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## Review

## Mucormycosis an added burden to Covid-19 Patients: An in-depth systematic review



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## ABSTRACT

As of 25th July, 2022, global Disease burden of 575,430,244 confirmed cases and over 6,403,511 deaths have been attributed to coronavirus disease 2019 (COVID-19). Co-infections/secondary infections continue to plague patients around the world as result of the co-morbidities like diabetes mellitus, biochemical changes caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) especially significant elevation in free iron levels, immune suppression caused by SARS-CoV-2, and indiscriminate use of systemic corticosteroids for the treatment of severe COVID-19 disease. In such circumstances, opportunistic fungal infections pose significant challenge for COVID-19 disease therapy in patients with other co-morbidities. Although COVID-19-associated Mucormycosis (CAM) has been widely recognized, currently extensive research is being conducted on mucormycosis. It has been widely agreed that patients undergoing corticosteroid therapy are highly susceptible for CAM, henceforth high index of screening and intensive care and management is need of an hour in order to have favorable outcomes in these patients. Diagnosis in such cases is often delayed and eventually the disease progresses quickly which poses added burden to clinician and increases patient load in critical care units of hospitals. A vast perusal of literature indicated that patients with diabetes mellitus and those with other co-morbidities might be highly vulnerable to develop mucormycosis. In the present work, the case series of three patients presented at Chest Disease Hospital

**Abbreviations:** AIDS, Acquired immunodeficiency syndrome; AMB, Amphotericin B; CAM, COVID-19-associated Mucormycosis; CD, Cluster of Differentiation; CDC, Center for Disease Control and Prevention; CDH, Chest Disease Hospital; CLSI, Clinical and Laboratory Standards Institute; CoH, coating protein family; COVID-19, coronavirus disease 2019; CT, Computerized Tomography; DM, Diabetes Mellitus; ECIL, European Conference on Infections in Leukemia; ELISpot, Enzyme-Linked ImmunoSpot; EMM, European Confederation of Medical Mycology; EUCAST, European Committee on Antimicrobial Susceptibility Testing; FISH, Fluorescence in situ hybridization; GRP78, glucose regulator protein 78; H1N1, Hemagglutinin Type 1 and Neuraminidase Type 1; H2N2, Hemagglutinin Type 2 and Neuraminidase Type 2; HIV-AI, human immunodeficiency virus associated immunodeficiency; ICU, Intensive Care Unit; IL, Interleukin; ITS, Internal Transcribed Spacer; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MRI, Magnetic resonance imaging; PCR, polymerase chain reaction; rRNA, Ribosomal ribonucleic acid; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus-2; SDA, Sabouraud dextrose agar; TNF- $\alpha$ , Tumor Necrosis Factor-alpha

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Srinagar, Jammu and Kashmir infected with CAM has been described with their epidemiological data in supplementary section. All these cases were found to be affected with co-morbidity of Diabetes Mellitus (DM) and were under corticosteroid therapy. Furthermore, given the significant death rate linked with mucormycosis and the growing understanding of the diseases significance, systematic review of the literature on CAM has been discussed and we have attempted to discuss emerging CAM and related aspects of the disease.

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## 1. Introduction

With persistent zigzagging in prevalence of COVID-19, researchers are actively involved in understanding the diseases and efforts are being made to contain disease [1–3]. As the prevalence of COVID-19 infections rises, healthcare professionals continue to face challenges in containing the allied co-infections, which potentially can lengthen hospital stays, worsen outcome of disease, decrease immunity and concurrently increase treatment costs [4]. Such infections may comprise of a variety of infectious agents which includes respiratory viruses, bacterial infection and fungal infections [5]. Lately, it has been observed from clinical settings that patients with COVID-19 suffer secondarily from mucormycosis, in this regard researchers have identified various risk factors for secondary infection of mucormycosis, which includes diabetes mellitus, immune-suppressing therapy and pathological alteration in pulmonary architecture due to SARS CoV-2 [6]. In context to this statement, there

are reports of the co-infection of respiratory viruses such as influenza and opportunistic invasive fungal infections that have led to poor prognosis and henceforth significant fatality rates [7].

Recently researchers have identified hyperactivation of the cytokine response which mediates inflammation and causes disruption in immune cells henceforth results in significant decline in cluster of differentiation CD4 and CD8 which renders patients susceptible for secondary infection [8]. Researchers indicate that activation of antiviral immunity in tissue micro-environment of infected individuals can facilitate the formation, growth and development of different classes of bacteria [9]. Furthermore, prolonged stay under mechanical ventilation has been identified as potential risk factor for development of mucormycosis [10]. In patients with active infection caused by pathogens (e.g., SARS CoV-2) an increased incidence of fungal infection (e.g. aspergillosis [11], cryptococcosis, candidiasis [12,13], Coccidioides [14], Geotrichum [15] Fusarium [16], Mucorales [17] Mucorales+Aspergillus [18] Pneumocystosis [19],

Mucormycosis, Histoplasmosis) was detected [20]. Finally it has been observed that excessive use of steroid drugs make patients susceptible to mucormycosis which can occur anytime during hospitalization or after hospitalization [21].

## 2. Methodology

### 2.1. Search strategy

The systematic review was conducted as per guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [22] (Fig. 1). In order to include eligible studies on COVID-19 and mucormycosis, we conducted extensive search from inception of COVID-19 till today on electronic database which include Medline, Pubmed, Embase, googlescholar, Web of Science, Elsevier, Scopus, Cochrane library, and Academic Search Complete by means of Boolean Operators (AND, OR) using terms “COVID-19” OR “SARS-CoV-2”) AND (“mucormycosis” OR “mucorale” OR “Zygomycosis” OR “black fungus” OR “secondary fungal infections” OR “Absidia” OR “Rhizomucor” OR candidiasis OR comorbidities). We further cross checked the reference lists from relevant publications and literature was exclusively restricted to English language only. A detailed search strategy is given in (Fig. 1), furthermore hand searching of research articles was conducted to extract information that was relevant to design of the current manuscript.

### 2.2. Analysis (inclusion and exclusion criteria)

#### 2.2.1. Inclusion

- i. Case studies, meta-analysis, observational studies and clinical trials with confirmed diagnosis of CAM were included in present study.
- ii. Patients with confirmatory diagnosis of COVID-19 either prior or at the time of mucormycosis.

- iii. Studies dealing with published records of COVID-19 and mucormycosis co-infection.

#### 2.2.2. Exclusion

- i. We excluded studies where confirmatory diagnosis for CAM was not adopted and clinical outcome was not reported.
- ii. Patients with no confirmed protocol adopted for diagnosis of COVID-19 were excluded from the study.
- iii. Unpublished data, preprints, thesis, paper where full text was not available and papers published in language other than English were excluded from search strategy.
- iv. Newspaper columns, preprints, editorials comments and incomplete reported data were excluded.

### 2.3. Screening and extraction

Articles and studies included by inclusion criteria were screened independently by four authors (N.N.Shah, S.U.Nabi, S.Muzamil and G.A.Rather) for any duplicate record and articles that failed to fulfill the above mentioned inclusion criteria were excluded. These authors selected articles independently based on title and abstract in first phase and then in second phase they selected articles from first phase based on full text reading. Eligible studies were assorted as per the predetermined criteria in excel file under subheadings of title, authors, year of publication, country and type of study, outcome, comorbidity and number of patients, epidemiology, clinical manifestation, risk factors, diagnostic methods and treatment guidelines and from these studies we drafted the present manuscript.

### 2.4. Synthesis

On preliminary screening literature from above mentioned sources total of 1549 results were retrieved, 554 were found to

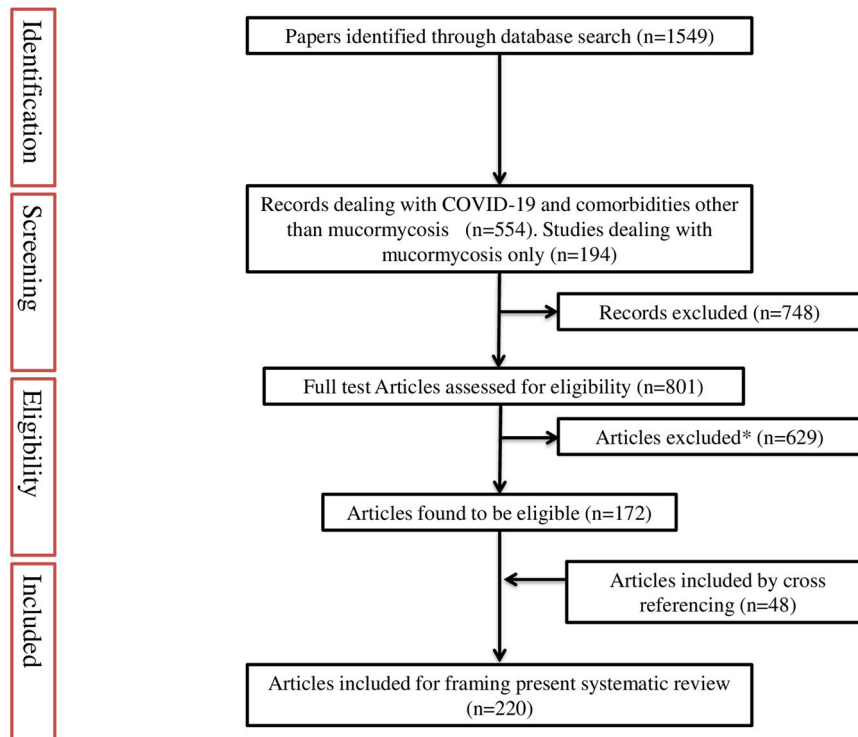


Fig. 1. : PRISMA methodology with flow chart of various step adopted for synthesis of present manuscript.

report COVID-19 and other comorbidities (other than mucormycosis) while 194 were found to report about various mucormycosis only so we excluded these results (748). From remaining 801 results 93 duplicate records, 134 non availability of full text results, 178 results with no documented diagnostic criteria, 49 in languages other than English and 175 (unpublished, thesis and preprints) were excluded from study. To the remaining 172 articles, 48 articles were added from reference list and finally 220 articles were included in synthesis of the present systematic review (Fig. 1).

### 3. Secondary infections and viral pandemics

Throughout the course of history, whenever global pandemics have spread, co-infections occurred simultaneously. A comparative account of the influenza pandemic of the Spanish flu (1918, H1N1), Asian flu (1957, H2N2), and influenza A (2009, H1N1) pandemic reveals that secondary bacterial co-infections had also been significant complication posing severe repercussions [23]. Bacterial pneumonia was reported in 90 % of cases in 1918 pandemic autopsies and 75 % of cases in 1957 autopsies in comparison with 55 % of cases in 2009 flu [24]. The literature points out to plethora of examples ranging from the classic necrotizing *Streptococcus pneumoniae* super infection in those patients in whom lungs have been damaged by influenza A [25].

Results from preclinical and clinical studies have indicated that secondary infections during pandemics usually viral pandemics increase morbidity and mortality in patients [26]. Recent study has indicated that prevalence of secondary infection in viral pandemics range from 11% to 35%. For instance during swine flu (2009), there were significantly higher proportion of bacterial pneumonia (29–55 %) in patients suffering from viral swine flu [27]. There are many published reports of co-infection during 1918 influenza pandemic and bacterial pneumonia was reported from 90% of cases that died during 1918 influenza pandemic and in majority of these cases *S. pneumoniae* was found most prevalent [28]. In Asian influenza Pandemic (1957) post mortem samples from patients that died during 1957 revealed bacterial infection in 80% of cases which increased the hospitalization rate of patients infected with secondary infection [29]. A cohort study was conducted in 140 patients in Sheffield City General Hospital and majority of the patients were found to be suffering from secondary infection of *S. pneumoniae* and *H. influenzae* [30]. In 1968 Hong Kong pandemic, England and Wales observed 55 % increased incidence of pneumonic deaths in which bacterial pneumonia was the major contributor [31]. Similarly in Atlanta hospital there was threefold increase in prevalence of bacterial co-infection during pandemic [32].

The co-circulation of influenza virus and the SARS CoV-2 in contemporary times have the potential to give rise the new strains of fatal viruses [33]. These viral pandemics and COVID-19 has been reported to be associated with immune abnormalities which includes significant reduction in CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocyte counts and significantly increased levels of inflammatory cytokines Interleukin (IL)-2R, IL-6, IL-10, tumor necrosis factor-alpha (TNF- $\alpha$ ). Corroborating with the statement, various reports have indicated that viral epidemics like COVID-19 induced immunological dysregulation, increased risk of subsequent infections like that of mucormycosis that is increasingly maiming COVID-19 patients as well as decreasing recovery rate [34,35]. A vast perusal of case studies and data from across centers suggest that inhalation of fungal spores as the most prevalent mode of transmission of secondary infections like mucormycosis. However, traumas with contaminated soil, intravenous transmission and direct implantation into damaged skin (burns) have also been reported as modes of potential transmission of secondary infections in immune compromised patients suffering from viral infection during pandemic [36]. Following the nasal invasion, these fungal infections spread swiftly to surrounding areas

including the orbit and occasionally to the central nervous system [37].

#### 3.1. Mucormycosis

Mucormycosis is a severe, life threatening infection caused by filamentous fungi classified under subphylum Mucoromycotina; Class Glomeromycetes; Order mucorales [38]. Along with *Aspergillus*, it is considered as one of the most invasive fungi that increasingly infects immune compromised individuals. Patients with associated risk factors and under immunosuppressant therapy are particularly vulnerable to developing opportunistic infection [39]. Mucormycosis is a comprehensive term that includes variety of infections caused by filamentous fungi belonging to the class Glomeromycetes as per the revised classification (previously classified under class zygomycetes). *Rhizopus arrhizus* has been reported to be the most prevalent mucorales clinical isolate (44 %) in the world, followed by *Rhizopus microsporus* (22 %), *Mucor indicus* (2.6 %), *Rhizomucor pusillus* (3.7 %), *Mucor circinelloides* (9.5 %), *Cunninghamella bertholletiae* (3.2 %), *Cunninghamella echinulata* (1 %), and *Apophysomyces elegans* (0.5 %) [38]. In the recent past mucormycosis was synonymously used with the term zygomycosis, however as per the phylogenetic reanalysis of the class zygomycetes, all infectious agents causing mucormycosis have been classified under the subphylum Mucoromycotina, and those causing Entomophthoromycosis have been classified under the subphylum Entomophthoromycotina (earlier both were classified under zygomycetes [40]. It is mostly commonly found in cases of organ transplant and patients with malignant tumors [41].

The favorable condition for establishment of mucormycosis includes neutropenia, immune deficiency, organ transplantation, significantly higher levels of iron in plasma, and other co-morbidities (diabetes mellitus) [42]. Among the most prevalent infectious agents *Rhizopus* spp, *Lichtheimia* spp and *Mucor* spp, (previously of the genera *Lichtheimia* and *Absidia*) are the most commonly reported pathogens in CAM [43]. In Spain, *Lichtheimia* spp. was identified as the leading cause of CAM, whereas in India, *Mucor* spp is the leading cause of the CAM [44]. This indicates the regional diversity and the urgency to understand local epidemiology. CAM is becoming more common over the world, but it is most prevalent among diabetic individuals in India and China [45,46]. The increased incidence of CAM in India has been attributed to increased incidence of diabetes mellitus in India, which is supposed to be an important risk factor for emergence of CAM [22]. Furthermore studies across the world have postulated that increased incidence of CAM in COVID-19 patients may be attributed to hypoxic conditions created by pulmonary tissue damage by SARS-CoV-2, increased use of steroids, acidic conditions created by ketoacidosis and inflammation induced hyperferritinemia [39].

The fungal co-infection gained prominent importance in 2020 owing to the significantly higher incidence rate in immune-compromised COVID-19 patients [47]. The degree of severity of infection (mucormycosis) depends on region of body infected, degree of immune suppression, age and other co-morbidities present in patients [42]. Researchers have postulated that recent outbreak of COVID-19 caused by double/triple variant of SARS-CoV-2 plays catalytic role in emergence of CAM [21,48]. The mean time interval between COVID-19 diagnosis and emergence of CAM was found to be 15 days, however mucormycosis developed in patients up to 90 days post infection with COVID-19. Researchers have proposed likelihood association between mucormycosis and COVID-19. As per recently published study it has been proposed that SARS-CoV-2 infection acts on multiple pathways which cause conditions favorable for emergence of CAM [22]. (i) SARS-CoV-2 causes damage to pancreatic islets and induces cytokine storm which causes hyperglycemia which is further enhanced by concurrent steroid therapy. (ii) SARS-CoV-2



causes endothelitis which promotes mucorale adhesion. (iii) SARS-CoV-2 causes impairment in phagocytosis which subsequently causes immunosuppression and hence makes favorable conditions for proliferation of mucorales [49].

#### 4. Forms of mucormycosis in COVID-19 patients (CAM)

Vast perusal of case studies reveal that particular mucorale species may show specific association with site infected like in the French Retro Zygo study, *Rhizopus arrhizus* was found in 85% cases of naso-cerebral forms, compared to only 17% cases of non-naso-cerebral forms [50]. The differential in virulence amongst mucorales species might explain this observation. The clinical presentation of mucormycosis has been found to be linked with underlying diseases. Individuals with DM are more likely to develop Naso-cerebral mucormycosis, whereas patients with hematological cancers are more likely to develop pulmonary mucormycosis [51]. Invasive mucormycosis has been categorized into six distinct clinical syndromes based on infection Pulmonary, rhino orbital, cutaneous, gastrointestinal and disseminated mucormycosis, and a miscellaneous form involving the bones, breasts, kidneys etc. Rhino-orbital syndrome is the most prevalent manifestation of mucormycosis, followed by pulmonary, cutaneous, and disseminated disorders. GI tract complications and renal mucormycosis are very rare [52].

- (i) Rhino-orbital mucormycosis is the most prevalent type of clinical presentation affecting patients with Diabetes Mellitus, malignancy, solid organ or hematopoietic stem cell transplant. *Rhizopus arrhizus* is the most prevalent infectious agent causing rhino-orbital cerebral mucormycosis [53]. The infection commonly develops from the paranasal sinuses, which lead to bone loss and eventually spreads to the orbit, eye, and brain. There may be unilateral facial edema, proptosis, and a palatal or palpebral fistula that develops into necrosis [54].
- (ii) Pulmonary mucormycosis is the second most widespread infections among different clinical presentations of mucormycosis. Inhalation of sporangiospores in immune compromised patients seems to be the primary cause of pulmonary infections. Research indicates that pulmonary mucormycosis has been recognized in patients with a history of neutropenia or hematological malignancies [55]. Pulmonary mucormycosis is contagious and spreads to other organs like to abdomen through the diaphragm. Additionally, Iron metabolism is well established to have a role in mucormycosis etiology. As a result, patients with an iron excess, such as those receiving deferoxamine chelation treatment, are more vulnerable to pulmonary mucormycosis [56].
- (iii) Cutaneous mucormycosis are the common forms of Mucormycosis in immune-compromised patients, occurring primarily as a result of cutaneous disruption [57].
- (iv) Gastrointestinal mucormycosis cases were rarely reported; however, since the last 2 decades, the incidences have been increasingly reported. The stomach, colon, and ileum are the most prevalent sites of gastrointestinal mucormycosis. Previously, the gastrointestinal mucormycosis was mostly encountered in premature neonates, and it was frequently associated with broadly disseminated illness. Other uncommon occurrences of gastrointestinal mucormycosis have been linked to other immune compromising diseases such as Acquired immunodeficiency syndrome (AIDS), organ transplantation and systemic lupus erythematosus in the past [50]. The symptoms of gastrointestinal mucormycosis depend on the location of the infection. However, the most frequent symptoms are non-specific abdominal discomfort and distention, as well as nausea and vomiting. Fever and hematochezia are other allied

complications. Additionally, the intra-abdominal abscess has also been recognized [58]

- (v) Disseminated and miscellaneous mucormycosis is the most serious form of mucormycosis, disseminated mucormycosis occurs when an immune impaired person develops severe fungemia, which leads to hematogenous spread to multiple bodily organs, including major visceral organs. Miscellaneous mucormycosis can infect any region of the body including bones and urinary tract which can have severe implications in the afflicted area [59].

#### 5. Pathogenic pathways and associated risk factors of CAM

Usually spore of fungi are cosmopolitan and are mostly present in air with primary mode of inoculation has been reported to be through inhalation or ingestion of spores or through injured skin. These often result in several infections that may be of rhino-cerebral, pulmonary, gastrointestinal, or cutaneous in nature [60]. Apart from these potential routes, medical devices like ventilators, bandages and other medical equipment act as potential source of fungal infections and this equipment can serve as point sources of infection which culminates into fungal outbreak in immunosuppressant patients [22]. Transmissions of infection through medical devices are more common in patients admitted in transplantation unit [61]. The other possibility of contracting fungal infection in COVID-19 patients includes period of discharge from the hospital, patients discharged from hospital are having significantly higher levels of iron in post discharge period which make conditions favorable for establishment of fungal infections [62]. mucormycosis is known for its angioinvasivity, which results in vascular thrombosis and eventually, tissue necrosis. mucormycosis is found to be predisposed by ketoacidosis and deferoxamine highlighting the relevance of acidifying ketone bodies, hyperglycemia and iron in mucorales pathogenicity [63]. Fungi after internalization involves germination of spores, escaping immune system due to pathophysiological or immune compromised conditions using growth conditions of host like increased iron levels, acidic conditions and high glucose levels. Once these opportunistic organisms get established they initiate pathological response which culminates into multiple organ failure [64]. Multiple organ failure is caused due to dissemination of fungal hyphae to various organs of body.

The intricate relationship between a spore-coating protein family (CoH) on the surface of *Rhizopus* spp. and endothelium glucose regulator protein 78 (GRP78) expressed on the surface of endothelial cells has been linked to angioinvasion. This contact causes host cell damage, which leads to fungal hematogenous spread. Increased levels of blood glucose and glucose byproducts promote fungal growth and enhance the expression of GRP78 and CoH, allowing *Rhizopus* to infiltrate host tissues more easily and which indicates why diabetic and deferoxamine treated individuals are more susceptible to mucormycosis [65]. An interesting fact to note here is that among various risk factors AIDS, liver cirrhosis, cancer, hematological diseases, high doses of steroids or solid organ transplant make patients vulnerable to mucormycosis. Decreased monocyte and neutrophil counts may be critical ones as they are potentially known to limit the spore germination [66]. Recent outbreak in India may be due to the high doses of steroids and uncontrolled blood sugar level in COVID-19 patients.

##### 5.1. Use of corticosteroids in COVID-19 and emergence of Mucormycosis

In United Kingdom clinically controlled randomized clinical trial was conducted and results of the trial supported use of corticosteroids in critically ill COVID-19 patients. Similarly observational clinical studies reported reduction in mortality, early weaning of ventilators and reduction in hospitalization in patients treated with

corticosteroids [67]. Most of the available literature about this disease has been drawn mainly from case reports and case studies, henceforth making it almost improbable to conduct large-scale clinically controlled randomized clinical trials in Indian conditions. This can be one reason for uncommon diagnosis and delayed treatment [68]. Moreover, due to the clinical and radiological resemblance of the aspergillosis to mucormycosis and lack of proper diagnostic devices make early identification obscure [8,69].

Despite the advantageous results obtained from various studies conducted across the globe, the major side effects observed included the emergence of secondary bacterial and fungal infections in patients under prolonged use of corticosteroids [70]. Although corticosteroids reduced cytokine storm in COVID-19 patients, henceforth causes reduction in pulmonary damage. Contrarily to this it has been observed that chronic use of corticosteroids cause increase in viral load and immune suppression hence paving way to secondary infection [71]. In COVID-19 patients' current use of systemic corticosteroids to treat severe COVID-19 has been reported to trigger various bacterial and fungal infections, and mucormycosis is one of them [72]. Mucormycosis is challenging to treat for a variety of reasons like uncommon diagnosis, several risk factors like diabetes mellitus, cancer, and neutropenia [69]. As per various reports, the number of mucormycosis infections has alarmingly risen to more than 12,000, mostly in patients recovering from COVID-19, with high frequency in the western states of Maharashtra and Gujarat [73]. This severe infection is normally very rare and has a mortality rate of about 50% [74]. At this time, it is impossible to forecast when the mucormycosis pandemic will cease, while better knowledge of the rare disease and of patients' sensitivity may assist physicians in India to detect instances earlier [75]. Recently, Meta-analysis has revealed therapeutic role of corticosteroids in amelioration of clinical symptoms in COVID-19 patients but in some of patients' treatment with corticosteroids resulted in immunosuppression and subsequently mucormycosis was reported in them. [76].

### 5.2. COVID-19 infection mediated immunosuppression and CAM

Clinical studies have suggested that SARS-CoV-2 infection cause disturbances in immune regulation by diminishing phagocytic activity of T lymphocytes especially CD4<sup>+</sup> and CD8<sup>+</sup> T cells which are main arsenals of immune system involved in elimination of infections [77]. Recent studies have proposed significantly reduction in number of T cells in critically ill COVID-19 patients, henceforth renders these patients susceptible to opportunistic pathogens [78]. To support these propositions a cohort study was conducted in critically ill COVID-19 patients and these patients were found to have immune paralysis [48]. So researchers have speculated that in critically ill COVID-19 patients SARS-CoV-2 itself promotes immune suppression state [79]. Hence parallel correlation between incidence of COVID-19 and mucormycosis is quite evident. In addition to this SARS-CoV-2 cause damage to micro-vasculature and endothelial framework which causes development of micro thrombi, necrotic changes in pulmonary epithelium and cellular infiltration into pulmonary parenchyma, these conditions make it favorable for secondary infection to establish [80]. Some researchers have postulated that prominent role is being played by mechanical ventilation as per them prolonged mechanical ventilation make pulmonary architecture favorable for establishment of mucormycosis [8].

### 5.3. Sequel of high free iron circulating in COVID-19 patients

In patients suffering from critical form of COVID-19, significantly higher levels of ferritin have been observed causing hyperferritinemia. In this direction [81] reported that viral protein fragments of SARS-CoV-2 cause hyper-transcription of genomic determinants involved in iron homeostasis and of the particular importance

hepcidin the main iron regulator genes in peripheral lymphocytes are overexpressed. The hepcidin causes accumulation of iron inside macrophages and enterocytes and promotes ferritin movement inside cells and hence lead to cellular necrosis [82]. Owing to this iron overload is treated with deferoxamine, which causes alteration in immune network and renders patients susceptible to secondary infections [83]. Presence of significantly higher levels of free iron in blood plasma contributes to the development of mucormycosis, usually low iron levels in body are supposed to be beneficial in containing secondary infections like mucormycosis [84]. High levels of free iron from host are assimilated by mucorales using iron permeases or siderophores present in their body. These receptors cause reduction of ferric (Fe<sup>+3</sup>) iron into ferrous (Fe<sup>+2</sup>) iron, later on ferrous form of iron is incorporated in to multicopper oxidase/ferrous permease complexes. From preclinical studies these complexes are significantly activated in virulent mucormycosis and degree of their expressivity is directly related to pathogenesis of the disease [85]. Recently it has been observed that few species of mucormycosis obtain iron from degradation of haeme components of hemoglobin, henceforth explains their angioinvasive nature [61].

### 5.4. Hyperglycemia fuels mucormycosis

Diabetes mellitus is characterized by hyperglycemia and prolonged hyperglycemia cause glycosylation of cellular macromolecules transferrin and ferritin, henceforth decrease their affinity for iron binding. In addition to this, acidic conditions caused by accumulation of ketone bodies cause release of iron from chelation compounds. Presence of free iron in blood plasma increases virulence factors of opportunistic fungi, henceforth these erratic conditions promote growth of secondary infections in COVID-19 patients [86]. In addition to this COVID-19 patients are associated with comorbidities which make conditions favorable for secondary infections like mucormycosis [87,88]. Meanwhile study from India proposed that uncontrolled Diabetes Mellitus, immune dysfunction and chronic use of steroids as main contributing factor for emergence of mucormycosis [87]. This statement is further supported by the clinical fact that neutrophils being very crucial for limiting fungal infection. In addition to this it is an established fact that diabetes mellitus causes significant reduction in chemotaxis and phagocytosis because of the excessive accumulation of free radicals [88]. In order to understand the pathogenic pathway involved in increased incidence of mucormycosis in diabetes mellitus preclinical studies have identified up regulation of glucose-regulated protein (GRP78) in diabetic patients which mediates internalization of fungal mucorales inside endothelial cells and hence results in proliferation of mucormycosis in diabetic patients [89]. Table 1 summarizes the pathological condition of CAM in patients with diabetes mellitus and hyperglycemia.

## 6. Present updated guideline for diagnosis of mucormycosis

As per Center for Disease Control and Prevention (CDC), clinical condition of CAM has been reported from different parts of world and researchers are grabbing to find an early biomarker of the disease [77]. The diagnosis of mucormycosis depends on the location of the suspected patient, availability of trained personnel, radiological techniques, histological and mycological examination. Patients that are diagnosed to be infected with mucormycosis should be immediately referred to a center with the highest level of treatment [90]. There are various fungal diseases that resemble COVID-19 in symptoms (pyrexia, dyspnea and cough) hence Laboratory investigation is a necessary tool in order to determine whether person has mucormycosis and/or COVID-19 [91,92]. COVID-19 and mucormycosis infection can coexist in some people [93]. People suffering from severe COVID-19 admitted to intensive care unit (ICU)

**Table 1**  
Cases of CAM presented with co-morbidities of hyperglycemia and Diabetes Mellitus as promoting factor for CAM reported from different parts of the world.

Year/References	Number of patients	Most common Comorbidity/Risk factor identified	Treatment	Outcome
2021/India[100]	2826	Diabetes Mellitus (DM)	Amphotericin B, aggressive and surgical debridement	Treatment reduced mortality rate from 52 % to 39 %
2021/United Kingdom[55]	1	Not identified	Surgical intervention and antifungal treatment	Death
2021/India[53]	49	Uncontrolled DM and concurrent steroid use	Intravenous liposomal AMB	Statistically reduced mortality
2021/United States of America[38]	10	Diabetic ketoacidosis (3) cancer (2)	Intravenous liposomal AMB	Death 5
2021/India[57]	31	Uncontrolled DM	Intravenous liposomal amphotericin B	Recovering 5
2021/Iran[44]	3	DM (2) and Lymphoma (1)	Antifungal drugs and surgical intervention	Reduction in 75 day all-cause mortality
2021/Italy[92]	5	Uncontrolled DM and concurrent steroid use	Surgical intervention followed by Antifungal therapy	Death 2
2022/India[50]	11	Hyperglycemia and steroids use	Amphotericin B and surgical debridement	Recovering 1
2021/India[104]	6	Diabetes Mellitus and chronic corticosteroid use	Liposomal Amphotericin B and surgical intervention	Discharged 3
2021/India[132]	6	Uncontrolled hyperglycemia	Antifungal therapy	Control of fungal infection
2021/India[85]	1	Type 1DM	liposomal amphotericin B	Only 1 mortality occurred
2021/Brazil[108]	287	Improper glucocorticoid use	Isavuconazole	All six patients survived
2021/USA[81]	41	Uncontrolled DM	Antifungal therapy	Survived
2022UK[40]	59	Hyperglycemia (HbA1c > 7%)	Surgical intervention, intravenous liposomal Amphotericin B and Posaconazole	Case-fatality rate reduced to 45.7 %
				90.24 % patients survived
				74.6 % patients survived and 25.4 % died

are more prone to co-infections. Aspergillosis and invasive candidiasis are the most prevalent fungal illnesses in COVID-19 patients. These fungal co-infections are linked with severe outcome with grave prognosis [94].

It is critical to be aware of the likelihood of fungal co-infection to avoid diagnostic and treatment delays and prevent severe disease and death from these infections. For diagnosis, a fluid sample from the patient's respiratory system may be taken for laboratory testing; alternatively, a tissue biopsy or a CT scan of the patient's infected organs (e.g., lungs, sinuses) may be performed [78]. An overview of various promising diagnosis methods for mucormycosis is shown in Fig. 2. There are various centers where advanced diagnostic equipment and techniques are not available like polymerase chain reaction (PCR), matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), or even computerized tomography (CT) and serology (Early markers of mucormycosis).

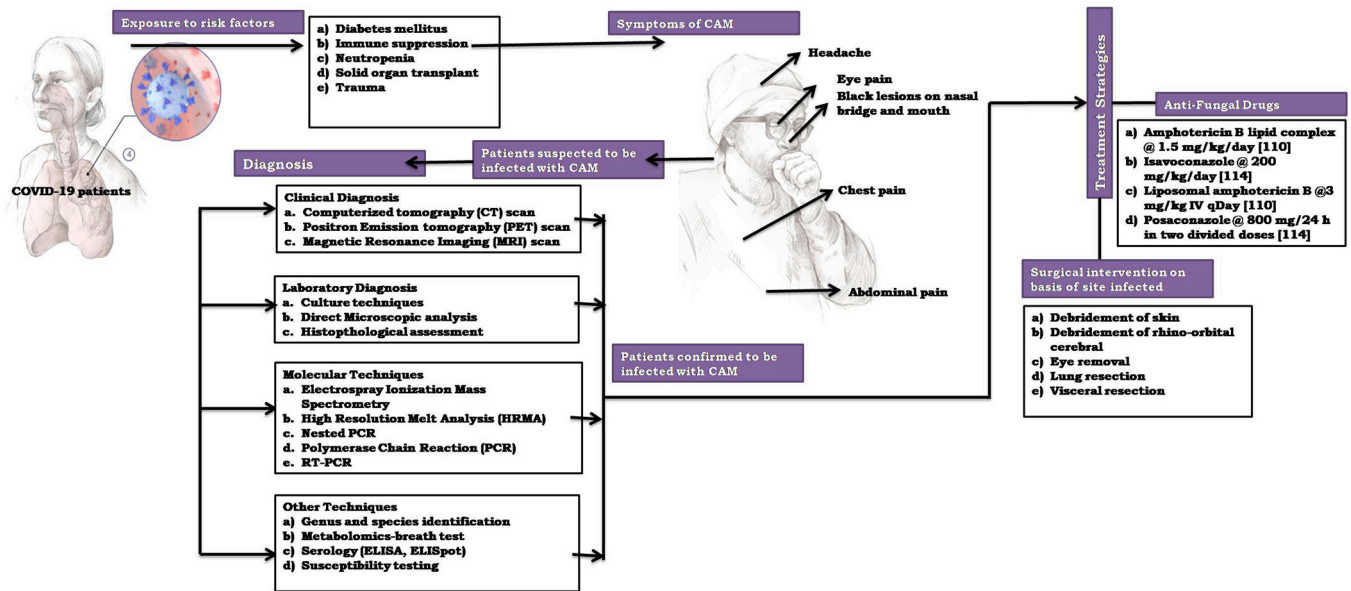
It has been suggested by the researchers at places where advanced facilities are not available investigators should rely on cultural and non-cultural examinations [13]. The definitive conformational methods include direct microscopic examination in presence of optical brighteners, morphological/cultural examination on fungal specific media (Sabouraud agar and potato dextrose agar) and DNA sequencing for identification of species. One of the major limitations of cultural examination includes low yield and negative results does not exclude absence of CAM [95]. Furthermore, intensive clinical evaluation can be employed for supplementing diagnosis of CAM [81]. In this direction [96] conducted retrospective study in CAM patients and these patients demonstrated pyrexia, dyspnea, myalgia, confusion, blackish appearance of skin, and headache during coinfection. Similarly [96] found similar symptomatic profile in CAM patients with addition of alterations in plasma osmolality and reduction in SpO<sub>2</sub> levels. Additionally, [96,97] reported increased incidence of prolonged pyrexia, ophthalmoplegia, lymphopenia, leukopenia, and leucocytosis in COVID-19 patients coinfecting with mucormycosis. Based on these findings researchers have hypothesized that overlapping symptoms observed in CAM indicate interaction between SARS-CoV-2 and fungus species involved which is yet to be established [21]. However differential symptoms of CAM need to be explored which needs large scale survey and mandatory registration of symptoms observed in CAM patients [20].

### 6.1. Radio-graphical testing for diagnosis of CAM

Radiographic testing is non-invasive imaging scans method that uses X-rays or gamma rays to diagnose. Predominantly CT scan and MRI of the infection suspected organ/s is being used [49]. Different radiological diagnostic approaches are being used in various cases, such as cranial and/or pulmonary CT scan, which is recommended for suspected CAM patients who face discomfort, ophthalmoplegia, proptosis, sinusitis and newly diagnosed amaurosis [98]. If sinusitis is detected, endoscopic examination is strongly suggested to diagnose mucormycosis. If an eye or brain problem is suspected in a COVID-19 patient, MRI should be performed because of its significantly higher sensitivity. These scans should be repeated frequently to track the status of CAM in COVID-19 patients [99].

Radiological features that have been found to be characteristic of CAM include pansinusitis or ethmoid sinusitis, nodular lesions, halo signs, reverse halo signs and wedge shaped infiltrations [30]. Reverse halo sign observed in radiological finding of CAM patients are basically ground glass opacity in center surrounded by ring of consolidation. The most common finding in CAM patients on radiological examination was involvement of nasal cavity and middle turbinate /meatus and soft tissues involved includes Pre-maxillary and retroantral fat necrosis [100]. Florid sinusitis and bone erosion are supposed to be the early changes observed in





**Fig. 2.** : An overview of epidemiology which includes risk factors, symptoms, diagnosis, and treatment of Mucormycosis associated COVID-19 patients. An overview of various promising diagnostic protocols employed for diagnosis of mucormycosis. Adopted from [79,80].

radiological examination [101]. Progressive necrotic changes were observed in sphenopalatine foramen and pterygopalatine fossa with concurrent bony erosions [102]. In patients with central nervous system involvement, orbital cellulitis and optic neuritis were the common manifestations observed in CAM patients. Other common finding observed in CAM patients includes trigeminal neuritis and cavernous sinus thrombosis [103]. Recently researchers have found typical finding of “birds nest” appearance in pulmonary form of CAM [101]. However radiological scans have low specificity and sensitivity which limits their use in diagnosis of CAM. So researchers have proposed advanced imaging techniques which include (PET/CT) coupled with [18F]-fluorodeoxyglucose (FDG) which is supported by many studies which reported enhanced uptake of FDG by mucormycosis lesion and its diminished uptake after antifungal treatment [99, 100, 104]. Recently, REBOVC (REmember Basics Of Vicious CAM) a checklist has been developed by researchers to aid radiologists in diagnosis of CAM from radiological examinations [105]. The checklist includes six abnormal findings which are indications of spread of infection from anterior to posterior aspect. These six abnormal findings include (i) unilateral mucosal thickening and accumulation of transudate. (ii) Facial swelling and spread of infection to masticator spaces. (iii) Involvement of pterygoid processes, alveolar processes and skull base. (iv) Optic nerve involvement, inflammation of Preseptal optic globe. (v) Mycotic aneurysms (ophthalmic vein and ophthalmic artery). (vi) Secondary fungal invasion to brainstem and meningitis. Recently a novel type of finding called “vascular cut-off sign” has been reported from CAM patients, which is characterized by abrupt disappearance of branches of pulmonary artery [105]. Soft tissues exhibit some characteristics findings on MRI among them “hypo-tense rim” is most commonly found in CAM patients, which is supposed to be caused by accumulation of metal like iron and magnesium by causative fungus [88].

### 6.2. Histopathology and culture studies for diagnosis of CAM

Mucormycosis diagnosis requires specimen culture for the genus/species identification and antifungal susceptibility testing. A direct microscopy, particularly when combined with fluorescent brighteners and dilacerating agents like KOH, can be utilized to provide quick tentative diagnosis of mucormycosis [106]. Under microscopic examination, mucorales have aseptate or semi-septate, uneven,

tape-like hyphae, just as histological specimens’ even artificial septa can form as result of hyphal folding or development of one hyphae across another, just as they might in histological studies. The broad-angle of non-dichotomous branching (45–90 degree) and wider hyphal width (varying from 6 to 25 μm) are crucial diagnostic features when compared to other filamentous fungi [107]. Still, identification at the species or sub-species level is not attainable at this time. Therefore, the microscopic examination should be coupled with macroscopic and microscopic evidence of infection to reach likely diagnosis because culture is mistakenly negative in up to 50 % of mucormycosis patients. Thus, immunohistochemistry or PCR technologies on fresh or formalin-fixed paraffin-embedded tissue has been proven to be very specific, while there has been some variance in sensitivity reported, and these tests may not be generally available [108].

As per CDC, the major limitation in diagnosis of CAM includes absence of specific biomarker for diagnosis; hence they proposed utility of histopathology, KOH mount and Calcofluor stain as quick and reliable method for diagnosis of CAM [109,110]. Since mucor is endosaprophytic which makes it challenging task for researchers to culture in In-Vitro conditions, hence histopathology remains major tool for diagnosing CAM [111]. To supplement the diagnostic criteria of CAM, rule out procedure of using negative galactomannan and beta-D-glucan pointers can be used to narrow the diagnostic criteria [87]. In this direction researchers have reported utility of histopathology and cultural methods for diagnosis of CAM, for instance histopathology indicated confirmatory positive results in 62.2 % in case series of 28 suspected CAM patients, subsequently [80], reported confirmatory results of histopathology in 22.2 % patients in case series of ten patients. However both of these studies reported lower sensitivity and specificity of cultural methods employed for diagnosis of CAM. Recent studies have proposed use of Hematoxylin and eosin (H & E) staining method for diagnosis of CAM and the method has been used widely by clinicians and researchers to obtain confirmatory results [112]. In addition to identifying the fungal element the staining method identifies changes in tissue architecture and pro-inflammatory cellular infiltrations which is characteristic of mucormycosis invasion. In addition to H and E staining, Grocott-Gomori’s Methenamine Silver (GMS) and Periodic Acid Schiff (PAS) have been used to supplement confirmatory results obtained from other diagnostic methods [113]. Recently, Lactophenol

cotton blue has been used in limited number of CAM patients and very encouraging results have been obtained in identification *A. elegans* which gives characteristic appearance of dark thin walled sporangiophores [114]. Furthermore, KOH mount have been used to diagnosis fungal infections in CAM patients as characteristic findings of broad ribbon-like aseptate hyphae with wide-angle branching are observed in biopsy samples [75].

Cultural examination of mucormycosis offers an advantage of species and genus identification and offers an opportunity for antifungal susceptibility testing and henceforth formulation of an effective antifungal therapeutic protocol [115]. Identification is mostly based on colonial morphology and microscopic structure [93]. The major limitation of cultural examination is its low sensitivity as false negative results were obtained in almost 50 % of CAM cases. The low sensitivity is attributed to destruction of fungal hyphae during processing of samples, requirement of special cultural conditions and on-going antifungal treatment [116].

### 6.3. Susceptibility testing for diagnosis of CAM

Antifungal susceptibility testing of mucorales has been standardized by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). Commercial techniques have been investigated for mucorales and have produced, in some cases, contradictory results when compared to established procedures, particularly for amphotericin B and posaconazole [117]. Currently, E-test is generally postulated for use in mucormycosis with marginal strength only. The use of standard antifungal susceptibility testing methods to help in focussed antifungal treatment in mucorales is controversial, but it may be therapeutically effective in situations of treatment failure. On the other hand, researchers highly advise using these approaches solely to understand epidemiological of this disease [118].

### 6.4. Novel serological methods for diagnosis of CAM

Serology has been used to confirm diseases that are challenging to detect using conventional approaches. Although evidence-based results in concurrence with this statement is lacking, the *Aspergillus* galactomannan tests and panfungal  $\beta$ -D-glucan test do not sense antigen components of the mucorales, and positive test is supposed to deliver strong indication for excluding mucormycetes as pathogenic agents of infection [119]. Various methods like ELISA, immunoblots, and immunodiffusion tests have been used in the past to diagnose mucormycosis, with varying degrees of success. Some of the tests used had low sensitivity and specificity and cross-reactivity with *Candida* and *Aspergillus* species [120] and lacked clinical validation [121]. Recently a study was conducted on using enzyme-linked immunospot (ELISpot) assay in three patients with invasive mucormycosis and they discovered mucorales-specific T-cells, while these cells were not found in any of the control patients. However there is a need of extensive research to evaluate their utility as early markers of disease and their probably utility as diagnostic tool for diagnosis of mucormycosis [122]. A novel method of Metabolomics-Breath Test has been developed for diagnosis of mucormycosis by [43] in laboratory animal model. They analysed metabolomics profile of laboratory animals experimentally infected with three common species of mucormycosis found in CAM patients. Results of these preclinical studies indicated that breath profile of infected mice varied significantly compared to control mice in terms of metabolite sesquiterpene. Based on the results of this study it can be postulated that this method has potential to be used as non-invasive diagnostic method for early and rapid diagnosis of CAM and evaluate therapeutic response.

### 6.5. Updated molecular- techniques for diagnosis of CAM

In present times we are lacking clinically proven and standardized test as the use of molecular techniques on both fresh clinical material and histopathological sections for the diagnosis of mucormycosis are presently minimally supported because formalin destroys DNA; thus, samples which are collected from patients and are not preserved in paraffin preferred over paraffin-embedded tissue [123]. DNA finding in serum is extremely encouraging, but it is only supported with modest strength due to a lack of standardization. Most biomarker-based assays are based on the internal transcribed spacer (ITS) region selected by the international subcommittee on fungal barcoding as the pan-fungal barcode. On the other hand only few tests have been fabricated on principle of RNA detection. The most promising among them is an 18 S rRNA-based Fluorescence in situ hybridization (FISH) method combined with PCR *Candida* spp., *Aspergillus* spp., and mucormycetes were identified using fluorescent probes [124].

The sensitivity and performance of all nucleic acid-based procedures must be strongly reliant on the DNA/RNA extraction technique utilized. The volume and purity of genetic materials extracts are determined not only by the type of clinical samples collected but also by the procedure employed for extraction [125]. The extraction procedures or chemicals used must be modified based on the clinical material and its specific composition to reduce the chances of amplification inhibition (e.g., proteins, large amounts of human genetic material) and ensure sufficient intact nucleic acids of the pathogenic agent remain available for further processing [126].

### 6.6. Identification of fungal species in CAM

Some genera, like *Cunninghamella*, have been linked to higher patient mortality rates and more infectious in animal models, at present little evidence identifying the causative mucorales to the genus and/or species level help to advice in the selection of antifungal treatment [45]. Identification down to the species level, on the other hand, is critical for improving epidemiological understanding of the disease. The clinical picture, in particular, might alter depending on the species. Furthermore, species identification is useful in the examination of health-care-associated mucormycosis epidemics [127] As a result, genus and species level identification is strongly recommended for better understanding of mucormycosis pathogenesis and hence better targeting of disease. Identification at the genus level as a means of guiding therapy has only minimal support. Internal transcribed spacer (ITS) sequencing is widely proposed as the effective approach for molecular identification. MALDI-TOF (matrix aided laser desorption ionization-time of flight) identification supported to some extent since it mostly relies on in-house databases, which many diagnostic centers lack [123].

## 7. Updated guidelines for treatments of CAM

In 2017 European Conference on Infections in Leukemia (ECIL) is supposed to be the pioneer conference that stated treatment guidelines for mucormycosis, which were subsequently updated by European Confederation of Medical Mycology (EMM) for treatment of CAM, with former recommended liposomal Amphotericin B and later recommended Amphotericin B lipid complex for treatment of CAM [123,128]. Mucormycosis is a severe infection that requires the use of antifungal medications prescribed by a doctor. In addition, it can require surgery to cut away the infected tissue, leading to eventual loss of body parts like the upper jaw and even an eye. In some cases, mucormycosis treatment includes combination of surgical procedure and antifungal medicines [129]. A brief of optimal treatment approaches for mucormycosis is shown in Fig. 3. Both surgical and antifungal therapeutic interventions have been

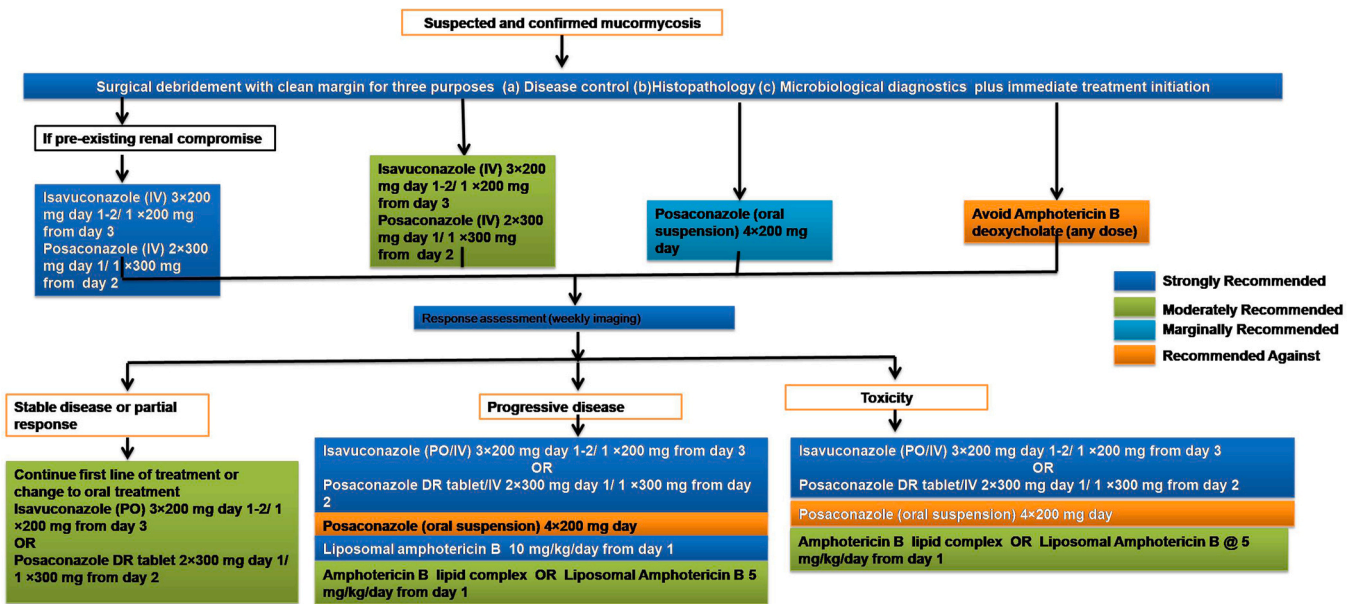


Fig. 3. Briefly summary of the optimal treatment approaches employed for treatment of mucormycosis. Adopted from [79,80].

recognized commonly because of infection's angioinvasive and necrotic character. Voriconazole is the sole novel medication that has efficacy against mucorales; however, it does not appear to give substantial benefits over traditional therapeutic regimen comprising of antifungal agents [130]. As of present times there are at least 11 different guidelines available for management of CAM and most of them are based on modified recommendations of European Confederation of Medical Mycology (ECMM) and Mycoses Study Group Education and Research Consortium [95]. Recently new guideline called Code Mucor has been developed for treatment of COVID-19-associated rhino-orbital-cerebral mucormycosis (CAROCM) and is specific for every sub stage of infection [131]. Most of these guidelines are based on controlling hyperglycemic condition and optimizing use of corticosteroids. Furthermore these guidelines postulate immediate radiology-guided surgical intervention and extensive surgical debridement of mucormycosis infected tissue including bone and if infection recurs procedure should be repeated. These propositions are supported by decreased mortality and disease progression in large sample size of 2826 CAROCM patients [101]. In addition to surgical procedures these guidelines recommended use of antifungal agents, for instance ICMR has recommended use of antifungal drug liposomal amphotericin B (AmB) @ 5 mg/kg/day for 4–6 weeks [101,132]. In case infection spread to CNS, a higher dose AmB has to be used in order to manage spread of infection and treat disease effectively and in case of non-availability of the liposomal amphotericin B, Deoxycholate and lipid complex of AmB has been suggested to be used in CAM patients [133]. Chronic use of antifungal drugs results in fever, significant increase in levels of kidney biomarkers and multiple organ dysfunctions, hence close examination of renal biomarkers and potassium levels should be undertaken while patients are under antifungal treatment [133].

### 7.1. Surgical treatment guidelines against CAM

Surgical treatment is crucial for managing mucormycosis, although disseminated infection can have several sites of infection. In addition, surgical interventions have been linked to increased cure and survival rates. Surgery is divided into several categories: epidermal tissue debridement, naso-orbital-cerebral mucormycosis debridement, Orbital exenteration, bone debridement, lung resection, and visceral resections [134]. Adapting surgical scope to the

progress of mucormycosis improves results and prevents healthy tissue loss. Liver resection with complete elimination of the mucormycosis is possible and results in a longer quality lifespan [99]. Furthermore, drainage of mucormycosis abscess followed by removing an infected area of the liver is possible. If a lung is resected, patients can have positive prognosis from emergency surgery to avoid bleeding and elective surgery, which has been demonstrated to improve survival. [135]. The surgical complication rate following visceral excision appears to be acceptable. In trauma patients, mucormycosis often appears as soft tissue infection. Therefore, early, radical, repetitive surgical debridement is recommended and can result in a permanent cure. When possible, the researchers strongly advise early comprehensive surgical treatment for mucormycosis, in addition to systemic antifungal therapy. As needed, resection or debridement should be performed [36]. As per guidelines of AIIMS surgical debridement should be undertaken in patients after stabilization [101], as per Code Mucor, aggressive debridement of infected tissue should be conducted and surgical should be performed in all cases of CAROCM [131]. Similarly European Confederation of Medical Mycology and the International Society for Human and Animal Mycology has recommended early surgical removal of infected tissue included bones if found to be infected [136]. Furthermore, Fungal Infection Study Forum has suggested extensive surgical debridement of pulmonary lobe of localized part of pulmonary tissue [137].

### 7.2. Antifungal drugs treatment guidelines against CAM

The absence of relevant pre-clinical and clinical studies makes it complicated for doctors to choose between the existing antifungal drugs for treating mucormycosis. Hence prospective interventional study of mucormycosis has been non-existent for several reasons. First, despite the disease's high mortality rate, it occurs less frequently than other opportunistic diseases. Another impediment to mucormycosis clinical trials is monotherapy dismal success rate [40,138]. In most cases, prevention is aimed at a wide spectrum of mucormycosis, such as candidiasis and aspergillosis. During prevention with posaconazole oral suspension, the breakthrough mucormycosis has been uncommon. Exposure to posaconazole sustained-release tablets or parental infusions may result in even lower invasive fungal infection rates [139]. Primary prevention with



posaconazole sustained-release tablets at moderate strength is recommended to avoid mucormycosis in immune-compromised patients or those with graft versus host disease, and oral suspension treatment is recommended at marginal strength [40].

Due to the poor prognosis rate, it may be unethical to randomly categorize patients in experimental arms of any "less intensive" treatment (i.e., monotherapy versus combination therapy, standard-dose versus high-dose monotherapy). For these reasons, there is no evidence to support primary prevention aimed solely at mucormycosis [140]. The decision of secondary prevention to avoid recurrence, particularly in immune-compromised patients, is common clinical question. Researchers defined secondary prevention as continued treatment in a patient who had been suspected to be infected with mucormycosis and responded to therapy or restarted treatment in a patient who had achieved successful disease control and was now immune-competent, but was scheduled for new period of immunosuppression in the absence of a consensus definition [9]. The evidence foundation for treatment decisions such as switching to posaconazole or isavuconazole to enable outpatient therapy is limited [94].

### 7.3. Recent updates in first-line antifungal monotherapy against CAM

Liposomal amphotericin B effectively cured mucormycosis with diverse organ involvement patterns in multiple case series. For the first-line therapy of mucormycosis, isavuconazole of moderate potency is indicated. For first-line therapy, the group had slight preference for posaconazole oral suspension and a moderate preference for posaconazole delayed-release tablets and infusion [141]. Primarily it has been recommended that treatment with liposomal amphotericin B (5–10 mg/kg per day) is strongly beneficial for all types of organ involvement. If significant nephro-toxicity occurs, the dose can be lowered as needed, although dosages less than 5 mg/kg per day are only indicated for those with moderate strength [142]. Doses should not be gradually raised over multiple days; rather, the entire daily dose should be administered on the first treatment day. A moderate strength amphotericin B lipid combination of 5 mg/kg per day is prescribed for patients without CNS involvement. When alternatives are available, the use of amphotericin B deoxycholate is discouraged. Lipid formulation Amphotericin B deoxycholate (AMB) in combination with other therapeutic drugs is found to have significantly more therapeutic merit compared to drug used alone (105).

#### 7.3.1. First-line combined antifungal treatment

In animal studies, several antifungal combinations have demonstrated the ability to improve survival rates while causing little antagonism. Some case series of CAM have encouraging results [143]. However, a historical control study and a propensity score analysis could not establish the benefits of antifungal combinations in case series with hematological malignancy. More than one fungal species can induce mixed infection in trauma patients, particularly those with blast injury, necessitating combination treatment with either posaconazole or voriconazole and liposomal amphotericin B [90]. Aside from the possibility for increased toxicity, medication interactions, expense and the disadvantages of combination therapy remain unknown. Mucor Code has recommended immediate induction therapy comprising of Liposomal Amphotericin B(5–10 mg/kgBW) and Deoxycholate, in case Amphotericin B is contraindicated Posaconazole IV should be used [137]. Similarly, European Confederation of Medical Mycology and the International Society for Human and Animal Mycology has recommended combinational therapy comprising of Amphotericin B, Itraconazole taken with acidic beverage and Iron chelators (deferasirox) for management for CAM [101]. Similarly Fungal Infection Study Forum has suggested slow infusion of Liposomal Amphotericin-B @ 5 mg/kgBW as

preferred treatment and higher dose @ 10 mg/kgBW if infection has spread to central nervous system. Indian Medical Association has recommended Liposomal Amphotericin-B as first line of treatment while as Isavuconazole and Posaconazole as second line of treatment [144]. Surprisingly no guideline promote use of combinational therapy for CAM patients except Himachal Pradesh National Health Mission which recommended use of triple regimen comprising of lipid polyenes, caspofungin and posaconazole, however these guidelines come with precautions that this regimen needs to be evaluated in large sample size before using this in actual clinical settings[101].

#### 7.3.2. Antifungal salvage treatment

Salvage treatment was characterized as receiving antifungals as a result of earlier antifungal intolerance or refractory disease. Renal toxicity might be a limiting issue for amphotericin B formulations, whereas liver toxicity is the most common azole toxicity [145]. Previous antifungals may have produced toxicity, or it may be predicted owing to post organ failure. Only two drugs have been shown to be effective in mucormycosis, therefore, salvage treatment usually entails switching to the other. In medical circumstances, intolerance or toxicity and refractory disease isavuconazole salvage therapies were effective [40]. As a salvage therapy, isavuconazole has a lot of support. The use of posaconazole sustained-release pills or parental injection for salvage therapy is strongly recommended. When available, it should be used over Posaconazole oral suspension, which has only limited role in salvage treatment. In case of first-line therapy failure with isavuconazole or posaconazole, the findings support the use of all three lipid-based amphotericin B formulations of strong to moderate strength [146].

## 8. Challenges in antifungal drugs to treat CAM

Beyond the challenging task of treating critically ill COVID-19 patients, treating two potentially fatal diseases is considerably more difficult. This situation is exacerbated in the COVID-19 infection conditions if mucormycosis causes the co-infection mainly produced by multidrug-resistant strains. The specifically antifungal medications are restricted, which results in dangerous medicinal interactions, toxic response and severe adverse effects on several organs [147]. Chronic clinical exposure to previously active new triazoles (e.g., posaconazole, voriconazole, and isavuconazole) or echinocandins (e.g., caspofungin, anidulafungin, and micafungin) can result in antifungal resistance, leading to treatment failures.

Another important consideration is the possibility of drug interactions during therapy [148]. Many different medicines are now being studied or used experimentally for COVID-19 treatment. The drugs which include immune modulators, cytokine receptor blocker, and glucocorticoids are commonly used in suppressing robust and deleterious inflammatory processes [149]. Furthermore, the over-suppressed immune system of a COVID-19 patient can support the advent of likely mucormycosis infection. Antifungal therapy failure can be directly linked to improper sample collection, lack of standard equipment for molecular tests, lack of early biomarkers of fungal components in diseased tissue and lack of competent specialists to identify the fungal agent accurately [150]. Regrettably, this actual picture may have a significant influence on the rising proportion of COVID-19 positive individuals who succumb to mucormycosis infections.

## 9. CAM studies from different parts of world

COVID-19 patients suffer from severe threat of bilateral pneumonia and co-infections with bacteria and fungus especially mucormycosis. Some mucormycosis cases have been identified in the COVID-19 patients, as listed in Table 2. The case fatality of COVID-19

**Table 2**  
Case series and case reports of COVID-19 associated mucormycosis (CAM) reported from different parts of globe.

Type of infection	Age in years	Medical history	Organs Involved	Clinical manifestation	Investigation	Diagnosis of CAM	Treatment for		Results and Outcome
							COVID 19	Other infections	
Rhino orbital Mucormycosis	60 years	Diabetes milletus	Signs of orbital cellulitis	swelling in the right preseptal, malar, premaxillary and retrobulbar regions with paranasal sinusitis	elevated serum creatinine (1.57 mg/dl)	Yes, suspected, Day 10	dexamethasone (4 mg twice daily)	Intravenous metropenem (3 gm/day) and vancomycin (2 g/day) with the addition of amphotericin B (0.5 mg/kg/day) and cessation of steroid therapy.	Died
Rhino orbital Mucormycosis	Male	Asthma	Conjunctival edema in right eye			Yes (suspected at admission)	Remdesivir plasma		Died on Day 26
Pulmonary Mucormycosis	33years	Hypertension	soft tissue necrosis along upper and lower eyelid	Rhinoorbital mucormycosis	hemoglobin value of 10.40 gm/dl	Yes, suspected	Convalescent intravenous dexamethasone		Discharged
Pulmonary Mucormycosis	55years	Diabetes	along upper and lower eyelid	Altered mentation, proptosis	C-reactive protein (CRP) 29.53 mg/l	Symptoms developed at Day 21	Amphotericin B (6 mg/ day)	Amphotericin B (formulation not mentioned)	
Pulmonary Mucormycosis	Male	Hypertension	Rhino-orbital cerebral	COVID ARDS	IL6 level of 3439 micrograms/ml		remdesivir (100 mg /day)	liposomal amphotericin B (3 mg/kg)	
Pulmonary Mucormycosis	Male	Renal disease	Kidney, Lungs, Paericardium Heart	<i>Rhizopus microspores</i>	Elevated serum creatinine (2.28 mg/dl)				
Pulmonary Mucormycosis	49 years	COVID	Pulmonary	COVID ARDS	Serum creatinine, 8.4 mg/dl	Yes, suspected	Remdesivir (100 mg /day)	liposomal amphotericin B (3 mg/kg)	Died on Day 21
Intestinal Mucormycosis	male	Acute lymphoblastic leukemia	mucormycosis with bronchopleural fistula and pneumothorax	Pulmonary mucormycosis <i>Rhizopus microspores</i>	and serum creatinine, given	Symptoms developed on Day 14	Tocilizumab		Died
Rhino Maxillary Mucormycosis	59years	leukemia	ileum	Gastro	Absolute neutrophil count, 0.06 109 / litre	Day 14	Dexamethasone (6 mg/ day)	Ceftriaxone	No recurrence after 1 year
Pulmonary Mucormycosis	50 years	Diabetes milletus	Jejunum	Mucormycosis	Elevated fasting blood sugar level	Yes, on the day of admission	(100 mg /day)	Azithromycin lipid formulation of amphotericin	Died on day 27
Pulmonary Mucormycosis	Female	Obesity	Not given	Neutropenic fever	decreased hemoglobin% (7 g %)	Yes, on the day of admission	Intravenous methylprednisolone 40 mg BD	Venofor 800 mg	
Pulmonary Mucormycosis	22 years	Hypothyroidism	Pain and swelling on right side of the mouth	Mucormycosis <i>Rhizopus oryzae</i>	HbA1c level was 8.3 %.	No, Autopsy diagnosis	Dexamethasone 4 mg BD	Surgical excision of the maxilla	
Pulmonary Mucormycosis	Male		Lungs, Heart, Brain	COVID (mechanically ventilated)	Lymphocyte count and serum creatinine, not provided		Not mentioned	Meropenem Linezolid Caspofungin	
Rhino orbital Mucormycosis	60 years	Diabetes milletus	Rhino-orbital	COVID	Elevated serum creatinine (1.57 mg/dl)	Yes, suspected, Day 10	Methylprednisolone 40 mg BD	Oseltamivir	Died
Sino orbital Mucormycosis	Male	Peripheral vascular disease	Sino-orbital	Mechanical ventilation required		Yes, after Day 18	Tocilizumab 400 mg	Amphotericin (0.5 mg/ kg/day)	Recovered on Day 38
Pulmonary Mucormycosis	38 years	High-grade fever	Periorbital	COVID	Lymphopenia (9.60 %)	Diagnosed on Day 25	Dexamethasone 4 mg BD	Surgical debridement	Recovered
Pulmonary Mucormycosis	Years	Shortness of breath	2.5 cm nodule in Right upper lobe	COVID ARDS pneumonia	Serum Interleukin 637.93 pg/ml		Inj.	Amphobectrin B 1 mg/dl	
Pulmonary Mucormycosis	Male	Hypertension	posterior segment with central cavitating necrosis		Neutrophil count 83.1 %		Remdesivir 100 mg/day	Liposomal amphotericin B	
Pulmonary Mucormycosis	72 years	Steroid-induced diabetes	with hypermetabolic soft tissues in right arm and thigh		Lymphocytic count 9.5 %		IV 800 mg/day	Liposomal amphotericin B	
Pulmonary Mucormycosis	Male	Hypothyroidism			HBA1c 6.3 %		Inj Dexamethasone 4 mg/day	Posaconazole 300 g/day	
Intestinal Mucormycosis	86 years	Hypertension	Gastric	COVID ARDS and diarrhea	Elevated serum creatinine (1.52 mg/dl)	Not suspected	Ramdevprir. Methylprednisolone 4 mg thrice/day		Died Day 5
Pulmonary Mucormycosis	Male	Ischemic cardiomyopathy	Stomach Intestines	COVID ARDS	Hypokalaemia Hypomagnesemia	Suspected D14	Convalescent Plasma Tocilizumab		Alive
					Elevated serum creatinine level (2.34 mg/dl)	Suspected D14		Azithromycin Ceftriaxone Liposomal treatment	(continued on next page)



Table 2 (continued)

Type of infection	Age in years	Medical history	Organs Involved	Clinical manifestation	Investigation	Diagnosis of CAM	Treatment for		Results and Outcome
							COVID 19	Other infections	
Mucormycosis Pulmonary	55 years Male	Diabetes mellitus End-stage renal disease	Lungs Lung Maxillary sinus thickening	COVID (mechanically ventilated)	Lymphopenia (5.3 %) Elevated serum creatinine (range 3–8 mg/dL)		Lopinavir–ritonavir Hydroxychloroquine	of Amphotericin B mg/kg/day Meropenem Linezolid liposomal preparation of Amphotericin B (5 mg/kg/day) Isavuconazole	Discharged on D54 Died Day 62
Mucormycosis	66 years male	Hypertension			Lymphopenia (6 %) Lymphopenia (400/1L) Renal failure dialysis required (creatinine levels not provided)				
Rhino orbital Mucormycosis	41 years	Diabetes mellitus diabetic ketoacidosis	sinuses, nasal passages, oral cavity	COVID-19 pneumonia Atelectasis and pneumonia on the left lobes	elevated serum creatinine (1.3 mg/dL (high) ast HbA1c 9.6 %) HbAc1 9.6 %	Yes, suspected, Day 1	dexamethasone steroids and hydroxychloroquine, dexamethasone (6 mg/day)	amphotericin B complex; cefepime and IV	Recovered Recovered
Rhino-sinusal Mucormycosis	62 years Male	type 2 diabetes mellitus disseminated cryptococcosis	rhinosinusal Left Maxillary Sinus with cellulitis	peripheral bilateral lung infiltrates and chronic sinusitis. COVID-19 pneumonia Mechanical ventilation Left maxillary sinusitis	HbAc1 9.6 % ast HbAc1 9.6 % (last HbAc 9.6 % creatinine level of 1.4 mg/dl)	Yes, suspected Day 1	antibiotic empirical therapy with ceftriaxone 1 gr q24h + azithromycin 500 mg q24h ceftriaxone 1 gr q24h Azithromycin 500 mg	three surgical debridements for the rhino-cerebral mucormycosis. insulin drip for diabetic ketoacidosis (DKA), Fluconazole isavuconazole piperacillin/tazobactam 4.5 mg IV Liposomal formulation of amphotericin B and an azole 6 subsequent ENT surgical debridement	
Rhinoorbital mucormycosis Pulmonary	33 Years Female	Asthma diabetes mellitus (previously undiagnosed)	Rhino-orbitocerebral Brain	COVID 19 Rhino-orbital mucormycosis Diabetes ketoacidosis	Elevated serum creatinine (2.28 mg/dL)	Yes (suspected at presentation)	Convalescent plasma Remdesivir	Amphotericin B Vancomycin	Died on day 26 Died on Day 20 admission developed
Mucormycosis Rhino Orbital	57 years Male	Hypertension diabetes mellitus	Eyes (opacification of left maxillary and ethmoid sinus)	Altered mentation, proptosis COVID 19 ARDS Pneumonia Pulmonary	Lymphopenia (5.9 %) Serum creatinine level 1.40 mg/dL hemoglobin 5.7 g/dL, total white blood cell of 17,100 WBC/mm3 ESR of 23 mm Not mentioned	Yes, suspected on day 4 Yes on Day 10	Dexamethasone (6 mg) Remdesivir Convalescent plasma	surgical resection of the involved tissue Amphotericin Liposomal formulation of Amphotericin B Cefepime Vancomycin	bradycardia and hypotension and expired due to massive hemoptysis and cardiopulmonary arrest. Died on day 31
Mucormycosis	86 Years male	Diabetes mellitus Hypertension Asthama	left maxillary and ethmoid sinus) Lungs	severe facial pain and paresthesia in the region of upper and lower jaw trigeminal neuralgia COVID ARDS (mechanically ventilated) Rhino orbital					

patients is usually high owing to the co-morbidities and associated secondary infections caused by various bacterial and fungal opportunistic pathogens [112]. Among many opportunistic pathogens aspergillosis and candidiasis have been mostly found in critically ill COVID-19 patients [13]. A recent meta-analysis has revealed that most of the cases of mucormycosis are from Indian subcontinent accounting almost 140 cases/million [87]. Researchers have attributed this to higher number of diabetic population living in India and use of under the counter drugs [151]. Among the patients with CAM, 57 % were diabetic, 18 % diabetic ketoacidosis and 57 % were suffering from uncontrolled diabetes mellitus [88]. Recently some researchers conducted cohort studies on 10 patients suffering from CAM among them 4 were diabetic and 5 were under intensive corticosteroid therapy for long time [152].

Several studies have been conducted across the globe in patients with CAM these studies have found significantly reduced levels of T lymphocytes and significantly higher levels of pro-inflammatory cytokines in these patients [153]. conflicting findings have been reported by other studies [154], according to them COVID-19 is not actual predisposing factor of mucormycosis but short term use of corticosteroid therapy is the main predisposing factor for emergence of CAM. Various meta-analysis studies have indicated that developed countries (0.005–1.7 %) have lower incidence of CAM compared to developing countries like India (0.14 %) [113]. These findings may be attributed to higher prevalence of co-morbidities like diabetes mellitus, hypertension and renal failure in developing countries like India [17]. Delayed treatment in CAM patients significantly increases case fatality rate in these classes of patients [17]. Although there are increasing incidences of CAM in COVID-19 patients but pathophysiology of the disease has largely remained ill understood. But based on the clinical studies and case series studies following probably mechanistic pathways have been proposed.

- i. COVID-19 causes extensive damage to pulmonary parenchyma, autopsy examination of patients that died of COVID-19 showed extensive necrosis of pulmonary epithelium which resulted in weakening of pulmonary epithelium and make these patients susceptible to secondary infections [80,155]
- ii. COVID-19 cause significant reduction in CD4<sup>+</sup>, CD8<sup>+</sup> T lymphocyte and elevation in pro-inflammatory cytokine levels which is utilized by opportunistic pathogens to cause secondary infection especially mucormycosis. Furthermore chronic intubation under mechanical ventilation adds risk for contracting secondary infections [8].
- iii. In patients with COVID-19, the most significant biochemical alteration includes significantly elevated levels of ferritin and free iron which acts as promoter for growth of mucormycosis. The situation is further complicated by presence of other co-morbidities like diabetes mellitus and use of medicinal preparations like corticosteroids which promote emergence of secondary infections. Recently RECOVERY trail was conducted in 2020; they found chronic use of high dose of corticosteroids results in fulminating of mucormycosis in COVID-19 patients.

## 10. Conclusions and future perspectives

The scientific world is concentrated on discovering antivirals and vaccines to treat/prevent COVID-19. Unfortunately, large population of patients are at risk for secondary infections, especially mucormycosis resulting from this novel type of COVID-19 infection. This finding emphasizes the need to focus on developing novel, specifically targeted and safe antifungal medications to battle mucormycosis and other fungal diseases in the COVID-19 patients [156]. The clinical presentation is non-specific, and early diagnosis of the mucormycosis by histopathology is time-consuming method. Direct culture investigation, molecular diagnostic techniques, PCR, and in

situ hybridization are all options for starting therapy. Therefore, medical professionals also emphasize the necessity of early mucormycosis identification techniques for optimal antifungal medication management. In the clinical setting, choosing the optimum drug regimen, dosage, method of administration and therapy duration is critical to therapeutic effectiveness and overcoming drug resistance [157]. The best strategy to deal with CAM is to use immunologic and metabolic profiling. The cases highlight the significance of being vigilant about opportunistic fungal pathogens commonly isolated from hospital and the environment. The clinical settings must consider future recommendations for expanding knowledge and appropriate intervention for CAM patients to promote effective mucormycosis infection reduction and robust surveillance at high-risk hosts during the COVID-19 pandemic [158].

## Conflict of interest

The authors declare no competing interests.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2022.10.011.

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