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SARS – COV-2 (COVID-19) Pandemic: A Critical Review on Novel Coronavirus Pathogenesis, Clinical Diagnosis and Treatment

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ABSTRACT

The 2019-nCoV is officially called SARS-CoV-2 and the disease is named COVID-19. The Novel coronavirus (SARS-CoV-2) caused pneumonia in Wuhan, China in December 2019 is a highly contagious disease. The World Health Organization (WHO) has declared it as a global public health emergency. This is the third serious Coronavirus outbreak in less than 20 years, following SARS in 2002–2003 and MERS in 2012. Currently, the research on novel coronavirus is still in the primary stage. It is currently believed that this deadly Coronavirus strain originated from wild animals at the Huanan market in Wuhan by Bats, snakes and pangolins have been cited as potential carriers. On the basis of current published evidence, we systematically summarize the epidemiology, clinical characteristics, diagnosis, treatment and prevention of COVID-19. This review in the hope of helping the public effectively recognize and deal with the novel coronavirus (SARS-CoV-2) and providing a reference for future studies.

Keywords: SARS-CoV-2, COVID-19, Coronavirus, pneumonia, Respiratory infection

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1. Introduction

There is a current worldwide outbreak of the novel coronavirus Covid-19 (coronavirus disease 2019; the pathogen called SARS-CoV-2; previously 2019-nCoV), which originated from Wuhan in China and has now spread to 7 continents including 102 countries. Governments show their concern to stop the outbreak spreading into a global health emergency. Coronaviruses, a large family of single-stranded RNA viruses, can infect animals and also humans, causing respiratory, gastrointestinal, hepatic, and neurologic diseases[1]. As the largest known RNA viruses, CoVs are further divided into four generation: alpha-coronavirus, beta- coronavirus, gamma-coronavirus and delta-coronavirus[2]. Now days there are six human coronaviruses (HCoVs) has been identified, including the alpha-CoVs HCoVs-NL63 and HCoVs-229E and the beta-CoVs HCoVs-OC43, HCoVs-HKU1, severe acute respiratory syndrome-CoV (SARS-CoV)[3], and Middle East respiratory syndrome-CoV (MERS-CoV)[4]. New coronaviruses appear to emerge periodically in humans, mainly due to the high prevalence and wide distribution of coronaviruses, the large genetic diversity and frequent recombination of their genomes, and the increasing of the human-animal interface activities[5,6].

2. Structure and genomes of Coronavirus

Coronaviruses (CoVs) have crown-like spikes on their surface and belong to the family Coronaviridae within the order Nidovirales. Corona viruses broadly infect vertebrates including humans, birds, bats, snakes, mice and other wild animals[7]. Corona viruses (HCoVs) have been identified since mid-1960s[8,9]. Four commonly detected HCoVs are 229E, OC43, NL63 and HKU1. In one study, 229E and OC43 accounted for approximately 15–29% of respiratory pathogens with relatively low virulence in humans[10]. Another epidemiological study in adults estimates that coronavirus causes about 15% of common colds[11]. Other significant causes of upper respiratory infections include influenza virus, rhinovirus, parainfluenza virus, Group A Streptococci, EBV and respiratory syncytial virus (RSV).

The three other strains of HCoVs, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have a different pathogenicity and lead to higher mortality rates in human populations. MERS-CoV was isolated from a male patient who died from acute pneumonia and renal failure in Saudi Arabia in 2012[12].

CoVs is the largest known genome among RNA viruses contains an envelope with a non-segmented, positive-sense, single-strand RNA, with size ranging from 26,000 to 37,000 bases[13]. All coronavirus genomes are arranged similarly with the replicase locus encoded within the 5' end and the structural proteins encoded in the 3' third of the genome arranged in the order hemagglutinin esterase (HE), if present (HE is only present in some beta coronaviruses), spike (S), small membrane (E), membrane (M) and nucleocapsid (N) and internal (I) protein, encoded within the N gene. The nucleo capsid protein complexes with the genome RNA to form a helical capsid structure found within the viral envelope. Trimers of the spike protein form the peplomers embedded in the envelope giving the virion its corona or crown-like morphology. In some coronavirus virions, the HE protein forms smaller spikes on the membrane. M and E are also transmembrane proteins involved in virus assembly[1].**Fig. 1**

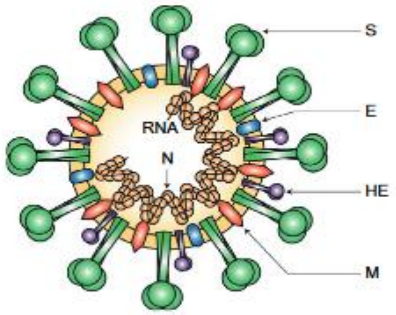

Fig.1: Coronavirus virion structure.

3. Genetic Diversity

The genome RNA shown in fig.2 is complexed with the N protein to form a helical cased within the viral membrane, HE, hemagglutinin-esterase; S, spike; E, small membrane envelope; M, membrane are all transmembrane proteins. The genomic structure of SARS-CoV-2 is 5′-UTR-orf1a-orf1ab-S (Spike)–E (Envelope)-M (Membrane)-N (Nucleocapsid)-3′UTRpoly (A) tail. Accessory genes are interspersed within the structural genes at the 3′ end of genome. The pp1a protein encoded by the orf1a gene and the pp1ab protein encoded by the orf1ab gene contains 10 nsps (nsp1-nsp10). The pp1ab protein also includes nsp12-nsp16.

The various CoVs of animal origin undergo evolution and genetic recombination, thereby resulting in mutated CoVs that may be highly pathogenic and potentially be more deadly to humans[15]. The mutation rate in the SARS-CoV genome was estimated to be $0.80–2.38 \times 10^{-3}$ nucleotide substitutions per site per year, which are similar to that of other RNA viruses[16]. The various CoVs of animal origin undergo evolution and genetic recombination either within the host species or upon jumping from one species to another. Such changes thus have the potential to lead to variants that have high pathogenic potential when transmitted to humans[17-19]. Recently, two mutations of the S protein and N protein SARS-CoV-2 may explain its zoonotic transmission[20]. Genomic alignment of 54 SARS-CoV-2 genomes identified two hotspots of hypervariability at positions 8789 (synonymous variant) and 28,151(Ser/Leu change), located in the polyprotein and ORF8 genes respectively[21].

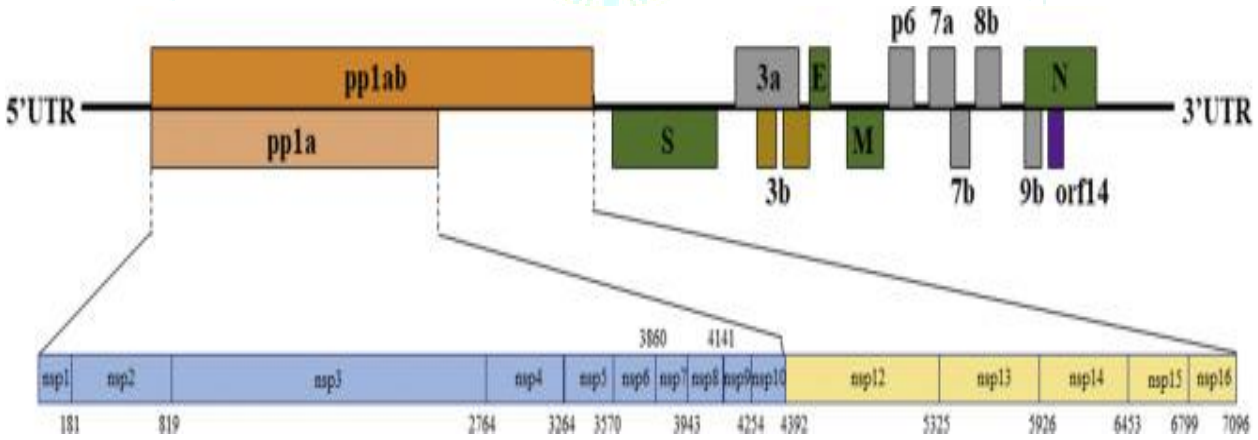

Fig. 2: Schematic diagram of the SARS-CoV-2 genome[14].

3. Mode of Transmission of Coronavirus

SARS-CoV originated from bats of the Hipposideridae family before dissemination to humans[22]. CoVs can transmit across species barriers. The earliest patients infected with SARS-CoV-2 in Wuhan ultimately caused the epidemic known as CoronaVirus Disease 2019 (COVID-2019)[23]. Some of these patients had a history of contact with a wholesale seafood market in the early stages, suggesting animal-to-person spread. Subsequently, a large number of patients reportedly did not have exposure to the markets, suggesting the development of person-to-person [24-27].

There are three ways to transmit the virus, including[28-30]:

1. Close person-to-person contact
2. Aerosol transmission
3. Transmission by touch

It also thought that the virus to be transmitted to other people by[31]

A respiratory droplet means coughing or sneezing. Droplet spread can occur when an infected person sneezes or coughs, whereupon virus containing droplets are propelled up to 3 feet through the air and are deposited on the mucous membranes of the mouth, nose, or eyes of persons who are nearby.

1. Transmission through the ocular surface is also possible.
2. Shaking hands with an infected person,
3. Touching an infected object/surface,

4. Frequent touching of the nose or mouth or coming into contact with a patient's excreta.
5. Another way is through "hidden transmission", in which asymptomatic infected individuals or carriers unknowingly transmit the virus to unsuspecting contacts.

4. The pathogenesis of COVID-19

Current understanding of the pathogenesis of HCoVs infection is still limited, especially for SARS-CoV-2. Before 2019, there were six CoVs that could infect humans and cause respiratory disease. HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 are sometimes attributed to the "common cold", but in rare cases can cause severe infections in infants, young children and elderly people. On the other hand, SARS-CoV and MERS-CoV can infect the lower respiratory tract and cause a severe respiratory syndrome in human. The new coronavirus SARS-CoV-2 is similar to SARS-CoV and MERS-CoV and can infect lower respiratory tract and cause severe pneumonia.

The origin of SARS-CoV-2 was thought to be wild animals in the Huanan Seafood Market in Wuhan. However, not all cases have an apparent connection with the Wuhan Huanan Seafood Wholesale Market. It is evident now that SARS-CoV-2 is capable of person-person transmission. We list the major pathogenic CoVs in Table 1 for better understanding of the pathogenesis of HCoV[32].

The term "cell pyroptosis" was first proposed in 2001[33]. In recent decades, there has been increasing evidence suggesting that "pyroptosis" is a novel inflammatory form of programmed cell death. In 2019, Chen et al. found that SARS-CoV Viroporin 3a triggered the activation of the NLRP3 inflammasome and the secretion of IL-1β in bone marrow-derived macrophages, suggesting SARS-CoV induced cell pyroptosis[34] The pathways involved in the activation of the signaling between NLRP3m IL-1β, IL-18 and GSDMD are illustrated in Fig. 3 and are a subject of study in samples from SARS-CoV-2 patients[35].

The COVID-19 may be linked to cell pyroptosis, especially in lymphocytes through the activation of the NLRP3 inflammasome. Morphological changes in lymphocytes and macrophages, nucleic acid and protein levels in classical and non-classical cells, detection of NLRP3 and GSDMD, and the role of inflammatory cytokines IL-1β and IL-18 requires further research.

Table 1: Partial list of important pathogenic human coronaviruses

Virus	Genus	Symptoms
HCoV-229E	*alpha*	mild respiratory tract infections
HCoV-NL63	*alpha*	mild respiratory tract infections
HCoV-OC43	*beta*	mild respiratory tract infections
HCoV-HKU1	*beta*	pneumonia
SARS-CoV	*beta*	severe acute respiratory syndrome, 11% mortality rate
MERS-CoV	*beta*	severe acute respiratory syndrome, 34% mortality rate
SARS-CoV-2	*beta*	severe acute respiratory syndrome, 2.6% mortality rate

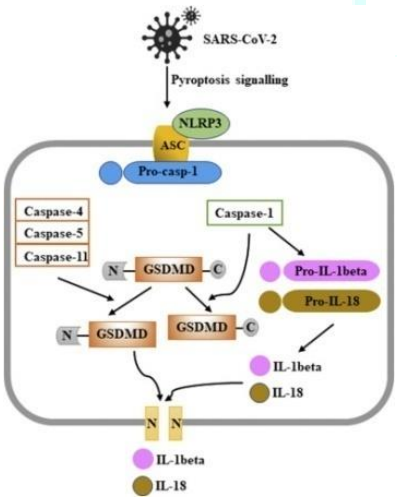

Fig. 3: A hypothesis of the relationship between SARS-CoV-2 and cell pyroptosis

5. Clinical Manifestation

The patients with SARS-CoV-2 shows a wide range of clinical manifestations are seen in from mild, moderate, to severe and rapidly progressive. Most of the patients with SARS-CoV-2 were normal and mild, and their mortality was lower than SARS-CoV and MERS-CoV.

5.1 Transmission: person-to-person, primarily via respiratory droplets (sneezing and coughing)

- Direct contact transmission: especially hand-to-face contact
- Fomite transmission: not documented but conceivably possible, especially with objects and surfaces that may have recently come into contact with infected individuals
- Transmission via mail and packaged (imported) goods: There is no evidence to suggest that mail and packaged (imported) goods pose a risk for the spread of COVID-19.
- Fecal-oral transmission: Evidence that both SARS-CoV and MERS-CoV are excreted fecally suggests that fecal-oral transmission is possible[36].

5.2 Incubation Period

The mean incubation period of CoVID-19 was a little bit different. The study with 138 patients, reported that the median durations from first symptoms to dyspnea, hospital admission, and Acute severe respiratory syndrome (ARDS)

were 5 days (range, 1-10), 7 days (range, 4-8), and 8 days (range, 6-12), respectively[37]. The mean time from symptom onset to hospitalization was between 2 and 8 days, but was shorter toward the later phase of the epidemic. The mean time from symptom onset to need for invasive mechanical ventilation (IMV) and to death was 11 and 23.7 days, respectively[38].

5.3 Symptom of CoVID-19

Symptom of CoVID-19 can range from no symptoms (asymptomatic) to severe pneumonia and death. The patients were initially diagnosed with the outbreak found that the most common symptoms were fever (98%), cough (76%), myalgia or fatigue (44%), and atypical symptoms included sputum (28%), headache (8%), hemoptysis (5%) and diarrhea (3%). About half of the patients had dyspnea (the median from onset to dyspnea was 8 days). Lymphocytopenia was observed in 63% of patients. All patients had pneumonia.

Complications included acute respiratory distress syndrome (29%), acute heart injury (12%), and secondary infections (10%); 32% of patients require to be treated in the ICU[39]. The patients presented ground-glass shadow on chest CT. Recent studies indicate that patients≥60 years of age are at higher risk than children who might be less likely to become infected or, if so, may show milder symptoms or even asymptomatic infection[40].

Before the first outbreak of SARS, a limited number of HCoVs such as HCoV-229E were frequently found to infect humans, and were widely circulating in human populations causing only mild illnesses like the common cold. However, SARS, MERS and SARS-CoV-2 present with a spectrum of disease severity ranging from flu-like symptoms to acute respiratory distress syndrome[41, 42].

6. Early Detection and Diagnosis

The SARS-CoV-2 infected cases have symptoms like fever, fatigue, dry cough, dyspnea etc., with or without nasal congestion, runny nose or other upper respiratory symptoms. Early identification of suspect cases is the key to inhibiting the spread of the virus. Rapid identification of the viral genome and the development of rapid diagnostic tests will facilitate the isolation of those who are confirmed as infected[43].

Diagnostic criteria[44-50]

The diagnostic criteria of suspected and confirmed cases were summarized in Table 2.

Table 2. The diagnostic criteria for suspected and confirmed cases

Case	Diagnostic criteria
Suspected case	Anyone with a history of epidemiology and any two of the clinical manifestations or anyone without epidemiological history and three of the clinical manifestations is considered to be a suspected case:
	(1) Epidemiological history:
	1) within 14 days before the disease onset, there is a travel history or living history in Wuhan or other areas with local cases
	2) within 14 days before the disease onset, there is contact with patients who had fever or respiratory symptoms from Wuhan or other areas with local cases
	3) a clustering of patients or a contact with patients infected with the SARS-CoV-2
	(2) Clinical manifestations:
	1) fever and/or respiratory symptoms
	2) with the above-mentioned imaging characteristics of pneumonia
	3) the total number of leukocytes in the early stage of the disease is normal or decreased, or the lymphocyte count is decreased
Confirmed case	Any suspected case with one of the following pathogenic features is reclassified as a confirmed case:
	(1) Positive results of SARS-CoV-2 nucleic acids by RT-PCR* of respiratory or blood specimens
	(2) DNA highly homologous to SARS-CoV-2 by genetic sequencing of viral genes in respiratory or blood specimens

*RT-PCR: real-time reverse-transcriptase polymerase-chain-reaction.

6.1 Physical examination

Patients with mild symptoms may not be present positive signs. Patients in severe condition may have shortness of breath, moist rales in lungs, weakened breath sounds, dullness in percussion, and increased or decreased tactile speech tremor, etc.

6.2 CT imaging examination

The imaging finding vary with the patient`s age, immunity status, disease stage at the time of scanning, underlying diseases, and drug interventions. *Chest X-ray examination* In the early stage of pneumonia cases, chest images show multiple small patchy shadows and interstitial changes[51], Severe cases can further develop to bilateral multiple ground-glass opacity, infiltrating shadows, and pulmonary consolidation, with infrequent pleural effusion.

6.3 Laboratory Diagnosis

Laboratory diagnosis mainly used to distinguished from other known viral virus of pneumonia, such as parainfluenza virus, influenza viruses, respiratory syncytial virus, rhinovirus, SARS-CoV,adenovirus, etc.; and also, from chlamydia pneumonia, mycoplasma pneumonia, and bacterial pneumonia. In addition, it should be distinguished

from non-infectious diseases, such as dermatomyositis,vasculitis, and organizing pneumonia[52]. So, laboratory diagnosis is necessary. Identification of CoVID-19 mainly includes virus isolation and viral nucleic acid detection. A variety of specimens (such as swabs, nasal swabs, nasopharynx or trachea extracts, sputum or lung tissue, blood and feces) were used for testing in timely manner, which gives a higher rate of positive detection of lower respiratory tract specimens[53]. The Patients suspected SARS-CoV-2 infection for diagnosis by real time RT-PCR method[54].

7. Prevention and Treatment of SARS-CoV-2 infection

Novel coronavirus infection is a new communicable disease which affects almost all populations with all age group[55]. SARS-CoV-2 infection has been classified as category B infectious disease legally but managed as category A infectious disease by Chinese government. It is paramount to implement infection control practices by infection source controlling, transmission route blocking, and susceptible population protection. The unprecedented flurry of activity by WHO and other global public health bodies has mainly focused on preventing transmission, infection control measures, and screening of travelers[56].

There is no clear, unified and effective treatment plan for COVID-19. Most guidelines emphasize early identification, early isolation, early diagnosis, and early treatment. The treatment and management of SARS-CoV-2 pneumonia mainly include the following aspects[57-61] Fig. 4.

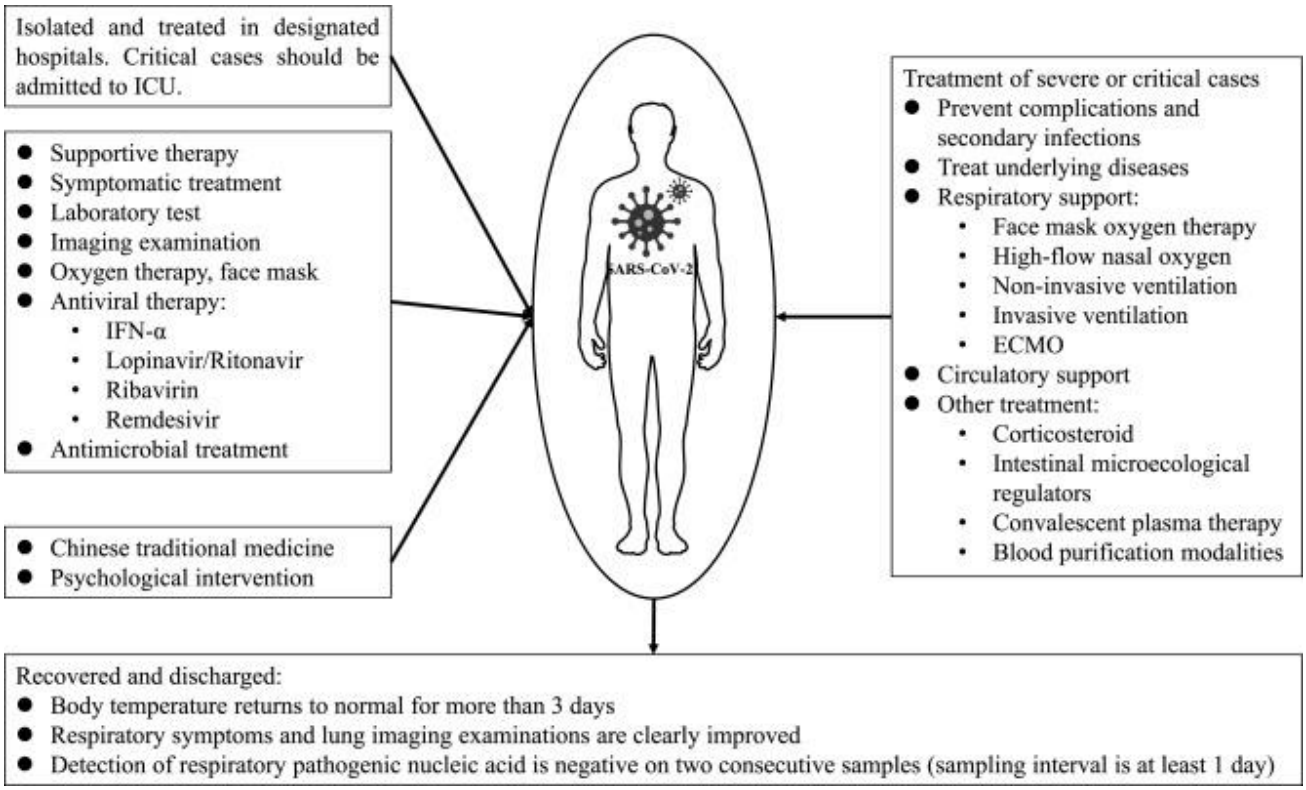

Fig. 4. The treatment and management of COVID-19 pneumonia. ICU: intensive care unit; ECMO: extracorporeal membrane oxygenation

7.1 General protective measures

1. Hand hygiene:

- Hands should be washed with soap and water or disinfected with a virucidal hand disinfectant after contact with potentially virus-contaminated objects and infected persons
- Avoid touching the face: i.e., the eyes, nose, and mouth.

2. Respiratory hygiene and cough etiquette

- Avoid coughing or sneezing in the direction of others!
- Use tissues and discard these after use.
- If tissues are unavailable, coughing and sneezing into the crook of the arm can help keep hands free of contamination.
- Maintain 3–6 ft (at least an arm's length) distance to coughing or sneezing persons.

3. Avoid exposure

- Avoid crowds of people (public transport, train stations, airports, mass events).
- Avoid travel to areas of outbreak.

4. Masks

- In individuals with confirmed and suspected infection: useful for preventing the diffusion of respiratory secretions, e.g., during patient transports
- In healthcare facilities (HCFs) or home care settings: crucial for health workers and persons taking care of an infected individual in close settings (in a HCF or at home)

- Surgical masks do not provide adequate protection in the setting of invasive diagnostics or those at high risk of exposure.
- N95 respirators and protective eyewear are recommended for health care personnel that are potentially exposed to airborne and fluid hazards (e.g., during invasive procedures).
- Confirmed COVID-19 patients and PUIs can use standard N95 respirators.
- If N95 respirator bottlenecks occur, unvalved N95 respirators may be used with a face shield.
- Respirators and masks should be used resourcefully with special consideration for HCF needs.
- In the general population: Surgical masks are most likely ineffective and may even pose an additional risk of infection.

7.2 Intensive care

- **Indications:** Admit to ICU and initiate intubation if any of the following are present:
  - Signs of respiratory failure
  - Dyspnea with hypoxemia
  - Tachypnea (RR > 30/min)
- **Airway management:** Considering health-care workers have an increased risk of developing COVID-19, especially during high-risk procedures such as intubation, aerosol-generating procedures should be avoided whenever possible[62-64].
  - Endotracheal intubation: Rapid-sequence induction is preferred, especially as it minimizes the spreading of infectious aerosols.
  - To avoid aerosolizing the virus, noninvasive ventilation, high-flow oxygen therapy, bronchoscopy, and nebulizer treatment should be avoided unless there is an absolute indication.
    - If NIPPV is indicated (e.g., COPD, asthma, DNI status): attempt with a helmet (vs. face mask) interface
- **Mechanical breathing:** ventilation with lower tidal volumes (LTV) as with ARDS[65]
  - Moderate tidal volume (6 mL/kg)
  - Plateau pressure < 30 cm H_2O
  - Permissive hypercapnia (target pH > 7.3)
  - PEEP and FiO_2 settings: adjust as needed according to ARDSnet protocol[66]
  - See therapy of ARDS for more information.

7.3 Medical therapy and Treatment

Early on, these patients are usually treated with conventional medications which had no clinical benefit, resulting in spread to health care personnel. For those with flu-like symptoms or even more severe disease, it would not be immediately evident that this is an atypical and virulent form of a coronavirus. For comparison, influenza has an estimated mortality rate of 0.07%–0.2%. A high index of suspicion is helpful but not foolproof.

The general strategies include bed rest and supportive treatment, including antiviral therapy[67], antibioics application, immunomodulating therapy[68], organ function support, respiratory support, bronchoalveolar lavage (BAL), blood purification and extracorporeal membrane oxygenation (ECMO)[69].

Potential target structures and agents: A variety of agents are being tested, and clinical studies are being conducted[70, 71].

- Inhibition of adhesion and invasion
  - Camostat (protease inhibitor)
- Inhibition of fusion
  - Chloroquine or less toxic hydroxychloroquine[72-75]
  - Hydroxychloroquine In combination with azithromycin is being intensely tested[76]
  - Umifenovir[77]
- Inhibition of protease
  - Lopinavir/ritonavir[78-80]
  - Darunavir/ritonavir (possibly in combination with umifenovir)
  - Remdesivir[81, 82]
- RNA polymerase inhibitors and nucleotide analogs
  - Favipiravir [83] (brand name: Avigan®; approved in Japan)
  - Remdesivir [81, 82]
  - Baloxavir marboxil
- Antibody therapy and biologicals [84]
  - Tocilizumab, especially in the phase of ARDS when IL-6 and CRP are increased [85]
  - Recombinant ACE2 (rhACE2, APN01) [86]
- Passive immunization through serum therapy: [87]
  - Immunized individuals (already had COVID-19) donate serum
  - Especially a potential option for risk groups

7.3.1 Antiviral therapy

To date, there is no anti-viral therapeutics that specifically targets human coronaviruses, so treatment is only supportive. *In vitro*, interferons (IFNs) are only partially effective against coronaviruses. *In vivo*, the effectiveness of IFNs combined with ribavirin requires further evaluation. A variety of other agents, including antiviral peptides and corticosteroids have been shown to be effective *in vitro* and/or in animal models. However, clinical evidence does not support the use of corticosteroid treatment for SARS-CoV-2 lung injury.

Antiviral agents and immunomodulators tested against SARS-CoV in animals and in vitro shown in Table 3[88]

Table 3. Antiviral agent(s) and/or immunomodulator(s) Used in Treatment of Covid- 19

Antiviral agent(s) and/or immunomodulator(s)	Study setting and methods (virus strain)	Main findings[a]
IFN-αB/D (hybrid IFN)	BALB/c mice (Urbani)	i.p. IFN-αB/D once daily for 3 days beginning 4 h after virus exposure reduced SARS-CoV replication in lungs by 1 $\log_{10}$ at 10,000 and 32,000 IU; at the highest dose of 100,000 IU, virus lung titers were not detectable
Ampligen [poly(I:C124)] (mismatched double-stranded RNA IFN inducer)	BALB/c mice (Urbani)	i.p. Ampligen at 10 mg/kg 4 h after virus exposure reduced virus lung titers to undetectable levels
Pegylated IFN-α as prophylactic treatment	Cynomolgus macaques (*Macaca fascicularis*) (patient 5668)	Significantly reduced viral replication and excretion, viral antigen expression by type 1 pneumocytes, and pulmonary damage; postexposure treatment with pegylated IFN-α yielded intermediate results
IFN-α2b (Intron A)	Vero (FFM-1, HK isolate)	Mean (SD) EC_{50} = 4,950 (890) IU/ml (SI of >2) for FFM-1 isolate; mean (SD) EC_{50} = 6,500 (980) IU/ml (SI of >105) for HK isolate
	Caco2 (FFM-1, HK isolate)	Mean (SD) EC_{50} = 1,530 (220) IU/ml (SI of >6.5) for FFM-1 isolate; mean (SD) EC_{50} = 880 (130) IU/ml (SI of >11.4) for HK isolate
IFN-β1b (Betaferon)	Vero (FFM-1, HK isolate)	Mean (SD) EC_{50} = 95 (17) IU/ml (SI of >105) for FFM-1 isolate; mean (SD) EC_{50} = 105 (21) IU/ml (SI of >95) for HK isolate
	Caco2 (FFM-1, HK isolate)	Mean (SD) EC_{50} = 21 (3.9) IU/ml (SI of >476) for FFM-1 isolate; mean (SD) EC_{50} = 9.2 (2.1) IU/ml (SI of >1,087) for HK isolate
IFN-γ1b (Imukin)	Vero (FFM-1, HK isolate)	Mean (SD) EC_{50} = 2,500 (340) IU/ml (SI of >4) for FFM-1 isolate; mean (SD) EC_{50} = 1,700 (290) IU/ml (SI of >5.9) for HK isolate
	Caco2 (FFM-1, HK isolate)	Mean (SD) EC_{50} = >10,000 IU/ml (SI NA) for FFM-1 isolate; mean (SD) EC_{50} = >10,000 IU/ml (SI NA) for HK isolate
IFN-β1a	Vero E6 (Tor2, Tor7, and Urbani)	IFN with p.i. IC_{50} = 50 IU/ml; IFN added postinfection IC_{50} = 500 IU/ml
IFN-β, IFN-α, IFN-γ	Vero, MxA-expressing Vero (FFM-1)	SARS-CoV strongly inhibited by IFN-β (with p.i.) and less so with IFN-α and IFN-γ; MxA does not interfere with viral replication
IFN-α, IFN-β	FRhK-4 (NM[f])	↓ intracellular viral RNA copies; IFN-α IC_{50} = 25 U/ml; IFN-β IC_{50} = 14 U/ml
IFN-α2b	Vero E6 (Tor2, Tor3, Tor7, and Tor684)	IC_{50} = ~500 IU/ml
Leu-IFN-α	FRhK-4 (HKU39849)	EC_{50} at 48 h = 5,000 μg/ml
	Vero E6 (HKU39849)	EC_{50} at 48 h = 19.5 μg/ml
IFN-α (p.i. for 16 h before viral inoculation)	FRhK-4 (HKU39849)	EC_{50} at 48 h = 39 μg/ml
	Vero E6 (HKU39849)	EC_{50} at 48 h = 19.5 μg/ml
IFN-β	FRhK-4 (HKU39849)	EC_{50} at 48 h = 200 μg/ml
	Vero E6 (HKU39849)	EC_{50} at 48 h = 106 μg/ml
IFN-β (p.i. for 16 h before viral inoculation)	FRhK-4 (HKU39849)	EC_{50} at 48 h = 625 μg/ml
	Vero E6 (HKU39849)	EC_{50} at 48 h = 19.5 μg/ml
IFN-β1b (Betaferon)	Vero E6 (2003VA2774)	IC_{50} = 0.2 IU/ml; IC_{95} = 8 IU ml
IFN-αn3 (Alferon)	Vero E6 (2003VA2774)	IC_{50} = 0.8 IU/ml; IC_{95} = 200 IU/ml
Human leukocyte IFN-α (Multiferon)	Vero E6 (2003VA2774)	IC_{50} = 2 IU/ml; IC_{95} = 44 IU/ml
IFN-β	Vero E6 (FFM-1)	IC_{50} = 110 IU/ml at 10 $TCID_{50}$; IC_{50} = 625 IU/ml at 100 $TCID_{50}$
Multiferon	Vero E6 (FFM-1)	IC_{50} = 540 IU/ml at 10 $TCID_{50}$; IC_{50} = 2,400 IU/ml at 100 $TCID_{50}$
IFN-α2b	Vero E6 (FFM-1)	IC_{50} = >3,125 IU/ml at 10 $TCID_{50}$; IC_{50} = >3,125 IU/ml at 100 $TCID_{50}$
IFN-α2a	Vero E6 (FFM-1)	IC_{50} = >3,125 IU/ml at 10 $TCID_{50}$; IC_{50} = >3,125 IU/ml at 100 $TCID_{50}$
IFN-alfacon1 (Infergen)	Vero (Urbani)	IC_{50} = 0.001 μg/ml
IL-4 and IFN-γ	Vero E6 (HKU39849)	IL-4 and IFN-γ downregulated cell surface expression of ACE2; ACE2 mRNA levels were also decreased after treatment
IFN-β and ribavirin	Caco2 (FFM-1)	Mean (SD) CI = 0.45 (0.07)

HR2-8 (HR2-derived peptide)	Vero 118 (NM)	EC_{50} = 17 μM
CP-1 (HR2-derived peptide)	Vero E6 (WHU)	IC_{50} ≈ 19 μmol/liter
HR1-1 (HR1-derived peptide)	Vero E6 (BJ01 and pseudovirus)	EC_{50} = 3.68 μM for wild-type virus assay; EC_{50} = 0.14 μM for pseudotyped virus assay
HR2-18 (HR2-derived peptide)	Vero E6 (BJ01 and pseudovirus)	EC_{50} = 5.22 μM for wild-type virus assay; EC_{50} = 1.19 μM for pseudotyped virus assay
HR2	Vero E6 (WHU)	CPE inhibition IC_{50} = 0.5-5 nM (synthetic HR2 peptide) and 66.2-500 nM (fusion HR2 peptide)
Peptides representing various regions of ACE2	TELCeB6, HeLa, and VeroE6 (pseudovirus)	IC_{50} = 50 μM (peptide aa 22-44); IC_{50} = 6 μM (peptide aa 22-57); IC_{50} = 0.1 μM (peptide aa 22-44 and 351-357) artificially linked by glycine
Peptides analogous to viral spike protein	Vero E6, L2 (Urbani)	Inhibit viral plaque formation by 40-70% at 15-30 μM; peptides analogous to regions of the N terminus or the pretransmembrane domain of the S2 subunit; inhibit viral plaque formation by >80% at 15-30 μM (peptides analogous to the SARS-CoV loop region)
siRNA, RL004, RL005	Vero E6 (Y3)	siRNA (600 pmol/liter) targeting conserved regions of SARS-CoV, ↓ virally induced CPE at 67 h
siRNA	FRhK-4 (HKU66078)	siRNA duplexes targeting regions in entire viral genome, ↓ virally induced CPE and viral production at 72 h
siRNA targeting viral RP	Vero (NM)	↓ virally induced CPE, ↓ viral production, ↓ viral protein synthesis at 1.5 or 3 μg of siRNA
RNA interference targeting viral RP	Vero E6, 293, HeLa (SARS-CoV-p9)	↓ expression of RP (293 and HeLa cells); ↓ plaque formation at 1 μg of siRNA
siRNA targeting S gene	Vero E6, 293T (BJ01)	↓ S gene expression in SARS-CoV-infected cells at 2, 3, and 4 μg of siRNA
siRNAs targeting S gene and 3′ untranslated region	Vero E6 (HK strain)	↓ viral antigen synthesis of 64% (by siSARS-S2), 51% (siSARS-S3), 40% (siSARS 3′ untranslated region) at 100 pmol of siRNA
Glycyrrhizin	Vero (FFM-1, FFM-2)	CPE assay mean (SD) CC_{50} = >20,000 mg/liter; EC_{50} = 300 (51) mg/liter (SI of >67)
	FRhK-4 (HKU39849)	EC_{50} at 48 h = >400 μg/ml
	Vero E6 (HKU39849)	EC_{50} at 48 h = 100 μg/ml
Mizoribine	Vero E6 (FFM-1)	IC_{50} = 3.5 μg/ml; CC_{50} = >200 μg/ml
	Vero E6 (HKU39489)	IC_{50} = 16 μg/ml
Ribavirin	Vero E6 (FFM-1)	IC_{50} = 20 μg/ml; CC_{50} = >200 μg/ml
	Vero E6 (HKU39489)	IC_{50} = 80 μg/ml
	FRhK-4 (HKU39849)	EC_{50} at 48 h = 50-100 μg/ml
	Vero E6 (HKU39849)	EC_{50} at 48 h = >200 μg/ml
Rimantidine	FRhK-4 (HKU39849)	EC_{50} at 48 h = 16 μg/ml
	Vero E6 (HKU39849)	EC_{50} at 48 h = 8-16 μg/ml
Lopinavir	FRhK-4 (HKU39849)	EC_{50} at 48 h = 16 μg/ml
	Vero E6 (HKU39849)	EC_{50} at 48 h = 8-16 μg/ml
Baicalin	FRhK-4 (HKU39849)	EC_{50} at 48 h = 12.5 μg/ml
	Vero E6 (HKU39849)	EC_{50} at 48 h = 100 μg/ml
Aurintricarboxylic acid	Vero (NM)	EC_{50} = 0.2 mg/ml; CC_{50} = 37.5 mg/ml; SI = 187
Reserpine	Vero E6 (HK strain)	EC_{50} = 3.4 μM; CC_{50} = 25 μM; SI = 7.3
Aescin	Vero E6 (HK strain)	EC_{50} = 6 μM; CC_{50} = 15 μM; SI = 2.5
Valinomycin	Vero E6 (HK strain)	EC_{50} = 0.85 μM; CC_{50} = 68 μM; SI = 80
Niclosamide	Vero E6 (Taiwan strain)	EC_{50} = 1-3 μM; CC_{50} = 250 μM
Nelfinavir	Vero E6 (FFM-1)	Mean (SD) EC_{50} = 0.048 (0.024) μM; CC_{50} = 14.75 (2.75) μM; SI = 302.1
Chloroquine	Vero E6 (FFM-1)	Mean (SD) IC_{50} = 8.8 (1.2) μM; CC_{50} = 261.3 (14.5) μM; SI = 30
	Vero E6 (Urbani)	Mean (SD) EC_{50} = 4.4 (1.0) μM; refractory to infection if pretreated with chloroquine (10 μM) for 20 h
Indomethacin	Vero E6 (Tor2)	IC_{50} = 50 μM
3C-like proteinase inhibitors		
Cinanserin (SQ 10,643)	Vero (NM)	IC_{50} = 5 μM
TG-0205221	Vero E6 (NM)	↓ viral load by 4.7 logs at 5 μM
Octapeptide AVLQSGFR	Vero (BJ01)	EC_{50} = 0.027 mg/liter; CC_{50} = >100 mg/liter; SI = >3,704

Peptidomimetic inhibitor	NM	IC_{50} = 45-70 μM
Calpain inhibitors, Val-Leu-CHO	Vero E6 (Urbani)	EC_{90} = 3 μM
Calpain inhibitors, Z-Val-Phe-Ala-CHO	Vero E6 (Urbani)	EC_{90} = 15 μM
Cyclopentenyl carbocyclic nucleosides	NM	EC_{50} = 47 μM for 1,2,3-triazole analogue (17c); EC_{50} = 21 μM for 1,2,4-triazole analogue (17a)
Nucleoside analogue inhibitor, β-d-*N*⁴-hydroxycytidine	Vero E6 (Urbani)	EC_{90} = 6 μM
Nitric oxide, *S*-nitroso-*N*-acetylpenicillamine	Vero E6 (FFM-1)	Mean (SD) IC_{50} = 222 (83.7) μM; SI = 3
Pyridine *N*-oxide derivatives	Crandel feline kidney (CRFK) and Vero (FFM-1)	Among 192 compounds tested, the oxide part on pyridine moiety was indispensable for antiviral activity with CC_{50} of 50-100 mg/liter
Stilbene derivatives	Vero E6 (NM)	Inhibited by compounds 17 and 19 at 0.5 mg/ml, and no significant cytotoxic effects were observed in vitro
Peptide-conjugated antisense morpholino oligomers (P-PMO)	Vero E6 (Tor2)	Several virus-targeted P-PMO (AUG1, AUG2, AUG3, 1AFBS, and 3UTR) consistently reduced CPE at a concn of 20 μM
20-mer synthetic peptides (S protein fragments)	FRhK-4 (GZ50)	IC_{50} = 24.9-113 μg/ml; IC_{90} = 0.9-15.9 μg/ml
Diverse small molecules,[b] MP576, HE602, and VE607	Vero (HKU39849)	EC_{50} = <10 μM
Adamantane-derived compounds[c]	FRhK-4 (NM)	IC_{50} = 0.5-3 μM; CC_{50} = >300 μM
Semisynthetic derivatives of glycopeptide		
Vancomycin	Vero E6 (FFM-1)	EC_{50} = 22->100 μM; CC_{50} = >80 μM
Eremomycin	Vero E6 (FFM-1)	EC_{50} = 14->100 μM; CC_{50} = 45->100 μM
Teicoplanin, ristocetin A, and DA-40926	Vero E6 (FFM-1)	EC_{50} = >80 μM; CC_{50} = >80 μM
***Lycoris radiata* (Chinese medicinal herb)**	Vero E6 (BJ001, BJ006)	Mean (SD) EC_{50} = 2.4 (0.2) μg/ml; CC_{50} = 886.6 (35.0) μg/ml
***Artemisia annua* (Chinese medicinal herb)**	Vero E6 (BJ001, BJ006)	Mean (SD) EC_{50} = 34.5 (2.6) μg/ml; CC_{50} = 1,035 (92.8) μg/ml
***Pyrrosia lingua* (Chinese medicinal herb)**	Vero E6 (BJ001, BJ006)	Mean (SD) EC_{50} = 43.2 (14.1) μg/ml; CC_{50} = 2,378 (87.3) μg/ml
***Lindera* sp. (Chinese medicinal herb)**	Vero E6 (BJ001, BJ006)	Mean (SD) EC_{50} = 88.2 (7.7) μg/ml; CC_{50} = 1,374 (39.0) μg/ml

- *a* i.p., intraperitoneal; EC_{50}, 50% effective concentration; SI, selectivity index; NA, not available; p.i., preincubation; NM, not mentioned; IC_{50}, 50% inhibitory concentration; $TCID_{50}$, 50% tissue culture infective dose; CI, combination index (combination index of <1 indicates synergism); CPE, cell culture cytopathic effect; aa, amino acids; RP, RNA polymerase; CC_{50}, 50% cytotoxic concentration; ↓, decreased.
- *b* MP576, HE602, and VE607 were validated to be inhibitors of SARS-CoV M^{pro}, Hel, and viral entry, respectively.
- *c* Bananin, iodobananin, vanillinbananin, and eubananin were effective inhibitors of the ATPase activity of the SCV helicase.

7.3.2. Immuno enhancement therapy

Synthetic recombinant interferon α has proven to be effective in treatment of SARS patients in clinic trials[89]. Pulmonary X-ray abnormal remission time was reduced by 50% in the interferon-treated group compared with the glucocorticoid-treated group alone. Interferon was also found to be an effective inhibitor of MERS-CoV replication[90]. Those findings suggested that interferon could be used in the treatment of COVID-19. Intravenous immunoglobulin might be the safest immunomodulator for long-term use in all ages, and could help to inhibit the production of proinflammatory cytokines and increase the production of anti-inflammatory mediators[91]. Moreover, Thymosin alpha-1 (Ta1) can be an immune booster for SARS patients, effectively controlling the spread of disease[92]. Intravenous immunoglobulin and Ta1 may also be considered as therapeutics for COVID-19.

A retrospective review analyzed 21 patients in which tocilizumab was added to standard COVID-19 therapy.(25) Preliminary data suggest tocilizumab may have clinical benefit as adjunctive therapy. Tocilizumab is Interleukin-6 (IL-6) Receptor-Inhibiting Monoclonal Antibody

that can cause Cytokine release syndrome may be a component of severe disease in COVID-19 patients[93].The Mechanism of Action of said that it inhibits IL-6-mediated signaling by competitively binding to both soluble and membrane-bound IL-6 receptors. IL-6 is a proinflammatory cytokine that is involved in diverse physiological processes such as T-cell activation, immunoglobulin secretion induction,

hepatic acute-phase protein synthesis initiation, and hematopoietic precursor cell proliferation and differentiation stimulation. IL-6 is produced by various cell types, including T- and B-cells, lymphocytes, monocytes, and fibroblasts.

7.3.3. Convalescent plasma therapy

When there are no sufficient vaccines and specific drugs, convalescent plasma therapy could be an effective way to alleviate the course of disease for severely infected patients[94]. Plasma collected from persons who have recovered from COVID-19 that may contain antibodies to SARS-CoV-2 Clinical trials are being conducted to evaluate the use of COVID-19 convalescent plasma to treat patients with severe or immediately life-threatening COVID-19 infections.Moreover, from the perspective of immunology, most of the patients recovered from COVID-19 would produce specific antibodies against the SARS-CoV-2, and their serum could be used to prevent reinfection. At the same time, antibodies can limit the virus reproduction in the acute phase of infection and help clear the virus, which is conducive to the rapid recovery of the disease[95].

Corticosteroids therapy: Corticosteroid therapy is not recommended for viral pneumonia; however, use may be considered for patients with refractory shock or acute respiratory distress syndrome.

7.3.4. Vaccine Development

Vaccines that have been developed are either not effective, or in some cases have been reported to be involved in the selection of novel pathogenic CoVs via recombination of circulating strains Vaccine development can be a challenge. It is noteworthy that almost 20 years after SARS, there is still no vaccine for coronavirus. After SARS, development of a vaccine appeared to be the best approach to prevent future SARS-CoV epidemics. However, there were many obstacles in SARS vaccine development. Firstly, researchers did not have a comprehensive understanding of the pathogenic mechanism of SARS-CoV. Secondly, animal models of SARS-CoV infection could not simulate human disease because of an incongruent pathogenesis. Thirdly, in order to test the efficacy, many people must be tested in areas where the virus is endemic. Although several candidate vaccines against SARS-CoV have been produced and tested, at present, unfortunately, there is no FDA approved vaccine against SARS[96].

8. Rigorous infection control

1. Contact tracing, strict isolation of actively ill patients and quarantine of close contacts should be implemented early[97].
2. Environmental hygiene in medical sectors and personal hygiene of health care workers should be maintained[98, 99].
3. Training in the use of personal protective equipment protects the safety of HCWs[100].
4. Establishing fever clinics, setting up designated hospital wards and SARS hospitals reduced human-to-human transmission[101].
5. Education of the public on communicable diseases and what measures to take on a personal basis to prevent spread.

9. Conclusion and Recommendation

Looking ahead, the most feasible options that should be further evaluated in clinical trials for the ongoing Covid 19 epidemic. The COVID-19 pandemic is spreading across the globe at an alarming rate. It has caused more infections and deaths as compared with SARS or MERS. The rapid spread of disease warrants intense surveillance and isolation protocols to prevent further transmission. No confirmed medication or vaccine has been developed. Current treatment strategies are aimed at symptomatic care and oxygen therapy. Prophylactic vaccination is required for the future prevention of COV-related epidemic or pandemic. To reduce the risk of transmission in the community, individuals should be advised or recommended to wash hands diligently, practice respiratory hygiene (eg, cover their cough), and avoid crowds and close contact with ill individuals, if possible. Facemasks are not routinely recommended for asymptomatic individuals to prevent exposure in the community. Social distancing is advised, particularly in locations that have community transmission. The strict control of cross-infection in medical institutions is also key to preventing the further spread of the epidemic.

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