoid arthritis treated with etanercept and tocilizumab. *Mod Rheumatol* Dec 2011;**21**(6):660—4.
43. Ogata A, Hirano T, Hishitani Y, Tanaka T. Safety and efficacy of tocilizumab for the treatment of rheumatoid arthritis. *Clin Med Insights Arthritis Musculoskelet Disord* 2012;**5**:27—42.
44. Sheng F, Han M, Huang Z, Zhang L. Interleukin 6 receptor inhibitor tocilizumab suppresses cytokine expression, inflammasome activation and phagocytosis in a cell model of sepsis. *Pharmazie* Nov 2 2016;**71**(11):636—9.
45. Ruiz-Limon P, Ortega R, Arias de la Rosa I, Abalos-Aguilera MDC, Perez-Sanchez C, Jimenez-Gomez Y, et al. Tocilizumab improves the proatherothrombotic profile of rheumatoid arthritis patients modulating endothelial dysfunction, NETosis, and inflammation. *Transl Res* May 2017;**183**:87—103.
46. Sprenkeler EG, Gresnigt MS, van de Veerdonk FL. LC3-associated phagocytosis: a crucial mechanism for antifungal host defence against Aspergillus fumigatus. *Cell Microbiol* Sep 2016;**18**(9):1208—16.
47. McCormick A, Heesemann L, Wagener J, Marcos V, Hartl D, Loeffler J, et al. NETs formed by human neutrophils inhibit growth of the pathogenic mold Aspergillus fumigatus. *Microb Infect* Nov 2010;**12**(12—13):928—36.
48. Wickrematilake G. Complicated rheumatoid nodules in lung. *Case Rep Rheumatol* 2020;**2020**:6627244.

49. Orciuolo E, Stanzani M, Canestraro M, Galimberti S, Carulli G, Lewis R, et al. Effects of Aspergillus fumigatus gliotoxin and methylprednisolone on human neutrophils: implications for the pathogenesis of invasive aspergillosis. *J Leukoc Biol* Oct 2007;**82**(4):839—48.
50. Baschant U, Tuckermann J. The role of the glucocorticoid receptor in inflammation and immunity. *J Steroid Biochem Mol Biol* May 31 2010;**120**(2—3):69—75.
51. Camargo JF, Husain S. Immune correlates of protection in human invasive aspergillosis. *Clin Infect Dis* Aug 15 2014; **59**(4):569—77.
52. Tappe B, Lauruschkat CD, Strobel L, Pantaleon Garcia J, Kurzai O, Rebhan S, et al. COVID-19 patients share common, corticosteroid-independent features of impaired host immunity to pathogenic molds. *Front Immunol* 2022;**13**:954985.
53. Mitaka H, Kuno T, Takagi H, Patrawalla P. Incidence and mortality of COVID-19-associated pulmonary aspergillosis: a systematic review and meta-analysis. *Mycoses* Sep 2021; **64**(9):993—1001.
54. Paramythiotou E, Dimopoulos G, Koliakos N, Siopi M, Vourli S, Pournaras S, et al. Epidemiology and incidence of COVID-19-associated pulmonary aspergillosis (CAPA) in a Greek tertiary care academic reference hospital. *Infect Dis Ther* Sep 2021;**10**(3):1779—92.
55. Salmanton-Garcia J, Sprute R, Stemler J, Bartoletti M, Dupont D, Valerio M, et al. COVID-19-Associated pulmonary aspergillosis, march-august 2020. *Emerg Infect Dis* 2021;**27**(4):1077—86.
56. Prattes J, Wauters J, Giacobbe DR, Salmanton-Garcia J, Maertens J, Bourgeois M, et al. Risk factors and outcome of pulmonary aspergillosis in critically ill coronavirus disease 2019 patients-a multinational observational study by the European Confederation of Medical Mycology. *Clin Microbiol Infect* Apr 2022;**28**(4):580—7.
57. Janssen NAF, Nyga R, Vanderbeke L, Jacobs C, Ergün M, Buil JB, et al. Multinational observational cohort study of COVID-19—associated pulmonary aspergillosis. *Emerg Infect Dis* 2021;**27**(11):7.
58. Chong WH, Neu KP. Incidence, diagnosis and outcomes of COVID-19-associated pulmonary aspergillosis (CAPA): a systematic review. *J Hosp Infect* Jul 2021;**113**:115—29.
59. Gangneux JP, Dannaoui E, Fekkar A, Luyt CE, Botterel F, De Prost N, et al. Fungal infections in mechanically ventilated patients with COVID-19 during the first wave: the French multicentre MYCOVID study. *Lancet Respir Med* Feb 2022; **10**(2):180—90.
60. Chong WH, Saha BK, Neu KP. Comparing the clinical characteristics and outcomes of COVID-19-associate pulmonary aspergillosis (CAPA): a systematic review and meta-analysis. *Infection* Feb 2022;**50**(1):43—56.
61. W. Chen, C. Yin, M. Zhong, B. Hu, X. Gao, K. Zhang, et al., Incidence and outcomes of patients with COVID-19 associated pulmonary aspergillosis (CAPA) in intensive care units: a systematic review and meta-analysis of 31 cohort studies, *Ann Palliat Med*, **11**(7), Jul 2022, 2202-2209.
62. Singh S, Verma N, Kanaujia R, Chakrabarti A, Rudramurthy SM. Mortality in critically ill patients with coronavirus disease 2019-associated pulmonary aspergillosis: a systematic review and meta-analysis. *Mycoses* Sep 2021;**64**(9):1015—27.
63. Pasquier G, Bounhiol A, Robert Gangneux F, Zahar JR, Gangneux JP, Novara A, et al. A review of significance of Aspergillus detection in airways of ICU COVID-19 patients. *Mycoses* Sep 2021;**64**(9):980—8.
64. Bretagne S, Sitbon K, Botterel F, Dellière S, Letscher-Bru V, Chouaki T, et al. COVID-19-Associated pulmonary aspergillosis, fungemia, and pneumocystosis in the intensive care unit: a retrospective multicenter observational cohort during the first French pandemic wave. *Microbiol Spectr* 2021;**9**(2):4.
65. Apostolopoulou A, Esquer Garrigos Z, Vijayvargiya P, Lerner AH, Farmakiotis D. Invasive pulmonary aspergillosis in patients with SARS-CoV-2 infection: a systematic review of the literature. *Diagnostics* Oct 10 2020;**10**(10).
66. Ebner J, Van den Nest M, Bouvier-Azula L, Fuszl A, Gabler C, Willinger B, et al. Routine surveillance of healthcare-associated infections misses a significant proportion of invasive aspergillosis in patients with severe COVID-19. *J Fungi (Basel)* Mar 8 2022;**8**(3).
67. Machado M, Valerio M, Alvarez-Uria A, Olmedo M, Veintimilla C, Padilla B, et al. Invasive pulmonary aspergillosis in the COVID-19 era: an expected new entity. *Mycoses* Feb 2021;**64**(2):132—43.
68. Kirchhoff L, Braun LM, Schmidt D, Dittmer S, Dedy J, Herbstreit F, et al. COVID-19-associated pulmonary aspergillosis in ICU patients in a German reference centre: phenotypic and molecular characterisation of Aspergillus fumigatus isolates. *Mycoses* Apr 2022;**65**(4):458—65.
69. Lackner N, Thome C, Ofner D, Joannidis M, Mayerhofer T, Arora R, et al. COVID-19 associated pulmonary aspergillosis: diagnostic performance, fungal epidemiology and antifungal susceptibility. *J Fungi (Basel)* Jan 18 2022;**8**(2).
70. Pelaez-Garcia de la Rasilla T, Gonzalez-Jimenez I, Fernandez-Arroyo A, Roldan A, Carretero-Ares JL, Garcia-Clemente M, et al. COVID-19 associated pulmonary aspergillosis (CAPA): hospital or home environment as a source of life-threatening Aspergillus fumigatus infection? *J Fungi (Basel)* Mar 19 2022; **8**(3).
71. Meijer EFJ, Dofferhoff ASM, Hoiting O, Buil JB, Meis JF. Azole-resistant COVID-19-associated pulmonary aspergillosis in an immunocompetent host: a case report. *J Fungi (Basel)* Jun 6 2020;**6**(2).
72. Tortorano AM, Esposto MC, Prigitano A, Grancini A, Ossi C, Cavanna C, et al. Cross-reactivity of Fusarium spp. in the Aspergillus Galactomannan enzyme-linked immunosorbent assay. *J Clin Microbiol* Mar 2012;**50**(3):1051—3.
73. Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med* 2018;**6**(10):782—92.
74. Verweij PE, Rijnders BJA, Bruggemann RJM, Azoulay E, Bassetti M, Blot S, et al. Review of influenza-associated pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert opinion. *Intensive Care Med* Aug 2020; **46**(8):1524—35.
75. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, 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* Sep 12 2020;**71**(6):1367—76.
76. Bartoletti M, Pascale R, Cricca M, Rinaldi M, Maccaro A, Bussini L, et al. Epidemiology of invasive pulmonary aspergillosis among intubated patients with COVID-19: a prospective study. *Clin Infect Dis* Dec 6 2021;**73**(11):e3606—14.
77. Kidd SE, Chen SC, Meyer W, Halliday CL. A new age in molecular diagnostics for invasive fungal disease: are we ready? *Front Microbiol* 2019;**10**:2903.
78. Buil JB, Zoll J, Verweij PE, Melchers WJG. Molecular detection of azole-resistant Aspergillus fumigatus in clinical samples. *Front Microbiol* 2018;**9**:515.
79. H. Lamberink, A. Wagemakers, K.C.E. Sigaloff, R. van Houdt, N.A. de Jonge and K. van Dijk, The impact of the updated EORTC/MSG criteria on the classification of hematological patients with suspected invasive pulmonary aspergillosis, *Clin Microbiol Infect*, **28**(8), Aug 2022,1120-1125.

80. Chong GL, van de Sande WW, Dingemans GJ, Gaajetaan GR, Vonk AG, Hayette MP, et al. Validation of a new Aspergillus real-time PCR assay for direct detection of Aspergillus and azole resistance of Aspergillus fumigatus on bronchoalveolar lavage fluid. *J Clin Microbiol* Mar 2015;**53**(3):868—74.
81. Scharmann U, Kirchhoff L, Hain A, Buer J, Koldehoff M, Steinmann J, et al. Evaluation of three commercial PCR assays for the detection of azole-resistant Aspergillus fumigatus from respiratory samples of immunocompromised patients. *J Fungi (Basel)* Feb 11 2021;**7**(2).
82. Giacobbe DR, Prattes J, Wauters J, Dettori S, Signori A, Salmanton-Garcia J, et al. Prognostic impact of bronchoalveolar lavage fluid galactomannan and Aspergillus culture results on survival in COVID-19 intensive care unit patients: a post hoc analysis from the European confederation of medical Mycology (ECMM) COVID-19-associated pulmonary aspergillosis study. *J Clin Microbiol* Mar 24 2022:e0229821.
83. Blot SI, Taccone FS, Van den Abeele AM, Bulpa P, Meersseman W, Brusselaers N, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. *Am J Respir Crit Care Med* Jul 1 2012;**186**(1):56—64.
84. van Arkel ALE, Rijpstra TA, Belderbos HNA, van Wijngaarden P, Verweij PE, Bentvelsen RG. COVID-19-associated pulmonary aspergillosis. *Am J Respir Crit Care Med* Jul 1 2020;**202**(1):132—5.
85. Hatzl S, Reisinger AC, Posch F, Prattes J, Stradner M, Pilz S, et al. Antifungal prophylaxis for prevention of COVID-19-associated pulmonary aspergillosis in critically ill patients: an observational study. *Crit Care* Sep 15 2021;**25**(1):335.
86. Van Ackerbroeck S, Rutsaert L, Roelant E, Dillen K, Wauters J, Van Regenmortel N. Inhaled liposomal amphotericin-B as a prophylactic treatment for COVID-19-associated pulmonary aspergillosis/aspergillus tracheobronchitis. *Crit Care* Aug 19 2021;**25**(1):298.
87. Ghuman H, Voelz K. Innate and adaptive immunity to Mucorales. *J Fungi (Basel)* Sep 5 2017;**3**(3).
88. Abdel-Aziz M, Azab N, Abdel-Aziz NM, Mucormycosis Abdel-Aziz DM. A potential head and neck problem in COVID-19 patients. *Laryngoscope Investig Otolaryngol* Feb 2022;**7**(1):67—9.
89. Reiterer M, Rajan M, Gomez-Banoy N, Lau JD, Gomez-Escobar LG, Ma L, et al. Hyperglycemia in acute COVID-19 is characterized by insulin resistance and adipose tissue infectivity by SARS-CoV-2. *Cell Metabol* Nov 2 2021;**33**(11):2174—2188 e5.
90. Anand VK, Alemar G, Griswold Jr JA. Intracranial complications of mucormycosis: an experimental model and clinical review. *Laryngoscope* Jun 1992;**102**(6):656—62.
91. Avatef Fazeli M, Rezaei L, Javadirad E, Iranfar K, Khosravi A, Amini Saman J, et al. Increased incidence of rhino-orbital mucormycosis in an educational therapeutic hospital during the COVID-19 pandemic in western Iran: an observational study. *Mycoses* Nov 2021;**64**(11):1366—77.
92. Mishra Y, Prashar M, Sharma D, Akash Kumar VP, Tilak T. Diabetes, COVID 19 and mucormycosis: clinical spectrum and outcome in a tertiary care medical center in Western India. *Diabetes Metabol Syndr* Jul-Aug 2021;**15**(4):102196.
93. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: a systematic review of cases reported worldwide and in India. *Diabetes Metabol Syndr* Jul-Aug 2021;**15**(4):102146.
94. Sen M, Honavar SG, Bansal R, Sengupta S, Rao R, Kim U, et al. Epidemiology, clinical profile, management, and outcome of COVID-19-associated rhino-orbital-cerebral mucormycosis in 2826 patients in India - collaborative OPAI-IJO Study on Mucormycosis in COVID-19 (COSMIC), Report 1. *Indian J Ophthalmol* Jul 2021;**69**(7):1670—92.
95. Moorthy A, Gaikwad R, Krishna S, Hegde R, Tripathi KK, Kale PG, et al. SARS-CoV-2, uncontrolled diabetes and corticosteroids-an unholy trinity in invasive fungal infections of the maxillofacial region? A retrospective, multi-centric analysis. *J Maxillofac Oral Surg* Sep 2021;**20**(3):418—25.
96. Pakdel F, Ahmadikia K, Salehi M, Tabari A, Jafari R, Mehrparvar G, et al. Mucormycosis in patients with COVID-19: a cross-sectional descriptive multicentre study from Iran. *Mycoses* Oct 2021;**64**(10):1238—52.
97. Sharma S, Grover M, Bhargava S, Samdani S, Kataria T. Post coronavirus disease mucormycosis: a deadly addition to the pandemic spectrum. *J Laryngol Otol* May 2021;**135**(5):442—7.
98. Sen M, Lahane S, Lahane TP, Parekh R, Honavar SG. Mucor in a viral land: a tale of two pathogens. *Indian J Ophthalmol. Feb* 2021;**69**(2):244—52.
99. Bhattacharyya A, Sarma P, Kaur H, Kumar S, Bhattacharyya J, Prajapat M, et al. COVID-19-associated rhino-orbital-cerebral mucormycosis: a systematic review, meta-analysis, and meta-regression analysis. *Indian J Pharmacol* Nov-Dec 2021;**53**(6):499—510.
100. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in wuhan, China. *Clin Infect Dis* Jul 28 2020;**71**(15):762—8.
101. Li W, Li M, Ou G. COVID-19, cilia, and smell. *FEBS J* Sep 2020;**287**(17):3672—6.
102. M. Hoenigl, D. Seidel, A. Carvalho, S.M. Rudramurthy, A. Arastehfar, J.P. Gangneux, et al., The emergence of COVID-19 associated mucormycosis: a review of cases from 18 countries, *Lancet Microbe*, **3** (7), July 2022, e543-e552.
103. Bhadania S, Bhalodiya N, Sethi Y, Kaka N, Mishra S, Patel N, et al. Hyperferritinemia and the extent of mucormycosis in COVID-19 patients. *Cureus* Dec 2021;**13**(12):e20569.
104. Tabassum T, Araf Y, Moin AT, Rahaman TI, Hosen MJ. COVID-19-associated-mucormycosis: possible role of free iron uptake and immunosuppression. *Mol Biol Rep* Jan 2022;**49**(1):747—54.
105. Gumashta J, Gumashta R. COVID19 associated mucormycosis: is GRP78 a possible link? *J Infect Public Health* Oct 2021;**14**(10):1351—7.
106. Nagalli S, Kikkeri NS. Mucormycosis in COVID-19: a systematic review of literature. *Inf Med* 2021;**29**(4):504—12.
107. Singh K, Kumar S, Shastri S, Sudershan A, Mansotra V. Black fungus immunosuppressive epidemic with Covid-19 associated mucormycosis (zygomycosis): a clinical and diagnostic perspective from India. *Immunogenetics* Apr 2022;**74**(2):197—206.
108. Kushwaha S, Khanna P, Rajagopal V, Kiran T. Biological attributes of age and gender variations in Indian COVID-19 cases: a retrospective data analysis. *Clin Epidemiol Glob Health* Jul-Sep 2021;**11**:100788.
109. Pal R, Singh B, Bhadada SK, Banerjee M, Bhogal RS, Hage N, et al. COVID-19-associated mucormycosis: an updated systematic review of literature. *Mycoses* Dec 2021;**64**(12):1452—9.
110. Kumar A. Mucormycosis in COVID-19 recovered patients. *J Med Virol* Apr 2022;**94**(4):1272—3.
111. Walsh TJ, Gamaletsou MN, McGinnis MR, Hayden RT, Kontoyiannis DP. Early clinical and laboratory diagnosis of invasive pulmonary, extrapulmonary, and disseminated mucormycosis (zygomycosis). *Clin Infect Dis* Feb 2012;**54**(Suppl 1):S55—60.
112. Yadav T, Tiwari S, Gupta A, Garg PK, Khera PS, Rajagopal R, et al. Magnetic resonance imaging in coronavirus disease - 2019 associated rhino-orbital-cerebral mucormycosis (CAROCM) - imaging analysis of 50 consecutive patients. *Curr Probl Diagn Radiol* Jan-Feb 2022;**51**(1):112—20.
113. Kondapavuluri SK, Anchala VKR, Bandlapalli S, Gorantla R, Danaboyina AR, Kondapavuluri BK, et al. Spectrum of MR imaging findings of sinonasal mucormycosis in post COVID-19 patients. *Br J Radiol* Nov 1 2021;**94**(1127):20210648.

114. Desai SM, Gujarathi-Saraf A, Agarwal EA. Imaging findings using a combined MRI/CT protocol to identify the "entire iceberg" in post-COVID-19 mucormycosis presenting clinically as only "the tip. *Clin Radiol* Oct 2021;**76**(10):784 e27—e784 e33.
115. L. Millon, D. Caillot, A. Berceanu, S. Bretagne, F. Lanternier, F. Morio, et al., Evaluation of serum Mucorales PCR for the diagnosis of Mucormycoses: the MODIMUCOR prospective trial, *Clin Infect Dis*, **75** (5), Sep 14 2022, 777-785.
116. Mulakavalupil B, Vaity C, Joshi S, Misra A, Pandit RA. Absence of Case of Mucormycosis (March 2020-May 2021) under strict protocol driven management care in a COVID-19 specific tertiary care intensive care unit. *Diabetes Metabol Syndr* Jul-Aug 2021;**15**(4):102169.
117. Tissot F, Agrawal S, Pagano L, Petrikkos G, Groll AH, Skiada A, et al. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. *Haematologica* Mar 2017;**102**(3):433—44.
118. Marty FM, Ostrosky-Zeichner L, Cornely OA, Mullane KM, Perfect JR, Thompson 3rd GR, et al. Isavuconazole treatment for mucormycosis: a single-arm open-label trial and case-control analysis. *Lancet Infect Dis* Jul 2016;**16**(7):828—37.
119. Seidel D, Simon M, Sprute R, Lubnow M, Evert K, Speer C, et al. Results from a national survey on COVID-19-associated mucormycosis in Germany: 13 patients from six tertiary hospitals. *Mycoses* Jan 2022;**65**(1):103—9.
120. Patel A, Agarwal R, Rudramurthy SM, Shevkani M, Xess I, Sharma R, et al. Multicenter epidemiologic study of coronavirus disease-associated mucormycosis, India. *Emerg Infect Dis* Sep 2021;**27**(9):2349—59.
121. Aranjani JM, Manuel A, Abdul Razack HI, Mathew ST. COVID-19-associated mucormycosis: evidence-based critical review of an emerging infection burden during the pandemic's second wave in India. *PLoS Neglected Trop Dis* Nov 2021;**15**(11):e0009921.