

## CORTICOSTEROIDS AND SECONDARY INFECTIONS: AN INSIGHT INTO CORONAVIRUS DISEASE-2019

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### ABSTRACT

Coronavirus disease 2019 (COVID-19), which is caused by novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged at Wuhan in China in December 2019 and has rapidly spread throughout the world. The droplets expelled during face-to-face exposure, mainly through talking, coughing, or sneezing, are the most common mode of transmission. So far, children have not been affected frequently without deaths. However, the course of this virus in the future is unknown. The diagnosis is mainly made through reverse transcription-polymerase chain reaction (RT-PCR) and serology testing. Treatment with dexamethasone at an early phase of developed acute respiratory distress syndrome (ARDS) caused by SARS-CoV-2 alters the pulmonary and systemic inflammatory response and decreases mortality. Corticosteroid therapy is associated with a sizable reduction in the duration of mechanical ventilation and hospital mortality. One of the major risk factor associated with corticosteroid therapy is associated with acquiring secondary infections. Pulmonary epithelial damage and inflammatory disease are the predisposing risk factors for pulmonary aspergillosis due to the release of danger molecular patterns during severe COVID-19. Galactomannan and culture testing of bronchoalveolar lavage fluid are the most sensitive diagnostic measures for aspergillosis in intensive care unit (ICU). Finally, the treatment of coronavirus associated pulmonary aspergillosis is complex. The only way one can prevent the spread of infection by following precautions such as frequent hand washing, wearing a mask in public places, social distancing, and by avoiding unnecessary gatherings.

**Keywords:** Coronavirus disease 2019, SARS-CoV-2, Acute respiratory distress syndrome, Dexamethasone, Pulmonary aspergillosis.

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### INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus which is named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The virus originated in Wuhan City in Hubei Province in central China and it is currently spreading all across the globe causing a pandemic. The China Health Authority alerted the World Health Organization (WHO) to several cases of pneumonia of unknown origin on December 31, 2019 [2]. On January 30, 2020, the WHO declared the Chinese outbreak of COVID-19 to be a Public Health Emergency of International Concern and as a pandemic on March 11, 2020.

### ORIGIN AND HISTORY

Since the virus appears with a crown-like projection on its surface, therefore the term Corona means a crown. It was first isolated in the late 1960s from patients suffering from the common cold [3]. Bees, palm civets, poultry, and animals are the main reservoirs of the virus. Until the outbreak of the SARS in 2002 in China [4], these viruses were thought to transmit infection only among animals. Middle Eastern countries later witnessed an outbreak of Middle East respiratory syndrome coronavirus [5]. Coronavirus is an enveloped (E-protein) single-stranded (positive-sense) RNA virus (+ssRNA) with club-shaped glycoprotein projections (S-protein). The spherical or pleomorphic form of the virus is 80-120 nm in size. The spikes are made of hemagglutinin-esterase [6].

SARS-CoV-2 is a member of the family Coronaviridae and it is subdivided into four genera (alpha, beta, gamma, and delta coronaviruses). SARS-CoV-2 belongs to betacoronavirus [7]. Based on the genome sequence studies of SARS-CoV-2, RaTG13 (a bat coronavirus), and SARS-CoV, they found that the virus is more related to RaTG13 with 96.2% overall genome sequence identity and also found that no evidence of recombination events detected in the genome of SARS-CoV-2.

Altogether, these findings suggest that bats might be the original host of this virus [8,9].

In a recently published study, it has further established that sarbecovirus lineage is responsible for the pandemic of COVID-19 and concluded that the SARS-CoV-2 itself is not a recombinant of any sarbecoviruses detected to date and has been circulating unnoticed in bats for decades [10].

### EPIDEMIOLOGY

Globally, 37 601 848 confirmed cases of COVID-19 with a death rate of 2.8% have been reported as of 13/10/2020. In India, a total of 7 175 880 cases has been confirmed with a death rate of 1.53% as of 13/10/2020. The worst-hit countries due to COVID-19 are the USA, India, and Brazil. At the end of 2019, cases have been reported on all continents, except for Antarctica [11]. The reported cases underestimate the overall burden of COVID-19 since only a tiny proportion of acute infections are being diagnosed and documented. Seroprevalence findings within the United States and Europe have shown that the rate of exposure to SARS-CoV-2, expressed as seropositivity, exceeds the level of reported cases by 10-times or more after accounting for possible false positives or negatives [12].

COVID-19 has better outcome results in children than in adults with the exception of those hospitalized by a rare pediatric inflammatory system syndrome at intensive-care facilities [13]. Clinical presentations in pregnant women are generally moderate, with few cases of serious illness and fatal outcomes [14].

### MODE OF TRANSMISSION

The possible origin of SARS-CoV-2 and the first mode of disease transmission are not yet identified [15]. The SARS-CoV-2 is alleged to have originated from an animal host (zoonotic origin) with the further

human-to-human transmission; however, a study is needed to elucidate whether any intermediate hosts have facilitated the transmission of the virus to humans [16]. The Huanan South China Seafood Market sells live animals such as snakes, bats, poultry, and marmots. This may be the juncture where zoonotic (animal-to-human) transmission occurred. A study has elucidated that humans became infected with SARS-CoV-2 through pangolins, acting as an intermediate host [17].

The droplets expelled during face-to-face exposure in the course of talking, coughing, or sneezing is the most common mode of transmission. Prolonged exposure to the infected individuals (being for at least 15 min within 6 feet) and shorter exposures to symptomatic individuals (e.g., coughing) are associated with a higher risk of transmission, whereas short exposures to asymptomatic contacts are less likely to result in transmission [18]. Contact surface spread (touching a surface with the virus on it) is another possible mode of transmission and reinforces the potential for transmission through fomites (objects such as a doorknob, cutlery, or clothing) [19]. A recent study established that the virus, suspended in a solution made to mimic human mucus, can stay as long as up to 28 days on the glass surfaces and plastic banknotes at 20°C; however, at higher temperatures (30°C and 40°C), the virus did not survive very long [20].

Aerosols (smaller droplets that remain suspended in the air) can also be transmitted, although it is unknown if this is a major source of infection in humans outside the laboratory setting. The risk of aerosol transmission, combined with droplet transmission, for example, during choir rehearsal and in restaurants, has been implied by some outbreak reports linked to indoor crowded spaces [21,22]. SARS-CoV-2 RNA has been found in blood and stool; however, fecal-oral transmission has not been reported [23]. SARS-CoV-2 transmission from people with infection but no symptoms, even those who developed symptoms later and were thus considered presymptomatic, has been well documented [24]. It is currently suspected that maternal COVID-19 is associated with a low vertical transmission risk. Maternal infection with SARS-CoV-2 occurred during the third trimester of pregnancy in the most documented studies, with no maternal deaths and a favorable clinical course in the neonates [25].

#### PATHOGENESIS AND CLINICAL MANIFESTATIONS

All ages are susceptible to COVID-19. The incubation period of COVID-19 is on average 5–6 days but can be as long as 14 days [26].

#### Virus entry and host cell invasion

The virus that is transmitted through respiratory droplets once, inside the body, S protein of the virus binds to host ACE-2 receptors and enters host cells through endocytosis [27]. After binding of SARS-CoV-2 to the ACE-2, the S protein undergoes activation by cleavage of the S1/S2 domain by the type 2 trans-membrane protease TMPRSS2 that causes conformational changes leading to viral and host cell membrane fusion [28]. Post membrane fusion, the virus enters the pulmonary alveolar epithelial cells and the viral contents are released in. Now inside the host cell, the virus undergoes replication through transcription and translation to synthesize new proteins [29]. Nucleocapsid (N) and membrane (M) proteins facilitate integration to the cellular endoplasmic reticulum. These newly formed nucleocapsids are then enclosed in the endoplasmic reticulum membrane and transported through exocytosis to the extracellular space and are ready to invade the adjacent epithelial cells as well as for providing fresh infective material for community transmission through respiratory droplets [27].

#### Asymptomatic phase and upper respiratory tract infection

SARS-CoV-2, which is obtained through respiratory aerosols, binds to ACE-2 receptors in the nasal epithelial cells in the upper respiratory tract [30]. The virus undergoes local replication and spread along with ciliate cell infection in the airways. This is the asymptomatic phase which lasts a couple of days and the immune response is limited during this phase. Compared to the throat, studies have shown higher viral loads in the nasal cavity, with no difference in viral burden between symptomatic and asymptomatic individuals [31].

Then, the virus migrates from the nasal epithelium to the upper respiratory tract through the conducting airways. There is a higher immune response during this stage involving the release of C-X-C motif chemokine ligand 10 (CXCL-10) and interferons (IFN- $\beta$  and IFN- $\lambda$ ) from virus-infected cells [32]. The majority of patients do not advance further than this phase as the expressed immune response is adequate to contain the spread of infection.

#### Cytokine storm, immune dysfunction, and progression to acute respiratory distress syndrome (ARDS)

Approximately one-fifth of all infected patients advance to the severe stage of COVID-19, characterized by cytokine storm and development of ARDS. The virus invades the lower respiratory tract and enters the type 2 alveolar epithelial cells through the host receptor ACE-2 and starts to undergo replication to produce more viral nucleocapsids. Many different cytokines and inflammatory markers are now produced by virus-laden pneumocytes, such as interleukins (IL-1, IL-6, IL-8, IL-120, and IL-12), factor- $\alpha$  (TNF- $\alpha$ ), IFN- $\lambda$  and IFN- $\beta$ , CXCL-10, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ), this is known as a cytokine storm. For neutrophils, CD4 helper T cells, and CD8 cytotoxic T cells, this cytokine storm acts as a chemoattractant, which then begins to get sequestered in the lung tissue. These cells are responsible for fighting off the virus but also responsible for the consequent inflammation and lung injury. High concentrations of pro-inflammatory CD4 T cells and cytotoxic granules CD8 T cells were also determined, suggesting antiviral immune responses and overactivation of T cells leading to dysfunction of the immune system. With the release of new viral particles, the host cell undergoes apoptosis, which will then infect the neighboring type 2 alveolar epithelial cells in the same way. It contributes to the destruction of both type 1 and type 2 pneumocytes due to the persistent injury caused by the sequestered inflammatory cells and viral replication, ultimately causing diffuse alveolar damage resulting in ARDS [33].

There are various clinical presentations of COVID-19 (Table 1). Fever, dry cough, shortness of breath, fatigue, myalgia, nausea/vomiting or diarrhea, headache, and rhinorrhea are common symptoms in hospitalized patients. Aches and pains, nasal congestion, conjunctivitis, sore throat, loss of taste or smell, or skin rash or discoloration of fingers or toes are other symptoms that can affect certain patients. Typically, these symptoms are mild and begin gradually. In about 3% of people with COVID-19, anosmia or ageusia can be the sole presenting symptom. Non-classical symptoms, such as isolated gastrointestinal symptoms, can also occur in patients [34-38].

#### RISK FACTORS, DIAGNOSIS, AND COMPLICATIONS

The potential for serious risk from COVID-19 rises with age, with older adults at the highest risk for those aged 85 or older. In fact, 8 out of 10 deaths associated with COVID-19 recorded in the United States were among adults aged 65 years and older.

Table 1: Clinical spectrum of COVID-19 disease [37,38]

Severity of disease	Presentation
Asymptomatic	No clinical symptoms, positive nasal swab test, and normal chest X-ray.
Mild illness	Fever, sore throat, dry cough, malaise, and body aches or nausea, vomiting, abdominal pain, loose stools.
Moderate illness	Symptoms of pneumonia (persistent fever and cough) without hypoxemia, significant lesions on high-resolution computed tomography of chest.
Severe illness Critical state	Pneumonia with hypoxemia (SpO <sub>2</sub> <92%) Acute respiratory distress syndrome, along with shock, coagulation defects, encephalopathy, heart failure, and acute kidney injury.

COVID-19 is a novel disease. Other factors, such as underlying health conditions, may increase the risk of serious illness. People of any age with the following conditions are at increased risk: Immunocompromised state from a solid organ transplant, cancer, chronic kidney disease, asthma (moderate-to-severe), chronic obstructive pulmonary disease, cystic fibrosis, obesity, serious heart conditions such as heart failure, coronary artery disease, or cardiomyopathies, hypertension, type 2 diabetes mellitus, cerebrovascular disease, sickle cell disease, thalassemia, HIV, and neurologic conditions such as dementia, liver disease, pregnancy, smoking, use of corticosteroids, or use of other immune weakening medicines. People living in rural communities and people with disabilities, developmental and behavioral disorders, experiencing homelessness, racial, and ethnic minority groups should be extra cautious [39]. In a recently published retrospective cohort analysis study, it is observed that blood group O is associated with a decreased risk for contracting SARS-CoV-2 infection, whereas people with blood type A and AB are most vulnerable to the infection. ABO blood group is only considered as a risk factor for SARS-CoV-2 infection but not for hospitalization or death from COVID-19 [40].

Usually, COVID-19 diagnosis is made using a polymerase chain reaction test through a nasal swab. However, due to the false-negative results of the SARS-CoV-2 polymerase chain reaction (PCR) test for nasal swabs, clinical, laboratory, and imaging findings can also be used to render a presumptive diagnosis.

#### RT-PCR and serology

Reverse transcription-PCR (RT-PCR) based SARS-CoV-2 RNA detection from respiratory samples (e.g., nasopharynx) is the standard for diagnosis. It amplifies viral genetic material obtained through nasal swab. Lower respiratory samples, such as bronchoalveolar lavage fluids, are more responsive than upper respiratory samples. Saliva may be an alternative specimen source [41]. Several serological tests and assays which include point-of-care and high throughput enzyme immunoassays can also aid in the diagnosis. IgM antibodies are measurable within 5 days of infection, with higher IgM levels during weeks 2–3 of disease, while IgG response is first seen around 14 days after onset of symptoms [42,43].

#### Laboratory findings

These include basic blood work. The laboratory abnormalities seen in COVID-19 include elevated serum C-reactive protein, lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase, and low albumin levels. Lymphopenia (absolute lymphocyte count  $<1.0 \times 10^9/L$ ) coupled with coagulopathy, slight prolongation of prothrombin, mild thrombocytopenia, and raised D-dimer values are the most common hematological abnormalities [44].

#### Imaging

The characteristic chest computed tomographic imaging abnormalities for COVID-19 are diffuse, peripheral ground-glass opacities [45].

Common complications among hospitalized patients with COVID-19 include pneumonia, ARDS, acute liver injury characterized by elevations in aspartate transaminase, alanine transaminase, and bilirubin, acute kidney injury, cardiac injury including elevated troponin, acute heart failure, dysrhythmias, and myocarditis; prothrombotic coagulopathy resulting in arterial thromboembolic and venous events, neurologic manifestations including impaired consciousness, acute cerebrovascular disease, and shock.

Rare complications among critically ill patients with COVID-19 include cytokine storm, rhabdomyolysis, multisystem inflammatory syndrome, macrophage activation syndrome, and pulmonary aspergillosis [46-48].

#### TREATMENT

There are currently no specific antiviral drugs or vaccines for SARS-CoV-2 management. In most cases, the treatment is primarily symptomatic and supportive. The following are the potential therapeutic options available for COVID-19.

Oxygen therapy through nasal cannula/simple face mask/venturi mask/non-rebreather mask in patients with mild cases ( $SpO_2$  levels of 94–97% in room air). In addition to oxygen treatment, antipyretics (acetaminophen) for fever and pain, oral fluids, and healthy diet on a symptomatic basis can be provided in mild cases [49]. High-flow nasal oxygen therapy (oxygen flow rate 30-40 L/min) can be provided to patients with moderate disease ( $SpO_2$  of 90–94% in room air) in those cases where it is not possible to maintain  $SpO_2 > 92\%$ . Non-invasive continuous positive airway pressure ventilation may also be used for respiratory failure management [50]. Patients with severe ARDS disease must be started on oxygen therapy immediately at 5 L/min. Endotracheal intubation and mechanical ventilation are considered in cases of impairment of oxygen saturation below 90% [49]. Extracorporeal membrane oxygenation (ECMO) for patients with refractory hypoxemia, despite endotracheal intubation and mechanical ventilation should be considered if feasible [51].

Broad-spectrum antibiotics, fluid resuscitation with isotonic crystalloids (normal saline [NS] and ringer's lactate), given at the rate of at least 30 mL/kg in the first 3 h and vasopressors for prevention/management of shock and peripheral hypoperfusion. Dobutamine is the drug of choice if the patient shows signs of poor perfusion and cardiogenic shock, despite the vasopressor and antibiotic support [49].

#### Other drug therapies

##### Antibiotics

They are effective in preventing or managing secondary bacterial infections and sepsis but in preventing viral infections. Azithromycin is effective in preventing pulmonary infections, in addition to having a significant anti-inflammatory effect on the airways [52].

##### Antiviral drugs

Most antiviral drugs undergoing clinical testing in patients with COVID-19 are repurposed, antiviral agents.

Remdesivir is a broad-spectrum antiviral agent that inhibits the action of viral RNA-dependent RNA polymerase [53]. It can be considered in patients with moderate illness at a loading dose of 200 mg intravenously over 1–2 h on day 1 followed by 100 mg intravenously for 5–10 days. Contraindications to the use of remdesivir include use in children, pregnant or lactating females, and patients with severe hepatic or renal impairment [49]. The recently concluded ACTT-1 trial final report on remdesivir for COVID-19 treatment deduced that remdesivir was superior to placebo in lowering recovery time and infection of the lower respiratory tract in adults who were hospitalized. The estimates of mortality were also lower in the drug group [54]. In contradiction to the ACTT-1 trial results, the interim WHO SOLIDARITY trial results concluded that remdesivir has little or no effect on hospitalized COVID-19 patients in terms of overall mortality, initiation of ventilation, and duration of hospital stay [55].

##### Lopinavir/ritonavir

LPV has been shown to inhibit the coronavirus protease activity. The effective dose of LPV is 400 mg orally every 12 h and was initially seen as a promising therapeutic choice for COVID-19, but no definitive benefit of LPV/RTV therapy compared to the routine management protocol was found in a recent randomized controlled trial (RCT) [56].

##### Favipiravir

It shows activity against RNA viruses by selective inhibition of the viral RNA-dependent RNA polymerase. It is used in mild-to-moderate cases with COVID-19. The recommended starting dose is 1800 mg twice daily on day 1 followed by 800 mg twice daily on day 14. It is associated with the rapid reduction of the viral load and an early symptomatic improvement [57].

##### Immunomodulators

These drugs act by modulating the inflammatory response in patients with COVID-19. Monoclonal antibodies directed against inflammatory mediators,

such as interleukin 1, interleukin 6, interferon-gamma, and complement factor 5a, all target the overactive inflammatory response following SARS-CoV-2 infection to prevent organ damage [58]. Of these, tocilizumab and sarilumab are best-studied interleukin 6 inhibitors. Tocilizumab is a humanized IgG1 monoclonal antibody. It may be considered in patients with moderate disease who have elevated inflammatory markers (IL-6) with a gradual increase in oxygen demand and patients with mechanical ventilation. The dosage is 8 mg/kg with a maximum dose of 800 mg at one time can be given slowly in 100 mL NS over 1 h and can be repeated once after 12–24 h if needed [59,60]. Tyrosine kinase inhibitors, such as imatinib, are also being investigated for their ability to inhibit vascular pulmonary leakage in individuals with COVID-19.

#### Chloroquine and hydroxychloroquine (HCQ)

Chloroquine, an anti-malarial drug (at the dose, 500 mg, every 12 h), blocks the virus infection by an increase in the endosomal pH required for virus/cell fusion. Efficacy in reducing exacerbation of COVID-19 pneumonia as well as accelerated viral and symptomatic clearance has been exemplified [61]. HCQ (200 mg every 12 h) is a chloroquine analog with an enhanced safety profile and anti-SARS-CoV effect. It is also shown to be associated with significant viral load reduction [62]. Adverse effects are common, most notably QT prolongation, with an increased risk of cardiac complications in an already vulnerable population [63].

On June 17, 2020, WHO announced the termination of HCQ drug from the Solidarity Trial, which is an international clinical trial to help find an effective treatment for COVID-19, launched by WHO and partners. The trials have shown that HCQ does not result in the reduction of mortality of hospitalized COVID-19 patients when compared with standard of care [64].

#### Corticosteroids

Small doses of methylprednisolone (not exceeding 0.5–1 mg/kg/d in moderate cases and 1–2 mg/kg/d in severe cases) are indicated in patients with deteriorating oxygen saturation. High doses should not be recommended due to immunosuppression by steroids, which delays viral clearance [65]. Recently, dexamethasone has also been found to be effective for decreasing mortality in severe and critically ill cases [66].

The panel reviewed eight RCTs (7184 patients) on July 17, 2020, to compare systemic corticosteroids to usual COVID-19 treatments. The RECOVERY study, the largest of the seven studies in which mortality data by subgroup (severe and non-severe) were available, assessed the effects of dexamethasone 6 mg administered once daily (oral or intravenous) for up to 10 days in 6425 hospitalized patients in the United Kingdom (2104 were randomized to dexamethasone and 4321 were randomized to usual care).

The WHO has issued two recommendations on the use of corticosteroids: A strong recommendation for systemic (i.e., intravenous or oral) corticosteroid treatment (i.e., 6 mg oral or intravenous dexamethasone daily or 50 mg intravenous hydrocortisone every 8 h) for 7–10 days in patients with severe and critical COVID-19 and a conditional proposal not to use corticosteroids in patients with non-severe COVID-19. This guidance was prompted by the publication of the preliminary report of the RECOVERY trial [67] on June 22, 2020, to assess the effect of dexamethasone in hospitalized patients with COVID-19 carried out by the University of Oxford.

Evidence from RCTs and prospective meta-analysis of RCTs performed by the WHO Rapid Evidence Evaluation for COVID-19 Therapies Working Group [68] and two previously published meta-analyses and pooled data on the safety of systemic corticosteroids were analyzed in different but related patient populations to establish the recommendations [69,70] (Table 2).

#### Primary outcome of RECOVERY trial

Mortality at 28 days was significantly lower in the dexamethasone group than in the usual care group, with deaths reported in 22.9% and 25.7%

patients, respectively. The mortality rate among patients receiving invasive mechanical ventilation and those receiving oxygen without invasive mechanical ventilation was lower in the dexamethasone group than in the normal treatment group, but there was no significant effect of dexamethasone in patients receiving no respiratory support at randomization. The higher mortality benefit in response to dexamethasone therapy was seen in patients with longer duration of symptoms who were more likely to be randomized to undergo invasive mechanical ventilation and has been associated with decreased 28-days mortality for those with symptoms for more than 7 days but not for those with a more recent onset of symptoms.

#### Secondary outcomes of RECOVERY trial

Patients in the dexamethasone group had a shorter duration of hospitalization than those in the usual care group and a greater probability of discharge alive within 28 days. The greatest discharge effect within 28 days was found in patients undergoing invasive mechanical ventilation than in patients who did not receive invasive mechanical ventilation at randomization. The number of patients progressing to pre-described secondary outcome of invasive mechanical ventilation or death was lower in the dexamethasone group. Analyses are underway on cause-specific mortality, renal dialysis or hemofiltration requirements, and ventilation periods.

Many COVID-19 treatment recommendations stated before the completion of the trial that the use of glucocorticoids has been either contraindicated or just not recommended [71]. But then, the RECOVERY study offers proof that dexamethasone therapy is effective in reducing the 28-days mortality rate in respiratory supporting patients with COVID-19. No gain (and the risk of harm) was observed in the study among patients who did not need oxygen.

#### Plasma therapy

Patients are treated with plasma collected from individuals within 2 weeks after recovery from COVID-19 [9]. Some important criteria for this technique are sufficient antibody titer in convalescent plasma, ABO compatibility, and cross-matching of donor plasma. The dose varies from 4 to 13 mL/kg, and a single dose of 200 mL is typically given slowly over 2 h. This therapy is considered in patients with severe disease [49].

#### Other supplementary therapies

Thromboembolic prophylaxis with subcutaneous low molecular weight heparin (e.g., enoxaparin 40 mg SC) is recommended for all hospitalized patients with moderate (once daily) to severe (twice daily) COVID-19 [72]. In addition, intravenous transplantation of ACE2-mesenchymal stem cells (MSCs) and blocking of Fc receptors with immunoglobulin (IVIG) are also potential therapeutic strategies for severe COVID-19 [73]. There are ongoing studies on the use of Ivermectin alone or in combination with doxycycline to mitigate the risk and also a suggested combinatorial therapy (Ivermectin, Famotidine, and Doxycycline) for the treatment of COVID-19 [74]. For patients diagnosed with COVID-19 suffering from fear and anxiety, psychological counseling must be provided [49].

In another recently published study, they demonstrated that nitric oxide (NO) can inhibit the viral replication by targeting the SARS-CoV-2 main protease *in vitro*. In patients with the SARS, NO which is a broad-spectrum antimicrobial and a potent vasodilator has proven to be effective in reducing SARS-CoV replication and hypoxia. It has been concluded, based on a few previous studies, that NO can be used for clinical use in the treatment of COVID-19 [75]. Vaccines save millions of lives each year. More than 100 vaccine candidates for COVID-19 are currently being developed, many of which are in the human trial process.

The infections that may worsen with corticosteroids should be considered. For example, *Strongyloides stercoralis* hyperinfection associated with corticosteroid therapy and with a 1/3<sup>rd</sup> mortality reduction conferred by dexamethasone in ventilated patients with

Table 2: Characteristics of trials included in the systematic review of the effects of systemic corticosteroids for COVID-19

	DEXA-COVID 19	CoDEX	RECOVERY	CAPE COVID	COVID STEROID	REMAP-CAP	Steroids-SARI	GLUCOCOVID	MetCOVID
ClinicalTrials.gov Identifier	NCT04325061	NCT04327401	NCT04381936	NCT02517489	NCT04348305	NCT02735707	NCT04244591	EudraCT number: 2020-001934-37	(NCT04343729)
Planned Sample Size	200	350	N/A	290	1000	N/A	80	180	420
Eligibility criteria	Intubation, mechanical ventilation, moderate to severe ARDS per Berlin Criteria and confirmed COVID-19	Intubation, mechanical ventilation, moderate to severe ARDS per Berlin criteria, onset of ARDS <48 h before randomization, and probable or confirmed COVID-19	Patients of all ages, including pregnant and breastfeeding women, hospitalized, SARS-CoV-2 infection (clinically suspected or laboratory-confirmed) and no medical history that might, in the opinion of the attending clinician, put the patient at significant risk if he/she were to participate in the trial	Minimal severity admitted to ICU or intermediate care unit, oxygen ( $\geq 6$ L/min) and probable or confirmed COVID-19	Oxygen ( $\geq 10$ L/min) and confirmed COVID-19	Admitted to ICU with high flow nasal oxygen with $\text{FIO}_2 \geq 0.4$ at $\geq 30$ L/min, noninvasive or invasive ventilatory support, or receiving vasopressors and probable or confirmed COVID-19	Admitted to ICU with $\text{PaO}_2$ : $\text{FIO}_2 < 200$ mm Hg on positive pressure ventilation (invasive or noninvasive) or high-flow nasal cannulae $> 45$ L/min and confirmed COVID-19	Symptom duration of at least 7 days, radiological evidence of lung disease in chest X-ray or CT scan, moderate-to-severe disease with abnormal gas exchange: PafI ( $\text{PaO}_2/\text{FIO}_2$ ) $< 300$ , or SAFI ( $\text{SaO}_2/\text{FIO}_2$ ) $< 400$ , or at least two criteria of the Brescia-COVID Respiratory Severity Scale; laboratory parameters suggesting a hyper-inflammatory state: serum C-reactive protein (CRP) $> 15$ mg/dL, D-dimer $> 800$ mg/dL, ferritin $> 1000$ mg/dL, or IL-6 levels $> 20$ pg/mL	Hospitalized patients with clinical and/or radiological suspicion of COVID-19 (history of fever and any respiratory symptom, for example, cough or dyspnea and/or ground-glass opacity or pulmonary consolidation on CT scan), aged 18 years or older at the time of inclusion, with $\text{SpO}_2 \leq 94\%$ at room air or in use of supplementary oxygen or under invasive mechanical ventilation
Drug given	Dexamethasone	Dexamethasone	Dexamethasone	Hydrocortisone	Hydrocortisone	Hydrocortisone	Methylprednisolone	Methylprednisolone	Methylprednisolone
Dose and administration	20 mg/d IV $\times$ 5 days, then 10 mg/d IV $\times$ 5 days	20 mg/days IV $\times$ 5 days, then 10 mg/days IV $\times$ 5 days	6 mg/days orally or intravenously	Continuous IV infusion $\times$ 8 days or 14 days (200 mg/d $\times$ 4 days 7 days; 100 mg/days $\times$ 2 days or 4 days; 50 mg/days $\times$ 2 days or 3 days)	200 mg/days IV $\times$ 7 days (continuous or bolus dosing every 6 h)	50 mg IV every 6 h $\times$ 7 days	40 mg IV every 5 days	40 mg IV q 12 $\times$ 3 days and then 20 mg q 12 h $\times$ 3 days	IV 0.5 mg/kg q 12 h $\times$ 5 days
Dose classification	High	High	Low	Low	Low	Low	High	High	High
Control intervention	Usual care	Usual care	Usual care	Usual care	Usual care	Usual care	Usual care	Usual care	Saline solution q 12 h $\times$ 5 days

(Contd...)

Table 2: (Continued)

	DEXA-COVID 19	CoDEX	RECOVERY	CAPE COVID	COVID STEROID	REMAP-CAP	Steroids-SARI	GLUCOCOVID	MetCOVID
Primary Outcome	60 days mortality	Ventilator-free days	28 days mortality	21 days treatment failure (death or persistent requirement for mechanical ventilation or high-flow oxygen)	Days alive without life support at 28 days	Composite of hospital mortality and ICU organ support-free days to 21 days	Lower lung injury score at 7 days and 14 days	Composite endpoint that included in-hospital all-cause mortality, escalation to ICU admission, or progression of respiratory insufficiency that required non-invasive ventilation	28 days mortality
Mortality outcome, days Serious adverse event definitions	28	28	28	21	28	28	30	In-hospital	28
	Secondary infections of pneumonia, or other similar Pulmonary embolism	Mortality, infections, and insulin use.	Cause-specific mortality, ventilation, dialysis, cardiac arrhythmia (in a subset), and other that were believed to be related to study treatment.	All SAEs excluding some listed in the protocol and excluding those expected adverse events which are related to the patient's disease or comorbidity	New episodes of septic shock (sepsis-3 criteria), invasive fungal infection, clinically important gastrointestinal bleeding, and anaphylaxis	Per ICH-GCP guidelines (events not already captured as a trial endpoint; e.g., mortality) When the event may reasonably have occurred because of study participation Australia, Canada, European Union, New Zealand, UK, US	Secondary bacterial infections, barotrauma, severe hypoglycemia, gastrointestinal bleeding requiring transfusion, and acquired weakness	Hyperglycemia	Sepsis or positive blood culture collected on day 7; insulin due to hyperglycemia
Location	Spain	Brazil	UK	France	Denmark		China	Spain	Brazil

Adapted from the World Health Organization: Corticosteroids for COVID-19. IV: Intravenous, ICU: Intensive care unit

COVID-19, there may be at increased risk of acquiring Coronavirus Associated Pulmonary Aspergillosis (CAPA).

### SECONDARY INFECTIONS IN COVID-19

Secondary infections are aided by exposure to a pathogen along with a compromised immune system as a result of the primary infection. The only choice for such patients is to strengthen their immune system and avoid the progression of infection that could result in septic shock or even death. Hospitals are a common source of pathogens that cause secondary infections [76]. Although secondary infections occur as a result of primary infection, multiple pathogens of viral, bacterial, or fungal source cause co-infections and occur simultaneously [77].

The secondary infections occurring in COVID-19 were respiratory, bloodstream, and urinary tract infections. A total of 52, 20, and 7 pathogens were confirmed in the respiratory, bloodstream, and urinary tract infection patients, respectively. Most of which were gram-negative, following by gram-positive bacteria, virus, fungi, and others (Table 3). The secondary infection rate in critical patients is much higher than that in severe patients with COVID-19 [78].

### CAPA

*Aspergillus* is an opportunistic fungal pathogen that can cause debilitating disease in immunocompromised hosts, including those with hematological malignancies. *Aspergillus* is an air-borne pathogen that places the lungs at the forefront of defense. Recently, severe viral pulmonary infections, including COVID-19 have shown to be associated with an increased risk for invasive pulmonary aspergillosis (IPA) [79]. Fungi belonging to genus *Aspergillus* are ubiquitous, thermotolerant (which can withstand temperature up to 37°C, spore survival temperature up to 50°C) and favorable for humid conditions. They mainly grow on decaying vegetations, basements of houses, saunas, etc. *Aspergillus* causes a wide range of lung diseases such as pulmonary aspergillosis [80]. The main reason for developing IPA is pulmonary epithelial damage and long-term systemic steroids [81].

### Prevalence of CAPA

Many of the prevalence studies conducted are from China. Research in Jiangsu Province found that 23.3% of patients had *Aspergillus*-positive throat swab [82]. Other research in China revealed that 27% of patients had a fungal infection and another study reported that 7.7% of patients developed IPA. However, none of these studies used standard diagnostic rules and defined definitions for the identification of CAPA. In fact, the gold standard test – galactomannan test for fungal infection is scarcely available in China [83]. It can also be concluded that these findings are far from the actual prevalence of the CAPA. However, recently, some studies and case series from Europe have reported a higher

prevalence of CAPA in COVID-19 patients with ARDS, ranging from 25% to 30% [84]. The development of CAPA was surprisingly rapid, with a median of 6 days and a span of 3–28 days after intensive care unit (ICU) admittance. Among 35 CAPA cases reported, the overall mortality rate was 63%; of particular importance was the 100% fatality rate of those with underlying diseases reported from the Netherlands, while the two patients without underlying conditions both survived [85]. Overall, the most prevalent *Aspergillus* spp. appeared to be *Aspergillus fumigatus* isolated among respiratory samples with a positive culture, followed by *Aspergillus flavus*.

### Risk factors

Risk factors that predispose patients with COVID-19 to develop secondary pulmonary aspergillosis are close to those reported for superinfections with influenza-IPA [81]. The most important risk factors include severe lung damage, the use of corticosteroids in those with ARDS, the widespread use of broad-spectrum antibiotics in ICUs [86], and the presence of comorbidities such as hypertension, coronary heart diseases, and diabetes increase the risk of infection overall [87], while the structural lung damage caused by COPD or asthma may particularly predispose patients to develop IPA [85]. It has also been proposed that toxicity due to antibiotics (disrupts gut microbiome steady-state composition, which allows fungi to thrive), cytokine storm due to viral antigen, and hypoxia-induced lung injury due to mechanical ventilation, high airway pressure can all result in pulmonary fibrosis. Patients with interstitial pulmonary fibrosis, who may require long-term systemic steroids, can develop IPA long after the acute phase has gone [88].

### Pathogenesis

Pathogenesis of CAPA is complex. A molecular understanding of physiology is needed to identify the mechanism by which the infection of COVID-19 promotes the development of IPA. The receptor and signaling pathways involved in the immune recognition of *Aspergillus* are well established [89].

Two possible mechanisms can be presumed and are likely to characterize the output of ARDS and, therefore, CAPA. The first involves the release of dangerous molecular patterns (DAMPs), signal molecules released by dying or damaged cells that act as endogenous danger signals to accelerate and boost the immune and inflammatory response that leads to lung injury [90]; DAMPs have also been shown to control inflammation and [91] intensify the inflammatory response by integrating with Toll-like receptors in experimental aspergillosis [92]. This underlying notion could help to explain fungal pathogenesis in intensifying inflammation conditions such as those seen in COVID-19 patients. The second potential mechanism involves the pathways needed to activate antiviral immunity. Hyperactivation of the IL-1 pathway induced by the SARS-CoV-2 infection establishes a highly permissive inflammatory environment that favors fungal pathogenesis. In addition to IL-1, increased levels of IL-6 have also been observed in epithelial cells after infection with *A. fumigatus*, with an effect on immune cell function indicating that infection can lead to increased cytokine levels in patients with severe COVID-19 [93].

All in all, decline in immune cells, especially T-lymphocytes (lymphocytopenia) and defective immune responses to SARS-CoV-2, has been shown to permit unregulated viral replication, resulting in hyper-inflammation, and serious complications such as ARDS [94], in addition to creating favorable conditions for the acquisition of secondary infections such as CAPA.

### Diagnosing CAPA

The diagnosis of pulmonary aspergillosis is challenging, with culture having low sensitivity [95]. At present, the optimal diagnostic algorithm for the diagnosis of CAPA is uncertain and is being actively investigated in an ongoing multinational exploratory trial in collaboration with the European Confederation of Medical Mycology (ECMM). To date, the

Table 3: Causative pathogen species in COVID-19

Secondary infections in COVID-19	
Causative pathogen	Organisms
Bacterium	<i>Mycobacterium intracellulare</i> <i>Chryseobacterium</i> <i>Pseudomonas aeruginosa</i> <i>Escherichia coli</i> <i>Stenotrophomonas maltophilia</i> <i>Enterococcus faecalis</i> <i>Staphylococcus</i> <i>Acinetobacter baumannii</i> <i>Enterococcus faecium</i>
Virus	<i>Mycoplasma hominis</i> HHV6 HSV1
Fungi	<i>Penicillium</i> <i>Aspergillus</i>

most prevalent approaches include attempting to recover *Aspergillus* spp. on culture media of bronchoalveolar fluid (BALF) and tracheal aspirate, as well as utilizing serologic biomarker testing such as the conventional Galactomannan (GM) which is a more sensitive test in patients with neutropenia, from BALF, tracheal aspirate and serum specimens. *Aspergillus* PCR, serum (1→3)-β-D-glucan, the *Aspergillus* galactomannan lateral flow assay, and the *Aspergillus*-specific lateral-flow device test are other diagnostic tests that may prove useful. Thus, the most promising diagnostic modalities tend to be BALF, tracheal aspirate culture, and traditional GM testing by BALF. Testing from blood samples may be safer and optimal [96].

A restricted role for bronchoscopy has been recommended in COVID-19 because it is an aerosol-generating procedure and it is recommended in critically ill patients with COVID-19 who are suspected of secondary infection, including fungal diagnostic workup [97].

#### Current treatment standards of CAPA

Whether antifungal therapy leads to any survival benefits in COVID-19 patients with IPA is not known, but still, once the diagnosis of CAPA is suspected, antifungal therapy should be started. Voriconazole remains the drug of choice [98]. However, in addition to its small therapeutic window and the need for therapeutic drug monitoring to ensure effectiveness and avoid drug-drug interactions, the use of voriconazole may be especially limited. It is one of the drugs most widely associated with major drug-drug interactions as it is metabolized by CYP2C19, CYP2C9, and CYP3A4 [99]. Isavuconazole and liposomal amphotericin B are the key alternative therapeutic choices for IPA in the ICU [100]. Compared to voriconazole, isavuconazole is associated with fewer adverse effects and shows a more favorable pharmacokinetic profile. However, it is also metabolized through CYP3A4, although drug-drug interactions are generally less a problem with isavuconazole than with voriconazole [101].

Liposomal amphotericin B is a broadly effective alternative treatment choice, but it sometimes complicates initiation or involves discontinuation of this antifungal agent in patients with renal insufficiency. For patients infected with SARS-CoV-2, which has demonstrated renal tropism and has been identified as a frequent cause of kidney injury, this concern is extremely crucial [102]. Although itraconazole is now rarely used for the treatment of invasive aspergillosis, some antiviral activity has been shown to be successful in the feline coronavirus model, specifically as a cholesterol transport inhibitor [103]. Furthermore, its latest oral SUBA formulation has excellent bioavailability [104], and itraconazole could therefore be an alternative choice for the treatment of IPA associated with COVID-19, although it shares the drug-drug interaction problems with other triazoles.

At present, in progress, new antifungal medications, namely fosmanogepix and olorofim, which may have similar effects without the same burden of drug-drug interactions and toxicity and may thus overcome the drawbacks of currently available antifungals and become the preferred treatment choices in the near future. If the documented high incidence of COVID-19-associated IPA in ICU patients is validated in larger studies, there may be a rationale for prophylaxis trials, for which not only triazoles and nebulized liposomal amphotericin [96], but also another novel antifungal currently under research, rezafungin (i.e., once weekly echinocandin with improved activity against *Aspergillus* spp.), maybe a candidate [105].

#### Photodynamic therapy

Most of the patients admitted to hospitals with COVID-19 infection have contracted bacterial co-infections (pneumococcal pneumonia caused by *Streptococcus pneumoniae* and infections caused by *Streptococcus aureus*).

Photodynamic treatment does not specifically destroy the virus yet destroys microorganisms in the respiratory tract using light and a photosensitizing chemical substance. The photodynamic therapy

generates reactive oxygen species and oxygen molecules which help in damaging the targets of the virus. Specific pharyngotonsillitis photodynamic therapy can not only help in reducing the number of microorganisms present in the oropharynx but also in preventing these microorganisms from penetrating the mucosa. Phagocytes (which engulf harmful foreign organisms) are also activated by this treatment, thereby preventing the proliferation of microorganisms in the mucosa.

A photodynamic therapy test against bacterial pneumonia was performed by the researchers. In this test, the scientists used a 780-nanometer wavelength extracorporeal illumination (light source) and used indocyanine green as a photosensitizer, which was supplied through nebulizers to the patients. The experiment concluded that in the group that obtained the photodynamic therapy, no deaths were reported, while 60% of participants in the control group died. Photodynamic therapy can be used to treat secondary infections in patients with COVID-19, either as a single therapy or as an antibiotic adjuvant [106].

#### PREVENTIVE MEASURES FOR COVID-19

The only way one can protect themselves from contracting the COVID-19 infection is by following preventive measures such as practicing good hand hygiene. Restrictions on close interaction between infected persons and healthy individuals is crucial for breaking the chains of the transmission of the virus. Identifying the suspected cases as soon as possible, testing, and also isolating the infectious cases is the best preventive measures for the spread of the virus so that they can be quarantined [107]. Incubation period, which means the time interval between exposure to virus and onset of symptoms of COVID-19 is 5-6 days on average, but can also be up to 14 days [108]. Thus, 14 days for the quarantine to be in a place from the last exposure to a confirmed case. If it is not possible for a person to be in quarantine in a separate space, then self-quarantine for 14 days at home is necessary. Support may be required during the use of physical distancing measures for avoiding the spread of the virus in those who are self-quarantined. Masks can be used as one of the preventive measures in which frequent hand washing, physical distancing whenever required, respiratory etiquette, environmental cleaning, and disinfection. Other precautions include indoor gatherings can be avoided as much as possible. Avoid keeping in touch with animals and their excretions or droppings. In areas with COVID-19 community transmission, it is advised that the health personnel's and caregivers working in the clinical sector should wear a medical mask continuously during every activity all through the entire shift [109] and should wear an N95, FFP2, or FFP3 respirator; where aerosol-generating procedures are performed [110]. Promoting safe individual practices at the workplace, engage workers in providing feedback on the preventive measures and their effectiveness by providing the videos, posters, and electronic message boards to enhance the awareness of COVID-19 [111].

#### IMMUNITY FOLLOWING SARS-COV-2 INFECTION

Specific antibodies and cell-mediated responses are induced following SARS-CoV-2 infection. Preliminary evidence demonstrates that some of these responses are safeguarding, but this remains to be established definitively. Besides, it is unclear if all infected patients have a protective immune response and if so, how long it would last to have a protective effect.

#### Humoral immunity

Protective immunity evidence following COVID-19 is emerging. There is emerging protective immunity evidence following COVID-19. Case series testing convalescent plasma for the treatment of COVID-19 reported neutralizing activity in the plasma of recovered patients, which seemed to be transmitted to recipients following a plasma infusion [112]. Similarly, in another study of 23 patients who recovered from COVID-19, spike protein and nucleocapsid protein receptor binding antibodies were identified by enzyme-linked immunosorbent

assay (ELISA) in most patients within 14 days of onset of symptoms; ELISA antibody titers were associated with neutralizing activity. Some results, however, indicate that the magnitude of antibody response may be correlated with disease severity and detectable neutralizing antibodies may not be mounted in patients with mild infection. In addition, the durability of neutralizing activity following infection is uncertain, as neutralizing antibodies decline over several months after infection [113,114].

#### Cell-mediated immunity

SARS-CoV-2 specific CD4 and CD8 T cell responses have also been identified in studies in patients who have recovered from COVID-19 and in individuals who have received an investigational SARS-CoV-2 vaccine, indicating the potential for a durable T cell immune response [115,116].

#### RISK OF REINFECTION

Overall, the short-term risk of reinfection (e.g., during the first few months following initial infection) tends to be minimal. However, occasional cases of probable reinfection have been reported. As an example, an asymptomatic case in Hong Kong identified two distinct strains, suggesting two distinct infections of SARS-CoV-2 on travel-related screening, 5 months after mild laboratory-confirmed COVID-19 [117]. The second infection, however, was asymptomatic, giving rise to the possibility that immunity from an initial infection could attenuate the seriousness of reinfection even if it is not prevented [118]. However, a recent published study mentioned that SARS-CoV-2 reinfection resulted in worse disease than the first infection in two patients, requiring oxygen support and hospitalization. This information is key in understanding the immunity following infection with SARS-CoV-2 [119].

Simply having a positive SARS-CoV-2 viral test after recovery does not inherently mean reinfection; a sequence that indicates a different strain at the time of presumptive reinfection is required to make a distinction. Specifically, the study from the Korea Centers for Disease Control and Prevention found that patients with COVID-19 who had a positive repeat RNA test after they had previously been cleared of isolation were unable to isolate the infectious virus in any of 108 patients examined [118].

#### CONCLUSION

As the world waits for a clinically approved vaccine, prevention is the best way to combat COVID-19. There are no standard therapeutic regimens to handle the spread of SARS-CoV-2. It is important to conduct large randomized trials for the use of repurposed drugs and more comparative shreds of evidence are to be established to reduce the morbidity and mortality in COVID-19 patients. As dexamethasone is on the list of essential medicines of the WHO, it is important to accentuate the need for future analyses of the evidence for corticosteroid use in COVID-19, such as its long-term effects and their use in non-severe patients, as they remain unclear and may be studied further. As additional therapies are under development for COVID-19, notably novel immunomodulators, it is important to ascertain how these interact with systemic corticosteroids; other endemic infections that may worsen with corticosteroids should be considered, especially CAPA in patients who are on invasive ventilation, which should emphasize the need for further fungal surveillance.

#### AUTHORS' CONTRIBUTIONS

All the authors contributed equally in the preparation of this manuscript.

#### CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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