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Research Paper

Review on COVID19- SARS CoV 2: Severe Acute Respiratory Syndrome Corona Virus 2

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Coronavirus disease 2019 (COVID-19) is primarily a respiratory disease and the chief causative agent is the novel coronavirus SARS-CoV-2.[1]The coronavirus belongs to Coronaviridae family which is one of the causative agent for acute upper respiratory tract infections. The mode of transmission is through droplet, which is similar to SARS-CoV-2 and cold viruses.[2] The current pandemic was announced on 11 March 2020 and the first case was reported from Wuhan, China. Since January 2020 there has been a global spread with Italy, Spain and United States having maximum number of cases with high mortality figures.

Recombination, mutator alleles, and mutational robustness are some of the evolutionary mechanisms that make Coronaviruses capable of expanding their host ranges, including humans. Therefore, understanding the virology of the Coronaviruses at a structural level is of utmost importance because the health threats from these zoonotic viruses are constant and long-term.[3]

I. The Origin and Evolution of SARS-CoV-2

Bioinformatics analyses showed that SARS-CoV-2 had characteristics typical of coronavirus family. It belongs to the betacoronavirus 2B lineage.[4] Early in the pneumonia epidemic in Wuhan, scientists obtained the complete genome sequences from five patients infected with SARS-CoV-2. These genome sequences share 79.5% sequence identity to SARS-CoV. Obviously, SARS-CoV-2 is divergent from SARS-CoV. It is considered to be a new betacoronavirus that infects human.[5] Scientists aligned the full-length genome sequence of SARS-CoV-2 and other available genomes of betacoronaviruses. Results indicate the closest relationship of SARS-CoV-2 with the bat SARS-like coronavirus strain BatCov RaTG13, with an identity of 96%. These studies suggest that SARS-CoV-2 could be of bat origin, and SARS-CoV-2 might be naturally evolved from bat coronavirus RaTG13.[6]

To date, 13 mutations in the spike protein have been identified. The mutation D614G should be paid special attention. In early February, the mutation Spike D614G began spreading in Europe. When introduced to new regions, it rapidly replaced the original strain to become the dominant strain.[7] The D614G mutation in the spike protein would increase infectivity. S^{G614} is more stable than S^{D641} and less S1 shedding are observed, so the SARS-CoV-2 with S^{G614} could transmit more efficiently.[8] One study shows that in multiple cell lines, the SARS-CoV-2 carrying the D614G mutation is eight times more effective at transducing cells than wild-type spike protein, providing evidence that the D614G mutation in SARS-CoV-2 spike protein could increase the transduction of multiple human cell types.[9] The D614G mutation could also decrease neutralization sensitivity to the sera of convalescent COVID-19 patients.[10]

II. Structure of SARS-CoV-2

Coronaviruses are large, enveloped, positive-stranded RNA viruses responsible for infecting a wide variety of mammalian and avian species.[11] These viruses contain spike-like projections of glycoproteins on their surface, which appear like a crown under the electron microscope; hence, they are referred to as coronaviruses. The coronavirus genome encodes several structural and nonstructural proteins. The structural proteins are responsible for host infection, membrane fusion, viral assembly, morphogenesis, and release of virus particles, among other functions, and the nonstructural proteins (nsps) facilitate viral replication and transcription.[12] The membrane (M), the envelope (E), and the spike protein (S) make up the structural proteins and are associated

with the envelope. Among these structural proteins, the trimeric S proteins protrude from the virus envelope and are the key machinery that facilitates virus entry into the host cell.[13]

The S proteins are clove-shaped, type-I transmembrane proteins and have 3 segments: a large ectodomain, a single-pass transmembrane, and an intracellular tail. The ectodomain of S proteins consist of the S1 subunit, containing a receptor-binding domain (RBD), and the membrane-fusion subunit (S2). The host-cell receptor recognition by the RBDs on S proteins is the initial step of viral infection, and the binding interactions between the coronavirus spike and its receptor is one of the most critical factors for host range and cross-species transmission. Human coronaviruses recognize a variety of host receptors; specifically, HCoV-229E recognizes human aminopeptidase N (hAPN),[14] MERS-CoV binds dipeptidyl peptidase-4 (DPP4)[15], HCoV-OC43 and HCoV-HKU1 bind certain types of O-acetylated sialic acid, and HCoV-NL63 and SARS-CoV recognize angiotensin-converting enzyme 2 (ACE2).[16]

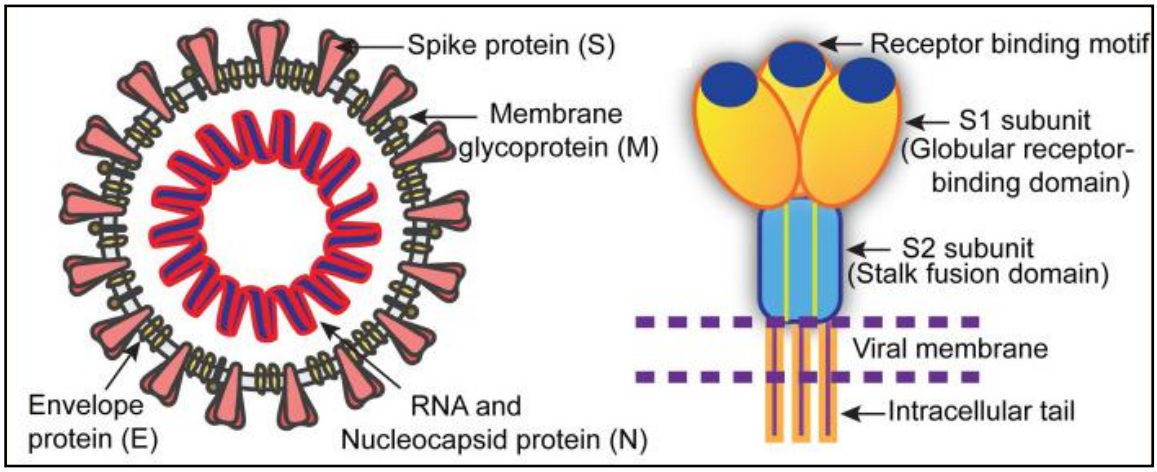

Fig 1: Schematic of the SARS-CoV-2 structure

Classification of Coronaviruses

The coronavirus study group of the International Committee on Taxonomy of Viruses has classified coronaviruses under the family Coronaviridae, subfamily Coronavirinae. Based on genotypic and serological characterization, Coronavirinae is divided into 4 genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus (Fig 2). Only 6 HCoV species that cause human disease were known before December 2019. Four of them cause common cold symptoms in immunocompromised individuals: these are HCoV-229E and HCoV-OC43, first identified in the mid-1960s[17]; HCoV-NL63, first identified in 2004[18]; and HCoV-HKU1, first identified in 2005.[19] The other 2 strains, which cause fatal illness, are SARS-CoV, first identified in 2003[20], and MERS-CoV, first identified in 2012.[21] SARS-CoV-2 has 96% nucleotide sequence identity to bat coronavirus RaTG13, a SARS-like coronavirus; therefore, it belongs to Betacoronavirus genera.

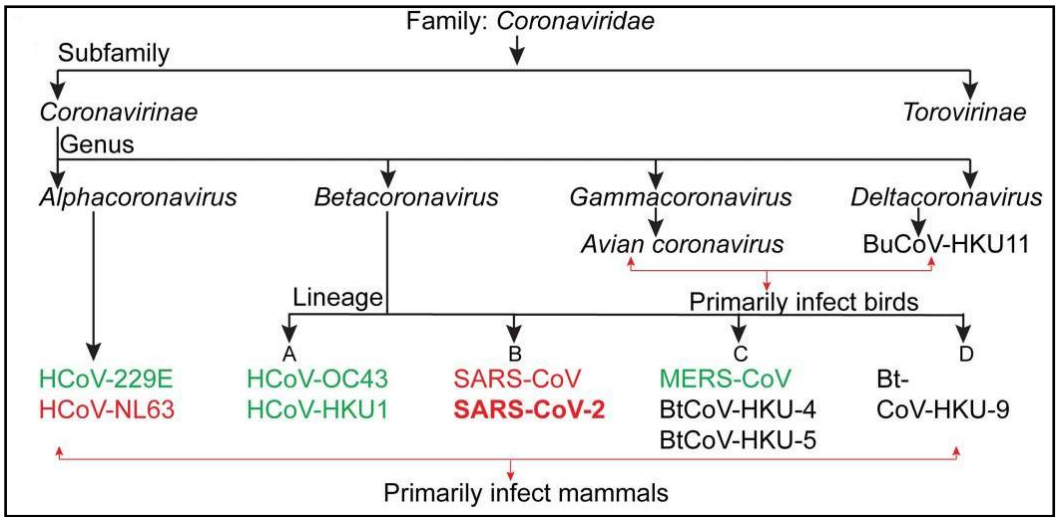

Fig 2: Classification of Coronaviruses: the 7 known HCoVs are shown in green and red. HCoVs in red bind the host receptor ACE2

Clinical Characteristics of COVID-19

The most common manifestations of COVID-19 are fever and dry cough. The majority of the patients showed bilateral pneumonia. Old males with comorbidities are more likely to be affected by SARS-CoV-2. The blood counts of patients showed leucopenia and lymphopenia. The content of IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNF-α in the plasma of ICU patients is higher than non-ICU patients.[22] COVID-19 is divided into three levels according to the severity of the disease: mild, severe, and critical. The majority of patients only have mild symptoms and recover.[22] Asymptomatic infection cases were also reported, but most of the asymptomatic patients went on to develop disease since the data of identification. Besides respiratory illness, COVID-19 disease could lead to myocardial injury and arrhythmic complications, neurological complications, such as myalgia, headache, dizziness, impaired consciousness, intracranial hemorrhage, hypogeusia, and hyposmia and even stroke.[23] Digestive symptoms and liver injury, hypercoagulability and thrombotic complications have also been reported. Critical patients could quickly progress to ARDS, hard-to-correct metabolic acidosis, septic shock, coagulation dysfunction, and multiple organ functional failure. Severe complications included ARDS, RNAaemia (detectable serum SARS-CoV-2 viral load), multiple organ failure, and acute cardiac injury. About 26.1% patients were admitted to the ICU because of complications caused by COVID-19. With proper diagnosis and treatments for COVID-19, most patients had a good prognosis. The elderly and the patients with underlying diseases have worse prognosis.[24]

Spike Glycoprotein

The Coronaviruses entry into host cells is mediated by spike glycoprotein (S protein) . The transmembrane spike glycoproteins form homotrimers that protrude from the viral surface. The spike glycoprotein is critical for the entry of the coronaviruses so it is an attractive antiviral target. S protein is composed of two functional subunits, including the S1 and S2 subunits. The S1 subunit consists of N-terminal domain (NTD) and receptor binding domain (RBD). The function of S1 subunit is bind to the receptor on host cell. S2 subunit contains fusion peptide (FP), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic tail (CT) (Fig 3). The function of S2 subunit is to fuse the membranes of viruses and host cells.[11]

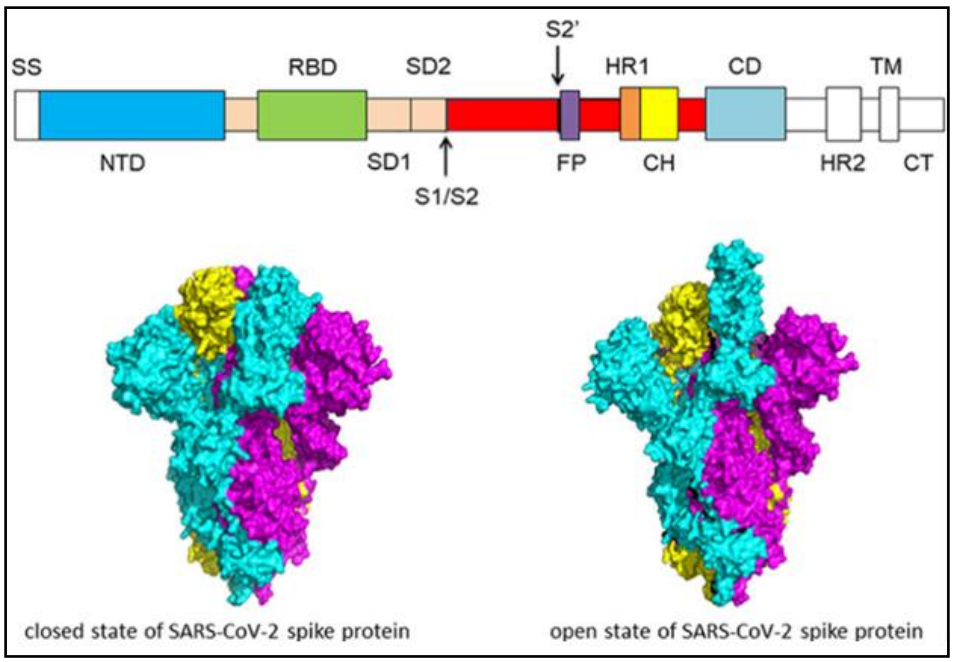

Fig 3: Schematic of SARS-CoV-2 spike protein primary structure.

Variants of SARS-CoV-2

Multiple variants of SARS-CoV-2 have been described, of which a few are considered variants of concern (VOCs), given their impact on public health. VOCs are associated with enhanced transmissibility or virulence, reduction in neutralization by antibodies obtained through natural infection or vaccination, the ability to evade detection, or a decrease in therapeutics or vaccination effectiveness. Based on the epidemiological update by the WHO, as of 11 December 2021, five SARS-CoV-2 VOCs have been identified since the beginning of the pandemic:

- **Alpha (B.1.1.7):** first variant of concern described in the United Kingdom (UK) in late December 2020

- **Beta (B.1.351)**: first reported in South Africa in December 2020
- **Gamma(P.1)**: first reported in Brazil in early January 2021
- **Delta (B.1.617.2):** first reported in India in December 2020
- **Omicron (B.1.1.529):** first reported in South Africa in November 2021

All five reported VOCs -Alpha(B.1.1.7); Beta(B.1.351); Gamma (P.1); Delta(B.1.617.2); and Omicron (B.1.1.529) have mutations in the RBD and the NTD, of which N501Y mutation located on the RBD is common to all variants except the Delta variant which results in increased affinity of the spike protein to ACE 2 receptors enhancing the viral attachment and its subsequent entry into the host cells. Along with NBD, RBD serves as the dominant neutralization target and facilitates antibody production in response to antisera or vaccines. Two recent preprints reported that a single mutation of N501Y alone increases the affinity between RBD and ACE2 approximately ten times more than the ancestral strain (N501-RBD). Interestingly the binding affinity of the Beta (B.1.351) variant and Gamma (P.1) variant with mutations N417/K848/Y501-RBD and ACE2 was much lower than that of N501Y-RBD and ACE2.[25]

Laboratory diagnosis of COVID-19

RT-PCR test

The most commonly used method for identifying genetic material from SARS-CoV-2 is real-time polymerase chain reaction (RT-PCR). This method involves reverse transcription of the genetic material of the virus (RNA) to complementary DNA (cDNA), followed by amplification of some regions of the cDNA. Probes (DNA/RNA marked sequences to identify the genetic target in the material) and primers (DNA/RNA sequences that promote replication of the genetic material found in the sample) were created after the SARS-CoV-2 genome was sequenced. Several serial amplification cycles are performed to identify these targets: the more cycles are needed, the lower the viral load of the material under study.[26] Four regions of the SARS-CoV-2 genome have been targeted: RdRp gene (RNA-dependent RNA polymerase), genes from structural proteins E (virus envelope) and N (virus nucleocapsid), and ORF1ab gene (open reading frame 1a and 1b).[26] Kits using different regions of the genome are commercially available.

Regardless of the method used, the sensitivity and specificity of the different RT-PCR kits are not 100%. This is considered the gold standard for diagnosis of SARS-CoV-2 infection, but its sensitivity is estimated to be approximately 70% and specificity, 95%.[27]Many factors can interfere with the results, whether related to the virus, to the method itself (the collection procedure and handling of the material), or even to the viral load of the sample (type of material collected, duration of symptoms, and disease severity).[28]

Serological Test

Serological tests identify the presence of humoral response to SARS-CoV-2. Antibodies of IgA, IgM, and IgG isotypes specific to different virus proteins are detected by enzyme-linked immunosorbent assay (ELISA) or chemiluminescence immunoassays (CLIA), and the latter has been shown to be more sensitive. It is known that the priority immune response to the virus is related to the cytotoxic activity of NK cells and CD8 + T lymphocytes. There is evidence of robust cellular response to SARS-CoV-2, regardless of the results of serological tests;[29] however, tests to evaluate the specific cellular immune response for SARS-CoV-2 are not yet commercially available.

Laboratory markers used in the diagnosis of COVID-19

Complete blood count – lymphopenia, eosinopenia, and neutrophil/lymphocyte ratio ≥ 3.13 are related to greater severity and worse prognosis. Thrombocytopenia is related to a higher risk of myocardial damage and a worse prognosis.[30] Lymphopenia results from a multifactorial mechanism that includes the cytopathic effect of the virus, induction of apoptosis, IL1-mediated pyroptosis, and bone marrow suppression by inflammatory cytokines.[31]

High values of C-reactive protein (CRP), ferritin, D-dimer, procalcitonin, lactic dehydrogenesis (DHL), prothrombin time, activated partial thromboplastin time, amyloid serum protein A, creatine kinase (CK), glutamic-pyruvic transaminase (SGPT), urea, and creatinine are risk factors for more severe disease, thromboembolic complications, myocardial damage, and/or worse prognosis.[31]

Immunological markers that may also represent risk factors for greater severity and/or worse prognosis are: decreased values of CD4 + T and CD8+ lymphocytes, and NK cells and increased values of IL6, IL-8, IL-10, IFN-γ, TNF-IL-2R, TNF-α, GM-CSF, and IL-1 β.[30]

Pharmacological management of COVID-19

Initially, early in the pandemic, the understanding of COVID-19 and its therapeutic management was limited, creating an urgency to mitigate this new viral illness with experimental therapies and drug repurposing. Since then, due to the intense efforts of clinical researchers globally, significant progress has been made, which

has led to a better understanding of not only COVID-19 and its management but also has resulted in the development of novel therapeutics and vaccine development at an unprecedented speed.

Currently, a variety of therapeutic options are available that include antiviral drugs (e.g., molnupiravir, paxlovid, remdesivir), anti-SARS-CoV-2 monoclonal antibodies (e.g., bamlanivimab/etesevimab, casirivimab/imdevimab,sotrovimab,bebtelovimab), anti-inflammatory drugs (e.g., dexamethasone), immunomodulators agents (e.g., baricitinib, tocilizumab) are available under FDA issued Emergency Use Authorization(EUA) or being evaluated in the management of COVID-19.[32]

The clinical utility of these treatments is specific and is based on the severity of illness or certain risk factors. The clinical course of the COVID-19 illness occurs in 2 phases, an early phase when SARS-CoV-2 replication is greatest before or soon after the onset of symptoms. Antiviral medications and antibody-based treatments are likely to be more effective during this stage of viral replication. The later phase of the illness is driven by a hyperinflammatory state induced by the release of cytokines and the coagulation system's activation that causes a prothrombotic state. Anti-inflammatory drugs such as corticosteroids, immunomodulating therapies, or a combination of these therapies may help combat this hyperinflammatory state more than antiviral therapies. Below is a summary of the latest potential therapeutic options proposed, authorized, or approved for clinical use in the management of COVID-19.

Antiviral Therapies

Molnupiravir is a directly acting broad-spectrum oral antiviral agent acting on the RdRp enzyme was initially developed as a possible antiviral treatment for influenza, alphaviruses including Eastern, Western, and Venezuelan equine encephalitic viruses. Based on a meta-analysis of available phase 1-3 studies, molnupiravir was noted to demonstrate a significant reduction in hospitalization and death in mild COVID-19 disease. Results from a phase 3 double-blind, randomized placebo-controlled trial reported that early treatment with molnupiravir reduced the risk of hospitalization or death in at risk unvaccinated adults with mild-to-moderate, laboratory-confirmed Covid-19.[33] Results from a phase 3 double-blind, randomized placebo-controlled trial reported that early treatment with molnupiravir reduced the risk of hospitalization or death in at risk unvaccinated adults with mild-to-moderate, laboratory-confirmed COVID-19.[33]

Paxlovid (ritonavir in combination with nirmatrelvir) is an oral combination pill of two antiviral agents which on an interim analysis of phase 2-3 data (reported via press release) which included 1219 patients, found that the risk of COVID-19 related hospital admission or all-cause mortality was 89% lower in the paxlovid group when compared to placebo when started within three days of symptom onset.[34] On December 22, 2021, the FDA issued a EUA authorizing the use of Paxlovid for patients with mild to moderate COVID-19.

Based on results from three randomized, controlled clinical trials that showed that remdesivir was superior to placebo in shortening the time to recovery in adults who were hospitalized with mild-to-severe COVID-19, the U.S. Food and Drug Administration (FDA) approved remdesivir for clinical use in adults and pediatric patients (over age 12 years and weighing at least 40 kilograms or more) to treat hospitalized patients with COVID-19.[35] However, results from the WHO SOLIDARITY Trial conducted at 405 hospitals spanning across 40 countries involving 11,330 inpatients with COVID-19 who were randomized to receive remdesivir (2750) or no drug (4088) found that remdesivir had little or no effect on overall mortality, initiation of mechanical ventilation, and length of hospital stay.[36] A recently published randomized double-blind placebo-controlled trial reported an 87% lower risk of hospitalization or death than placebo when at-risk non hospitalized patients with COVID-19 were treated with a 3-day course of remdesivir.[37]

Hydroxychloroquine and chloroquine were proposed as antiviral treatments for COVID-19 initially during the pandemic. However, data from randomized control trials evaluating the use of hydroxychloroquine with or without azithromycin in hospitalized patients did not improve the clinical status or overall mortality compared to placebo.[38] Data from randomized control trials of hydroxychloroquine used as postexposure prophylaxis did not prevent SARS-CoV-2 infection or symptomatic COVID-19 illness.[39] Hydroxychloroquine and chloroquine are currently not indicated for the treatment of COVID-19 in hospitalized and nonhospitalized patients

Lopinavir/ritonavir is an FDA-approved combo therapy for the treatment of HIV and was proposed as antiviral therapy against COVID-19 during the early onset of the pandemic. Data from a randomized control trial that reported no benefit was observed with lopinavir-ritonavir treatment compared to standard of care in patients hospitalized with severe COVID-19. [40] Lopinavir/Ritonavir is currently not indicated for the treatment of COVID-19 in hospitalized and nonhospitalized patients.

Ivermectin is an FDA-approved anti-parasitic drug used worldwide in the treatment of COVID-19 based on an in vitro study that showed inhibition of SARS-CoV-2 replication.[41] A single-center double-blind, randomized control trial involving 476 adult patients with mild COVID-19 illness was randomized to receive Ivermectin 300 mcg/kg body weight for five days or placebo did not achieve significant improvement or

resolution of symptoms.[42] Ivermectin is currently not indicated for the treatment of COVID-19 in hospitalized and non-hospitalized patients.

Vaccines [43]

Several vaccines have been manufactured so far with the nucleic acid (RNA and DNA) platform, adenovirus vector platform, the traditional platforms of vaccine preparation using inactivated or live attenuated viruses, protein subunit platform, adjuvant recombinant platform, nanoparticle-based vaccine platform and virus-like particle (VLP)-based platforms . Based on the nucleic acid platform, the mRNA vaccines BNT162b2 manufactured by BioNTech and mRNA-1273 developed by Moderna, Cambridge, MA, USA, have been found with 94% effectiveness. The adenovector vaccine ChAdOx1-S/AZD1222, manufactured by the University of Oxford/AstraZeneca, Cambridge, UK, showed around 70% efficacy after the first dose and 81.3% after the second. Another adenovirus vector vaccine, Sputnik V, developed by Gamaleya Institute in Moscow, was found with nearly 92% efficacy; and the Ad26.COV2.S vaccine from the Janssen Pharmaceuticals of Johnson & Johnson, Belgium, was found with nearly 70% efficacy.

	Pfizer / Moderna	Astra-Zeneca	Janssen Johnson and Johnson
1. Mechanism of action	mRNA	Adenovirus viral vector (replication deficient chimpanzee adenovirus)	Adenovirus viral vector (replication incompetent human adenovirus serotype-26)
2. Antigen	Full length spike protein	Spike protein	Spike protein
3. Doses	2 doses, 21 days apart	2 doses, 12 weeks apart	Only 1 dose
4. Side effects	Rare allergies, anaphylaxis and facial paralysis (Bell's palsy)	Rare thromboembolic events, blood-clots, pulmonary embolism and thrombocytopenia	Rare cases of blood clots, thrombocytopenia and Guillain-Barre syndrome
5. Overall efficacy	95%	70%	72%
6. Storage temperature	-70 degrees celsius. 2 to 8 degrees celsius for 5 days.	2 to 7 degrees celsius for 6 months	2 to 8 degrees celsius for 3 months

Preventive strategies [44]

The WHO has stated that education, isolation, prevention, controlling the transmission, and treatment of infected persons are the critical steps in controlling contagious diseases like COVID-19. It is possible to minimize the spread of infection by making the following recommendations.

Staying at home (home quarantine) and avoiding any direct contact with any healthy (possible asymptomatic patients) or infected person, which has been called shielding; avoiding nonessential travel; observing social distancing rules like avoiding crowded public places and maintaining at least two meters of distance between each person, especially if they are coughing or sneezing; avoiding shaking hands when greeting others; frequently washing hands for at least 20 s with soap and water or hand sanitizer with at least 60% alcohol, especially after touching common surface areas, using the bathroom, or shaking hands, avoiding touching eyes, nose, and mouth with unwashed hands; and disinfecting surfaces using household sprays or wipes.

It should be mentioned that due to the long incubation period and presence of asymptomatic patients, using a medical mask (especially N95) or a respirator (especially FFP3) could be recommended. Also, sterilizing the used respirator, only reusing it for a limited time, and proper disposal of the used masks, have been recommended. Although respirators (the protective classes, including FFP1, FFP2, and FFP3) are produced as single-use items, they could be used again for a limited time unless there is a risk for contamination through the deposition of infectious particles on the surface. When the respirator becomes soiled or wet with bodily fluids or it can no longer be appropriately fitted, or if breathing via the respirator becomes difficult, it should be discarded. Also, masks should be discarded after being used during an aerosol-generating procedure (AGP). Until now, manufacturers have had no reason to disinfect masks or to produce masks for repeated use. However, there is a vital need to be able to disinfect masks and reuse them. SARS-CoV-2 remains viable in the environment, including on the surface of different materials like cardboard, iron, or tissue for some time. This suggests that there is a risk for rapid contamination of the outer surface of respirators and surgical masks. Contamination of the respirator surface could be prevented through placing a medical mask over it, or wearing a face shield that can be cleaned. Because of the severe contamination of respirators and surgical masks in the COVID-19 pandemic, several methods could be considered for the sterilization of used masks, including steam, hydrogen peroxide, or radiation.

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