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# Present therapeutic and diagnostic approaches for SARS-CoV-2 infection

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## 19.1 Introduction

Coronavirus disease-2019 (COVID-19) disease is caused by the novel severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) and was declared as pandemic on March 11, 2019 by the World Health Organization (WHO) (Cucinotta & Vanelli, 2020). The COVID-19 infection started in China (Wuhan city) and later spread to every country in the world (Maxmen, 2021). The major challenge in controlling this virus is the rapid mutation it undergoes that gives rise to multiple genetic variants. According to the CDC website, there are five variants of interest (VOI) and four variants of concern identified till date. Variants of high consequence are yet to be reported and will be updated as soon as it is identified in any part of the world (SARS-CoV-2 Variant Classifications and Definitions, 2021). Recently, B.1.617 variant of SARS-CoV-2 spread is creating havoc in India in terms of spread and fatality rates. This strain was classified as VOI by WHO on May 10, 2017 (SARS-CoV-2 Variant Classifications and Definitions, 2021).

There are mainly two ways of combating the virus, that is, prevention and treatment. One means of prevention is restricting viral entry into our body using social distancing, wearing masks, avoiding gathering, etc. (Kretzschmar et al., 2021). The other means of preventing the disease is by neutralizing the virus once it makes entry into the body, that is, by vaccines. A lot of success has been achieved in the development of a vaccine for COVID-19. Currently, there are five vaccines that are approved for emergency use throughout the world by various regulatory agencies. They have been able to curtail the

infection from spreading to a considerable extent in countries like the United States and United Kingdom (Wouters et al., 2021).

Treatment of COVID-19 assumes significance when all the preventive measures fail to restrict the virus from entering an individual's body and the immunity developed is not sufficient for the elimination of the virus. There is a multifactorial challenge involved in finding the right therapy for this disease. Conventional drug discovery programs take an average of 15–18 years for a drug to be marketed (Mohs & Greig, 2017). Considering the intensity of the pandemic, this approach is not feasible at the moment. Furthermore, the rapid mutations the virus is undergoing (nine mutant varieties identified in 1 year) are also detrimental in the discovery of chemical entities that will act against the virus. Till date, only remdesivir is the only antiviral regulatory body's authorized drug for the treatment of COVID-19, which has shown a maximum success rate (Ita, 2021; Salian et al., 2021). The rest of the treatment majorly involves the management of symptoms associated with the disease. In addition to this, there are regulatory challenges, selection of dosing regimens for repurposing a drug, and overcoming problems associated (social, economic, and logistics) for conducting a clinical trial (Shi et al., 2021).

In addition to prevention and treatment, the diagnosis of COVID-19 also played a crucial role in the first wave of the pandemic. Diagnostic tests played an important role in preventing the further spread of COVID-19 infection by enabling the quick implementation of control measures that limit the spread via isolation and contact tracing of COVID-19 positive patients. A test that can accurately confirm the presence of a virus within minutes is the need of the hour. A rapid identification test will also lead to faster administration of drugs, which will speed up the recovery time of the patient (Chen et al., 2021; Yüce et al., 2021).

In this book chapter, we will discuss the various types of therapeutic approaches applied for the treatment of COVID-19 patients. There are more than 4000 drugs in clinical trials for treating this dreadful disease. It will be beyond the scope of this chapter to discuss all of them. Hence, we will be summarizing the types of COVID-19 treatment approved by the regulatory authorities across the globe along with their advantages and disadvantages. A detailed review of the diagnostic tests approved is also presented in this chapter. Special emphasis is also given to the advances made in the development of self-test kits and diagnostic tests that can identify the presence of viruses in minutes. In summary, the book chapter will be helpful for designing and implementing various strategies to deal with the current COVID-19 pandemic in terms of treatment and diagnosis.

## 19.2 Therapeutic approaches for the treatment of Coronavirus disease-2019

An effective cure for COVID-19 disease is still elusive for scientists worldwide. As discussed in the previous section, there are multiple challenges involved in the discovery of a successful treatment for COVID-19. Considering the huge number of molecules in clinical trials, it is very difficult to classify them based on any criteria like chemical, pharmacological, degree of success, etc. Therefore, we will review the drugs which are currently approved for use by various regulatory bodies worldwide. Such drugs can be grouped as follows:

1. drugs for symptomatic treatment;
2. immunotherapy-based therapy;
3. antiviral agents;
4. adjuvant therapy.

### 19.2.1 Drugs for symptomatic treatment

The most common symptoms associated with COVID-19 are fever, body ache, headache, and weakness. Paracetamol is the drug of choice with a maximum dose of 3000 mg per day. The WHO recommends that either paracetamol or ibuprofen can be used as an antipyretic in COVID-19 patients without any significant adverse effects [[The Use of Non-Steroidal Anti-Inflammatory Drugs \(NSAIDs\) in Patients with COVID-19, 2021](#)]. There is no conclusive proof that multivitamins improve fatigue conditions in patients. The second major common symptom is dry cough, which is recommended to be treated by over-the-counter cough suppressants. The OTC analgesics are used for the treatment of sore throat according to its severity [[Managing COVID-19 Symptoms \(Including at the End of Life\) in the Community: Summary of NICE Guidelines, 2020](#)]. A less severe symptom affecting the small COVID-19 infected individuals is loss of smell and/or taste. These olfactory dysfunctions are treated when they persist beyond 2 weeks. The treatment involves repeated inhalation of a set of common odorants like rose, jasmine, lemon, eucalyptus, etc. for 20–30 seconds 2–4 times a day until the condition improves ([Whitcroft & Hummel, 2020](#)). A very rare symptom affecting less than 0.5% of patients is skin rash and discoloration of fingers/toes. This feature is cured without any drug treatment. In severe cases, a dermatologist can be consulted. Recently, a few strains of the virus show diarrhea as one of the symptoms. There is no drug therapy recommended for the same and is treated by WHO-approved oral rehydration salts [[D’Amico et al., 2020](#); [Managing COVID-19 Symptoms \(Including at the End of Life\) in the Community: Summary of NICE Guidelines, 2020](#)]. The details of the drugs used for symptomatic treatment are presented in [Table 19.1](#). The most severe symptom is low blood oxygen levels leading to shortness of breath and/or chest pain. Oxygen cylinders or oxygen concentrators can be used to increase the oxygen level of an individual. The advantage of an oxygen concentrator over the oxygen cylinder is that it will never run out of gas as long as there is an uninterrupted power supply. However, these concentrators need to be used under medical supervision as an excess of oxygen can lead to toxicity, leading to death. In critical cases, patients are subjected to artificial ventilation ([Pulse Oximeters and Oxygen Concentrators: What to Know About At-Home Oxygen Therapy, 2021](#)).

### 19.2.2 Immunotherapy-based therapy

Immunotherapy utilizes a patient’s immune system to fight viruses from the body. A considerable amount of success has been achieved by this therapeutic strategy for the treatment of cancer and viral infections. Immunotherapy works by boosting a patient’s immune system by increasing the level of the desired antibody and/or immune cells. The major challenge in the discovery of novel immunotherapy against SARS-CoV-2 infection is

TABLE 19.1 Drugs used for the management of Coronavirus disease-2019.

Drug name/ treatment	Therapeutic category/chemical category	Purpose in COVID-19	Target	Mechanism of action	Previous usage	Approval status for COVID-19 treatment
Paracetamol	NSAIDs/amino phenol	Antipyretic	COX	Inhibits release of prostaglandins responsible for increase in body temperature	Antipyretic	Approved by all regulatory agencies
Chlorpheniramine maleate <sup>a</sup>	Antihistaminic/ propyl amines	Cough suppressants	H1 histamine receptor	Inhibits release of histamine which is one of the mediators for cough	Allergic condition, antitussive	Approved by all regulatory agencies
Levodropropizine <sup>a</sup>	Antitussives/ phenyl piperazine	Cough suppressants	H1 histamine receptor and $\alpha$ -adrenergic receptors	Inhibits airway sensory nerves and thereby in vitro release of neuropeptides from C-fibers is stopped	Antitussive	Approved by all regulatory agencies
Odorants (rose, lemon, eucalyptus, etc.)	Odorants/terpenes, essential oils	Treatment of olfactory dysfunction	Olfactory receptors	Increased cAMP level due to G-protein activation of adenylyl cyclase. This causes opening of ion channel and efflux of $\text{Cl}^-$ and influx of $\text{Ca}^{2+}$ in cilia, thereby leading to depolarization of olfactory neuron	Treatment of olfactory dysfunction	Approved by all regulatory agencies
ORS	Fluid replenishers/ glucose and inorganic salts	Treat Diarrhea	No target	stimulate Na and fluid absorption in the small intestine via a cyclic AMP- independent process	Treat dehydration	Approved by all regulatory agencies
Convalescent plasma	Immunotherapeutic agent/	Neutralizes SARS-CoV-2	No target	Antibodies present in the plasma will eliminate the virus	Treat diphtheria, scarlet fever and pertussis	Approved by all regulatory agencies
Bamlanivimab	immunotherapeutic agent/Polypeptides	Neutralizes SARS-CoV-2	CD6 receptor and overlapping epitope in the RBD of S protein	Blocks T lymphocyte activation, leading to suppression of inflammatory mediators. Blocks virus attachment into the human cell	Not used	Approved by USFDA and later revoked
Regdanvimab	immunotherapeutic agent/Polypeptides	Neutralizes SARS-CoV-2	RBD of SARS-CoV-2 S1 spike protein	Blocks virus attachment into the human cell	Not used	Approved in Republic of Korea and Europe

Etesevimab	Immunotherapeutic agent/polypeptides	Neutralizes SARS-CoV-2	overlapping epitope in the RBD of S protein	Blocks virus attachment into the human cell	Not used	Approved by all regulatory agencies
Casirivimab	Immunotherapeutic agent/polypeptides	Neutralizes SARS-CoV-2	Nonoverlapping epitope in the RBD of S protein	Blocks virus attachment into the human cell	Not used	Approved by all regulatory agencies
Imdevimab	Immunotherapeutic agent/polypeptides	Neutralizes SARS-CoV-2	Nonoverlapping epitope in the RBD of S protein	Blocks virus attachment into the human cell	Not used	Approved by all regulatory agencies
Tocilizumab	Immunotherapeutic agent/polypeptides	Neutralizes SARS-CoV-2	IL-6 receptor	Blocking of IL-6 binding, leading to immunosuppression	Not used	Approved by all regulatory agencies
Remdesevir	Antivirals/purine nucleotide analog	Inhibits growth of virus	RdRp	Replaces adenine nucleotides in the RdRp and inhibits RNA synthesis	Not used	Approved by all regulatory agencies
Favipiravir	Antivirals/purine analog	Inhibits growth of virus	RdRp	Prodrug of guanine analog, which binds to RdRp instead of guanine nucleotides and inhibits RNA synthesis	Influenza	Approved in India and European countries
Chloroquine/hydroxychloroquine (HCQ)	Antimalarial/quinolones	Inhibits growth of virus	ACE receptor of host cell	Prevents fusion of the virus with host cell. Transport of SARS-CoV-2 from early endosomes to endolysosomes	Malaria, amebiasis systemic HCQ for lupus erythematosus, rheumatoid arthritis	Approved by USFDA and retracted
Ivermectin	Antiparasitic/avermectin class	Cytotoxic to the virus	Importin alpha/beta-1 nuclear transport proteins	Prevents entry and exit of viral proteins from host nucleus, thereby Leading to cessation of viral replication	Onchocerciasis, strongyloidiasis, soil-borne helminthiasis and scabies	Approved in India
Lopinavir/ritonavir	Antiretroviral/peptidomimetic	Inhibits viral growth	3CLpro and PLpro	Cleavage of viral polyproteins is inhibited, leading to cessation of transcription and viral replication	AIDS	Approved by USFDA and retracted
Zinc	Essential minerals/inorganic substance	Cytotoxic to virus	RdRp	Binds to aspartic acid triad, 618D, 760D, and 761D and inhibits the enzyme, thereby suppressing the replication process	Daily supplement	Approved in India

(Continued)

TABLE 19.1 (Continued)

Drug name/ treatment	Therapeutic category/chemical category	Purpose in COVID-19	Target	Mechanism of action	Previous usage	Approval status for COVID-19 treatment
2-Deoxy-D-glucose	Antineoplastic agent/glucose analog	Cytotoxic to virus	Hexokinase and phosphoglucoisomerase	Inhibits glycolysis that leading to depletion of ATP within the virus and subsequently viral cell lysis	Not used	Approved in India
Doxycycline	Antibiotics/ tetracyclines	Bacteriostatic	50S ribosomal subunit of bacteria	Inhibits bacterial protein synthesis and thereby preventing growth of bacteria	Rheumatoid arthritis, Asthma, severe allergies, a few types of cancer	Approved in all countries
Azithromycin	Antibiotics/ macrolides	Bacteriostatic	50S ribosomal subunit of bacteria	Inhibits bacterial protein synthesis and thereby preventing growth of bacteria	Bacterial infections	Approved in all countries
Dexamethasone	Antiinflammatory/ steroids	Antiinflammatory	Glucocorticoid receptors	Inhibits translation of pro-inflammatory molecules	Rheumatoid arthritis, Asthma, severe allergies, a few types of cancer	Approved in all countries
Low molecular weight Heparin	Anticoagulant/ polysaccharide	Anticoagulant	Thrombin and antithrombin III	Inactivates thrombin and coagulation factor Xa, which leads to less fibrin generation and finally clot is not formed	Thrombosis, pulmonary embolism	Approved in all India
Vitamin C	Vitamins/furanose	Antiinflammatory	No specific target	Scavenges free radicals and prevents vascular/tissue injury	Scurvy, supplements	Approved in all India
Vitamin D3	Vitamins/ secosteroid	Ta boost immune system	D3 receptor on immune cells	Decreased production of inflammatory cytokines and increased production of antiinflammatory cytokines	Osteoporosis, Rickets, supplements	Approved in India

<sup>a</sup>The most widely used drugs for the treatment of cough are presented.

3CL<sup>pro</sup>, 3 Cysteine-like protease; ACE, angiotensin-converting enzyme; AIDS, acquired immune deficiency syndrome; COX, cyclooxygenase; HCQ, hydroxychloroquine; ORS, oral rehydration salt; PL<sup>pro</sup>, papain-like protease; RBD, receptor-binding domain; RdRp, RNA-dependent RNA polymerase; SARS-CoV-2, severe acute respiratory syndrome-Coronavirus-2; USFDA, United States Food and Drug Administration.

the dysfunctional and inadequate immune response to viral infections (AminJafari & Ghasemi, 2020; Masoomikarimi et al., 2021). Additionally, immune system invasion by SARS-CoV-2 also leads to an event of the exaggerated immune response, known as a “cytokine storm” (Mustafa et al., 2020). Hence, multiple immunotherapeutic approaches are applied for COVID-19 treatment, which is discussed underneath.

### **19.2.2.1 Convalescent plasma therapy**

Convalescent plasma therapy is a type of passive immunity which involves transfusing plasma of a person who has recovered from the disease to a critically ill COVID-19 patient who has impaired immunity (Pau et al., 2021; Simonovich et al., 2021; Wang et al., 2021). It has been successful in treating diphtheria, scarlet fever, and pertussis Table 19.1. In the case of Spanish flu and SARS, it has shown mixed responses. USFDA approved this therapy on April 3, 2020 with the following conditions (Sahu et al., 2020):

1. The donor should show no symptoms a minimum of 28 days before donation.
2. The donor should not show symptoms 14 days before donation and show a negative COVID-19 reverse transcription-polymerase chain reaction (RT-PCR) test from a nasopharyngeal swab.
3. The antibody titer level should be 1:160.
4. The ratio of partial pressure of arterial oxygen ( $\text{PaO}_2$  in mmHg) to fractional inspired oxygen ( $\text{FiO}_2$  expressed as a fraction) of patients should be less than 300.
5. Patients with dyspnea with respiratory rates more than or equal to 30 per minute and blood oxygen saturation, 93%.
6. The appearance of more than 50% lung infiltrates in patients within 24–48 hours.

The therapy has been successful in treating many critically ill patients. However, it has failed in many circumstances. The major challenges with this therapy are the adverse reactions post-infusion, large doses of plasma, and low antibody titer levels in recovered patients. In addition to this, donor availability and willingness, storage/transportation of plasma concentrates, and apheresis center capacity also hampers its timely and proper implementation (Altuntas et al., 2021; Pau et al., 2021).

### **19.2.2.2 Monoclonal antibodies**

Monoclonal antibodies (mAbs) are a group of antibodies that are produced against a particular epitope of an antigen by a specific set of B cells. They have been successfully used to treat cancer, autoimmune disorders, and a few infectious diseases. In the case of SARS-CoV-2 the epitope is the S1 spike protein that aids in virus attachment to the host cell via angiotensin-converting enzyme 2 (ACE2), leading to viral entry (Du et al., 2009; Taylor et al., 2021). They were able to reduce symptoms and lessen disease progression in patients with mild to moderate COVID-19 infection. The mAbs were found to be more effective for patients who have not developed antibodies against the virus (Owji et al., 2020; Taylor et al., 2021; Wang et al., 2020a,b). Initially, the FDA approved a combination of 700 mg Bamlanivimab/1400 mg etesevimab and 1200 mg Casirivimab/1200 imdevimab as emergency use authorization (EUA) to treat nonhospitalized patients who have the risk of disease progression. Recently, the FDA revoked EUA approval for Bamlanivimab as many strains of the virus were becoming resistant to the mAb (COVID-19 Treatment



[Guidelines, 2021](#)). Imdevimab binds the S protein receptor-binding domain (RBD) from the left side of the lower end, while casirivimab binds to the receptor from the top. In the case of Bamlanivimab, it binds an epitope on the RBD in both its open and closed conformation, whereas Etesevimab reversibly binds to the active conformation of the RBD ([Taylor et al., 2021](#)).

Bamlanivimab, an anti-CD6 IgG1 monoclonal antibody was approved in India for emergency use for the treatment of mild to moderate COVID-19 patients ([Eli Lilly Ties up with MSN Labs, Torrent Pharma for Sale of Covid-19 Drug Baricitnib in India, 2021](#)). It binds to the CD6 receptor and blocks the T lymphocyte activation, which in turn suppresses the pro-inflammatory cytokines and decreases the cytokine storm. It was successfully used in 2013 for the treatment of psoriasis and has is an example of repurposed anti-SARS-Cov-2 therapy ([COVID-19 Treatment Guidelines, 2021](#); [Kaplon & Reichert, 2021](#); [Kumar et al., 2021](#)).

Tocilizumab is another IL-6 receptor antagonist monoclonal antibody which is FDA authorized to cure COVID-19 patients with systemic inflammation and respiratory failure due to low levels of oxygen. The rationale for using this monoclonal antibody is that there is over secretion of IL-6 by bronchial epithelial cells in severe COVID-19 illness ([Zhang et al., 2008](#)). A single intravenous dose of 8 mg/kg body weight, not exceeding 800 mg, along with dexamethasone (6 mg daily for up to 10 days) is recommended by the FDA for the few hospitalized patients within the first 3 days of admission ([COVID-19 Treatment Guidelines, 2021](#)).

Regdanvimab is an anti-SARS-CoV-2-spike monoclonal antibody approved in the Republic of Korea for treating COVID-19 patients exhibiting mild to moderate symptoms [Drug information of Regdanvimab, Korean Ministry of Food and Drug Safety ([960mg \(Regdanvibam\) \(monoclonal antibody, recombinant gene\), 2021](#))]. Regdanvimab has been developed by selecting the most potent neutralizing antibody against RBD of SARS-CoV-2 S1 spike protein. A phage library was constructed by cloning the antibody variable regions from peripheral blood mononuclear cells isolated from a convalescent patient's blood. The scFv-Fc and the full-length IgG were then cloned and produced in CHO cells ([Kim et al., 2021](#)). A single intravenous dose of 40 mg/kg body weight in 90 minutes for patients above age 18 is recommended by the Korean Ministry of Food and Drug Safety.

Several immunotherapeutic agents like interferons, NK cells, anti-IL-6 mAbs (siltuximab), other mAbs, immunoglobulins, and active immunity approaches like the BCG vaccine are undergoing clinical trials worldwide. The results of these trials will guide future approval processes and determine the success of approaches in the treatment of COVID-19 infection ([Chen et al., 2020](#); [COVID-19 Treatment Guidelines, 2021](#); [Shetty, 2020](#)). The details of the antibodies approved by regulatory agencies used are highlighted in in [Table 19.1](#).

### 19.2.3 Antiviral agents

Drugs cytotoxic to the virus are the most desired therapeutic approach among medical practitioners. However, these agents demonstrate many side effects because they fail to distinguish between viruses and normal cells. Hence, the most effective target for the development of such drugs will be the one that is exclusively present in viral cells and/or overexpressed in them as compared to human cells. It is very difficult to find such a

macromolecular target that can guide antiviral drug discovery. Hence, an attempt is made to target the amino acid sequence of a protein's catalytic domain, which differs from the analogous human proteome (Adamson et al., 2021). The other criterion for discovering novel antiviral therapeutics is to target the viral replication process because the majority of the clinical manifestations of COVID-19 are due to the replication of SARS-CoV-2. The antiviral drugs act by preventing viral entry into the host cell via ACE2 receptor and transmembrane serine protease 2 (TMPRSS2). Since the virus is dependent on the host cell for its replication, it cannot proliferate further once its entry into the host cell is blocked. The other means of restricting viral replication is by targeting a protease enzyme and the RNA-dependent RNA polymerase (RdRp) enzyme. SARS-CoV-2 releases two proteases, that is, papain-like protease (PL<sup>Pro</sup>) and the 3-chymotrypsin-like protease (3CL<sup>Pro</sup>), which are equally crucial for the viral replication process. Additionally, PL<sup>Pro</sup> extracts ISG15 from host-cell proteomes to aid the virus in dodging innate response (Kandeel et al., 2021). An RdRp enzyme is required for replication and transcription of SARS-CoV-2. Hence, this enzyme is essential for the survival of the virus (Salian et al., 2021). The conserved catalytic domain of the enzyme makes it an attractive target in the discovery of antiviral drugs. The drugs (Table 19.1 and Fig. 19.1) which are approved for treating COVID-19 by regulatory agencies worldwide, along with the ones which were withdrawn after approval, are discussed under the following subsections.

### 19.2.3.1 Remdesevir

Remdesevir is an adenine nucleotide analog RdRp inhibitor that acts as a viral multiplication suppressor via premature cessation of RNA transcription (Kokic et al., 2021). Till date, it is the only FDA-approved antiviral drug for the treatment of COVID-19 in hospitalized patients. Additionally, it is authorized for emergency use to treat children below 12 years. The major disadvantage is that the patient needs hospitalization for the administration of the drug as it can be given by intravenous route only. However, it has not been able to significantly reduce the mortality rate (up to 3%) of COVID-19 patients (COVID-19 Treatment Guidelines, 2021). Nevertheless, till date, it is the best possible option amiable with doctors to treat a COVID-19 patient because it has tremendously improved the recovery time in critically ill COVID-19 patients.

### 19.2.3.2 Favipiravir

It is a small molecule RdRp inhibitor that is a purine nucleotide analog. Inside the tissues, it undergoes phosphoribosylation to its active form, favipiravir-ribonucleotide phosphate, which binds to the RdRp instead of purine nucleotides and thus inhibiting the enzyme activity (Furuta et al., 2017). Further, it has been found to induce lethal mutagenesis in vitro during influenza virus infection, making it a virucidal drug (Baranovich et al., 2013). It has been approved in India with a dose of 1800 mg twice daily on the first day of treatment, followed by 800 mg twice daily up to a total treatment course of 14 days. The advantage of this drug is that it can be administered orally and does not require hospitalization like remdesevir. However, it has not been effective in hospitalized patients with life-threatening symptoms (Dabbous et al., 2021).

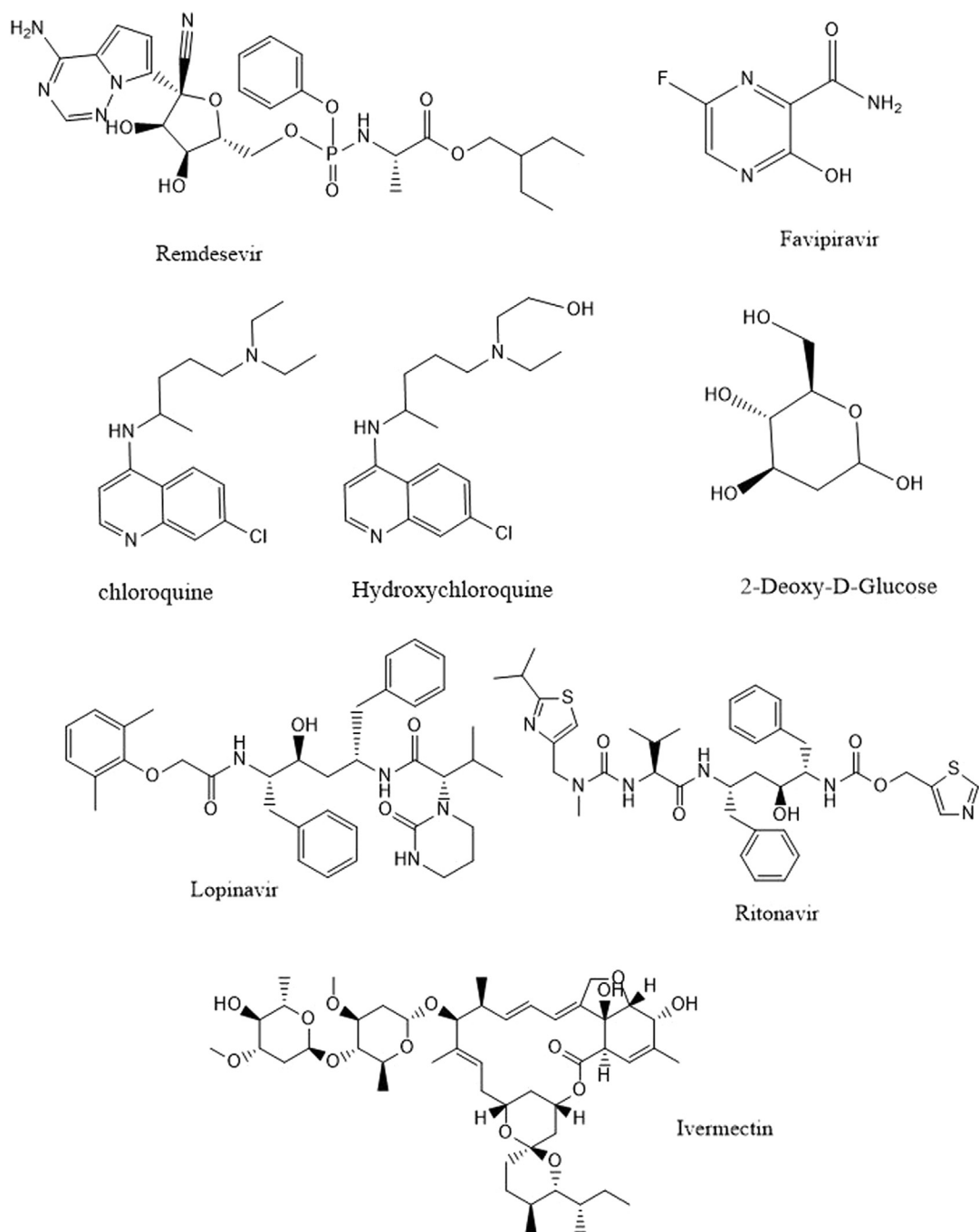


FIGURE 19.1 Structure of antiviral drugs used for the treatment of Coronavirus disease-2019.

### 19.2.3.3 Chloroquine/hydroxychloroquine

Chloroquine is originally marketed for the treatment of malaria. Its analog, hydroxychloroquine, was discovered as a less toxic alternative and demonstrated fewer drug–drug interactions. Additionally, hydroxyl chloroquine is approved for systemic lupus erythematosus and rheumatoid arthritis treatment (Oren et al., 2020; Rolain et al., 2007). They act by inhibiting the glycosylation of the host-cell ACE receptor, which hinders the binding of the virus to the cell. Additionally, they increase the endosomal pH, which prevents the fusion between SARS-CoV-2 and the host cell. Further, they also block the transport of SARS-CoV-2 from early endosomes to endolysosomes and thus prevent the release of the viral genome (Liu et al., 2020; Vincent et al., 2005; Wang, et al., 2020a,b). However, hydroxychloroquine was not effective in significantly reducing viral load in patients with or without azithromycin. Initially, the FDA-approved hydroxychloroquine as emergency use approval (EUA) for prophylaxis of healthcare workers in particular and treatment of hospitalized patients. Later, it was observed that its severe cardiac adverse effects and other toxicity outweigh its clinical benefits. Therefore, the regulatory bodies revoked its EUA approval for hydroxychloroquine (Bessière et al., 2020; COVID-19 Treatment Guidelines, 2021).

### 19.2.3.4 Ivermectin

Ivermectin is a Nobel Prize–winning antiparasitic drug which is used in the treatment of onchocerciasis, strongyloidiasis, helminthiasis, and scabies. In 2014, Gairdner Global Health Award was given for the discovery, development, and use of ivermectin and it is one of the drugs on the WHO essential medicines list (Formiga et al., 2021). It has shown promising results as an antiviral agent in various in vitro studies. However, it has not been approved for the treatment of any viral infections by the FDA and EMA outside clinical trials (COVID-19 Treatment Guidelines, 2021). In India and some developing countries, it is used to treat COVID-19 patients with mild symptoms at a dose of 12 mg, once daily, for 5 days (Clinical Guidance for Management of Adult COVID-19 Patients, 2021). The proposed mechanism of antiviral action of ivermectin is by suppression of importin alpha/beta-1 nuclear transport proteins. Experimental studies proved that the drug binds to host importin alpha, which prevents its association with importin beta-1 subunit and/or viral polypeptides that are transported by the carrier protein. This prevents the viral proteins from altering transcription and antagonizing the host's antiviral response (Caly et al., 2020; Martin & Jans, 2021). Further, it was shown by docking studies that ivermectin binds to a spike protein's ACE2 RBD. Practical studies need to be carried out to prove this hypothesis (Lehrer & Rheinstein, 2020). Moreover, the antiinflammatory properties of the drug can act as a booster for its antiviral action (Dinicolantonio et al., 2021; Portmann-Baracco et al., 2020).

### 19.2.3.5 Lopinavir/ritonavir

The success of a protease inhibitor in treating AIDS stimulated researchers to use the inhibitors of this protein as repurposed drugs to cure COVID-19. The multiplication of the SARS-CoV-2 virus is dependent on the breakage of polyproteins into an RdRp and a helicase by proteases. Two proteases are responsible for this

cleavage: 3-chymotrypsin-like protease (3CL<sup>Pro</sup> or M<sup>Pro</sup>) and papain-like protease (PLpro). There is a remarkable difference between the proteases of HIV and SARS-CoV-2. The HIV protease is a homodimer aspartyl protease that has two identical subunits of 99 amino acids each. The active site is located at the junction of two monomers, comprising Asp–Thr–Gly catalytic residues. 3CLpro or M<sup>Pro</sup>, on the other hand, is a cysteine protease comprising 306 amino acids, wherein His41 and Cys145 form the catalytic dyad. PLpro consists of 317 amino acids where the catalytic triad is C111, H273, and D293. Further, it was found that there are 96% and 86% similarities between 3CLpro and PLpro of SARS-CoV-2 and SARS-CoV, respectively (Ancy et al., 2020; Mahdi et al., 2020; Nutho et al., 2020). In spite of the difference between the amino sequence of SARS-CoV and HIV protease, HIV's protease inhibitors are effective in the treatment of patients suffering from SARS (Yamamoto et al., 2004). Moreover, the HIV protease inhibitors have shown promising results against similar analogs of *Leishmania* and *Plasmodium falciparum* (Sonoiki et al., 2017; Valdivieso et al., 2010). Therefore, the use of protease inhibitors for treating COVID-19 has been justified because of its success against SARS, Leishmaniasis, and malaria. It was initially approved as restricted public health emergency use in India. Later clinical data revealed that there is no benefit in its use for the treatment of COVID-19 patients (Bhattacharyya et al., 2020). It was never approved for use by FDA in any form and CDC, the United States recommends against the use of these drug combinations for treating COVID-19 patients (COVID-19 Treatment Guidelines, 2021).

#### 19.2.3.6 Zinc

Zinc has been revealed to increase cytotoxicity and induce apoptosis when used with a zinc ionophore like chloroquine/hydroxychloroquine. Experimental studies also proved that elevated intracellular zinc inhibits RNA virus replication. The antiviral effect of zinc can be attributed to its ability to suppress the RdRp enzyme (Suara & Crowe, 2004; te Velthuis et al., 2010; Zoghi et al., 2021). These studies prompted health authorities to permit investigation of zinc alone or in combination with hydroxychloroquine for the prevention and/or treatment of COVID-19. A dose not exceeding 50 mg of elemental zinc, twice daily, is allowed in clinical trials (te Velthuis et al., 2010; Thomas et al., 2021; Yao et al., 2021). However, the results of these studies are inconclusive to conclude recommending zinc as a treatment strategy. Further, zinc should be used with caution because long-term zinc intake as supplements can lead to the copper deficiency that causes severe hematologic and neurological adverse effects. Additionally, oral zinc decreases the absorption of polyvalent cation binding drugs (Kumar, 2006; Myint et al., 2018; Zinc Fact Sheet for Health Professionals., 2021). The harmful effects of long-term intake of zinc led regulatory bodies to recommend against using zinc supplementation above the recommended dietary allowance for the prevention of COVID-19, except in a clinical trial (COVID-19 Treatment Guidelines, 2021). However, ICMR of India has approved the usage of zinc (11 mg for men and 8 mg for women per day) as supportive management therapy for nonhospitalized patients (Clinical Guidance for Management of Adult COVID-19 Patients, 2021).

### 19.2.3.7 2-Deoxy-D-glucose

The synthetic glucose analog, 2-deoxy-D-glucose (2-DG) was developed as an anticancer agent based on the principle that nutrient and energy deprivation can have a cytotoxic effect on cells. It is a competitive inhibitor of the hexokinase enzyme of the glycolytic pathway, wherein it gets converted to 2-deoxy-D-glucose-6-phosphate (2-DG6P). Thereafter, the phosphorylated derivative of the drug molecule competitively binds to glucose-6-phosphate isomerase, an enzyme involved in the second step of glycolysis, where it converts glucose-6-phosphate to fructose-6-phosphate. This action of the 2-DG inhibits glycolysis and stops the generation of ATP within a cell, leading to cell death. It has been experimentally proved that cancer cells and SARS-CoV-2 demonstrate greater sensitivity to glucose deprivation-induced cytotoxicity than normal cells. The 2-DG treatment completely blocked SARS-CoV-2 replication and virus-induced ACE2 and IL-1b expression. Additionally, TNF- $\alpha$ , IL-6, and IFN  $\alpha/\beta/\lambda$  expression are blocked in SARS-CoV-2 infected monocytes treated with 2-DG (Codo et al., 2020; Pajak et al., 2020). These studies make this cheap, readily available, and easily administrable molecule (the drug comes in powder form in a sachet, which is taken orally by dissolving it in water) an attractive candidate for testing in COVID-19 patients. In clinical trials carried out in India, this drug showed quicker recovery of hospitalized patients and reduces patients' dependence on supplemental oxygen (A randomized, two treatment group clinical study to evaluate the effectiveness and safety of study drug 2-deoxy-D-glucose with SOC compared to soc alone in treatment of moderate to severe COVID-19 patients, 2021; DCGI Approves Anti-COVID Drug Developed by DRDO for Emergency Use, 2021). The molecule was developed by Defense Research and Development Organization (DRDO), Government of India, in collaboration with Dr. Reddy's Laboratories Limited, India. The Drugs Controller General of India (DCGI) has given emergency use approval for its generic drug as an adjunct therapy (90 mg/kg body weight/day) in moderate to severe COVID-19 patients (DCGI Approves Anti-COVID Drug Developed by DRDO for Emergency Use, 2021).

## 19.2.4 Adjuvant therapy

Adjuvant therapy plays a crucial role in the management of a disease. In COVID-19, the adjuvant therapies augment the effect of the antiviral drug and speed up the recovery of patients (Teimury & Khaledi, 2020). The various adjuvant treatment options for COVID-19 infection, approved by regulatory agencies worldwide are discussed under the following subsections and summarized in Table 19.1.

### 19.2.4.1 Antibiotics

Antibiotics were also considered as one of the strategies to combat this horrifying virus. The basis for administering them in COVID-19 is the same as that of their use in other viral infections, that is, to prevent opportunistic infections by bacteria and other viruses. Further, this approach helps the immune system be more tuned to fight exclusively against the virus (Ghazi et al., 2016). Doxycycline and azithromycin (Fig. 19.2) are the two antibiotics that have been prescribed in a few countries by doctors for the management of COVID-19 infection. Doxycycline belongs to the tetracycline class of antibiotics which

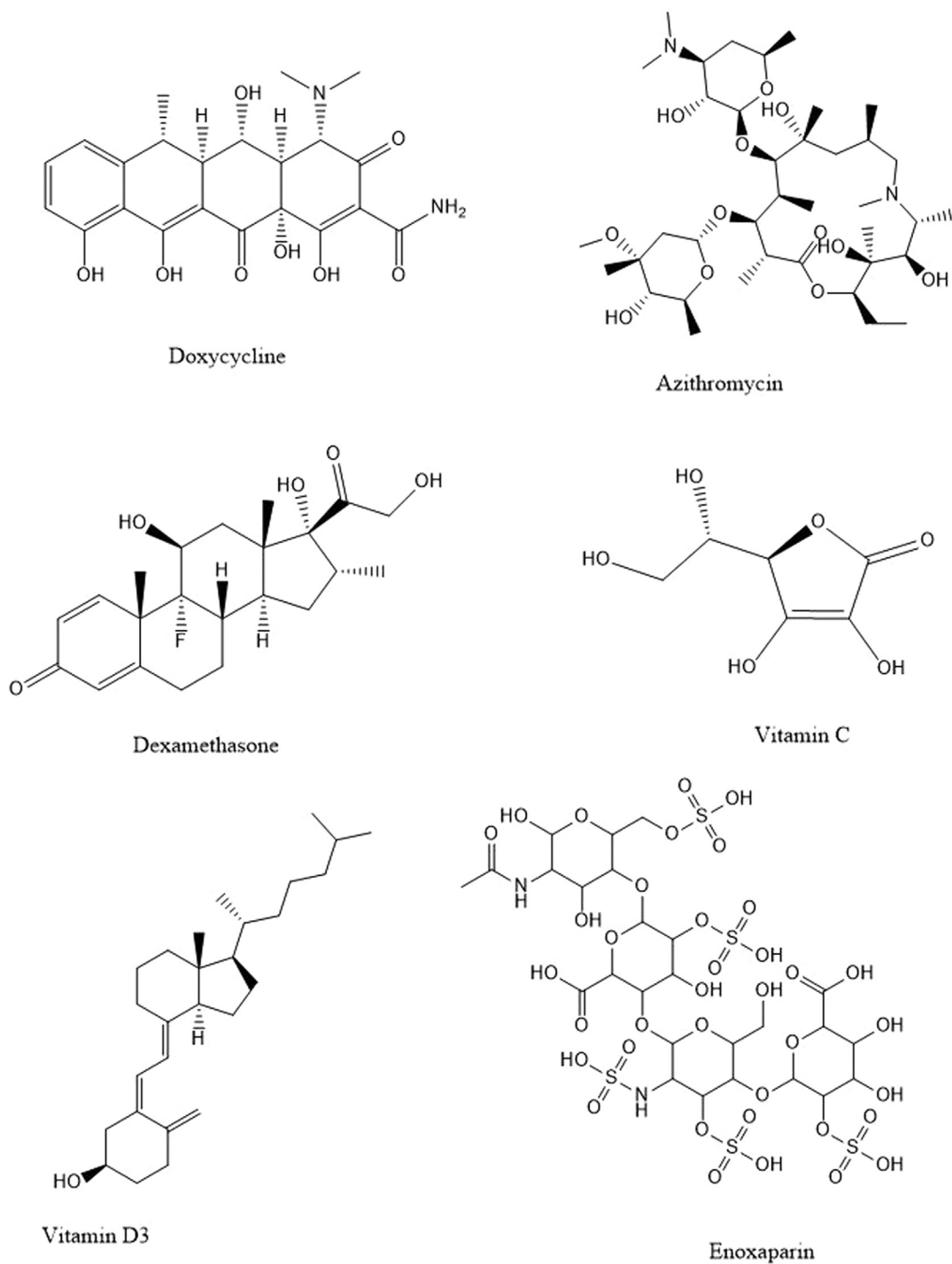


FIGURE 19.2 Structure of drugs used as adjuvant therapy in Coronavirus disease-2019.

exerts its bacteriostatic action by binding to the 30S ribosomal subunit and thereby inhibiting protein synthesis in bacteria (Gielen et al., 2010). In India, ICMR protocol suggests the use of 5 BD doxycycline tablets for 5–7 days (along with ivermectin 12 mg OD for 5 days) as supportive management to treat COVID-19 patients who are in home isolation (Clinical Guidance for Management of Adult COVID-19 Patients, 2021).

Azithromycin is a macrolide antibiotic that exerts its antibacterial action by binding to the 50S ribosomal subunit, leading to the cessation of polypeptide biosynthesis in a bacterial cell. Additionally, the drug molecule has demonstrated antiviral properties in various in vitro models by preventing the entry of virus into the host cells (Gielen et al., 2010; Gyselinck et al., 2021). Azithromycin was shown to have affinity at the spike protein–ACE2 binding point. Additional theoretical studies revealed that azithromycin suppresses a viral cofactor binding site due to its striking molecular similarity with GM1, a host-cell ganglioside that binds the ganglioside binding domain of the spike protein. Further, it is proposed that azithromycin, by virtue of its being a weak base, facilitates the viral efflux from the host cell. However, all these hypothesis needs to be validated by practical experimental studies. Moreover, the immunomodulatory action of azithromycin augments its use as an antiviral agent in the treatment of COVID-19 (Malek et al., 2020; Sultana et al., 2020). Clinical trials with azithromycin along with hydroxychloroquine/chloroquine were not found to be satisfactory even for emergency use approval by FDA and EMA (COVID-19 Treatment Guidelines, 2021).

#### 19.2.4.2 Glucocorticoids

Glucocorticoids are particularly useful in patients who have developed cytokine storm (hyper immune/hyper inflammatory response) to viral infection (Dequin et al., 2020; Horby et al., 2021; Tomazini et al., 2020). The overresponsive immune system causes extensive damage to the lungs in particular and other organs in general, leading to multi-organ failure, which may result in death. Glucocorticoids exert antiinflammatory action by binding to their receptor and this receptor–drug complex along with drugs binds to the DNA sequences responsible for translating inflammatory signaling molecules such as phospholipase A2, interleukins, TNF- $\alpha$ , GM-CSF, etc. (Ramamoorthy & Cidlowski, 2016). The numerous side effects associated with corticosteroids warrant their use with caution and in critically ill patients (Oray et al., 2016). Dexamethasone (Fig. 19.2, 6 mg once daily up to 10 days) is the drug of choice among all glucocorticoids because it demonstrates minimum mineralocorticoid activity. In the case of nonavailability of dexamethasone, prednisone (daily dose not exceeding 40 mg), methyl prednisolone (daily dose not exceeding 32 mg), and hydrocortisone (daily dose not exceeding 160 mg) being recommended. Additionally, hydrocortisone was administered to patients for managing septic shock. The FDA recommends dexamethasone in hospitalized patients requiring supplemental oxygen and mechanical ventilation (COVID-19 Treatment Guidelines, 2021).

#### 19.2.4.3 Anticoagulants

Coagulation of blood leading to blockage of vessels (thromboembolism) was found among many COVID-19 patients. An exaggerated inflammatory response (cytokine storm) as discussed under Section 19.2.4.2, that results in coagulation pathway activation and endothelial injury is believed to be the reason for embolism (Biswas et al., 2021;



Bonaventura et al., 2021; Cohen et al., 2006; Leizorovicz et al., 2004). The rate of venous thromboembolism in early studies was found high enough for health authorities to frame guidelines for the use of anticoagulants. WHO and FDA suggest the use of low-dose anticoagulants for preventing thrombosis in hospitalized patients. Low molecular weight heparin or enoxaparin (Fig. 19.2, less than 4000 IU daily) is the recommended prophylactic dose in hospitalized patients (COVID-19 Treatment Guidelines, 2021).

#### 19.2.4.4 Vitamin supplements

Vitamin C (ascorbic acid) and vitamin D3 (cholecalciferol) are the two vitamins that have been extensively studied for the therapeutic management of COVID-19. Vitamin C (Fig. 19.2) is useful in sepsis and respiratory infection, wherein they decreased the mortality rate (Fowler et al., 2019; Holford et al., 2020; Kashiouris et al., 2020). Further, the ability of vitamin C to maintain cellular immunity and vascular integrity along with its antioxidant and antiinflammatory properties made it an ideal candidate for examining its effect on COVID-19 patients (Ellulu et al., 2015; Mrityunjaya et al., 2020). Clinical data is insufficient for its recommendation against or for its use in COVID-19 patients (COVID-19 Treatment Guidelines, 2021; Zhang et al., 2008). However, a few countries like India have included it under supportive management for home isolation patients. A dose not exceeding 1000 mg is usually prescribed for 7–10 days (Clinical Guidance for Management of Adult COVID-19 Patients, 2021; Thomas et al., 2021). A higher dose can cause a number of dose effects like kidney stones, digestive distress, cramps, etc. (Sestili, 1983).

Vitamin D3 or cholecalciferol (Fig. 19.2) plays a major role in maintaining normal levels of calcium and phosphorous in the blood. Further, it helps in the absorption of calcium by bones, thereby aiding in forming and maintaining strong bones (Bikle, 2012). In addition to its classical role in maintaining bone homeostasis, it has been reported that vitamin D3 also mediates innate and adaptive immune responses (Aranow, 2011). This is mediated by its receptor on B cells, T cells, and APC, wherein the active metabolite of the vitamin is synthesized. Further, it was observed that deficiency of vitamin D3 is associated with increased autoimmunity and enhanced susceptibility to infection (Aranow, 2011; Martens et al., 2020). Moreover, it was also revealed that vitamin D3 supplements result in elevated T cell levels along with their enhanced activity (Fernandez Lahore et al., 2020). All the preceding discussion makes vitamin D3 an attractive drug to manage COVID-19 disease. A lot of clinical trials are going on with results from several such studies available in the public domain (Amrein et al., 2014; Murai et al., 2021; Putman et al., 2013). Till now, the results are inconclusive to recommend the use of vitamin D3 for the prevention and/or treatment of COVID-19 infection (COVID-19 Treatment Guidelines, 2021).

### 19.3 Diagnosis of severe acute respiratory syndrome-Coronavirus-2 pathogenesis

SARS-CoV-2 is an RNA virus and hence all RNA detection technologies are used for the correct detection of the infection in the host. The most accepted and widely used test is, hence, the RT-PCR test, where the RNA is reverse transcribed into DNA and this DNA is then checked for certain genes specific to the SARS-CoV-2 virus. With time, several

other fastidious kits are also being used and other test methods like the antibody and antigen tests are also used to detect the spread of the virus among asymptomatic patients as well. Diagnosis of Covid-19 is an important step to containing the viral infection and also understanding its epidemiology. Early detection blocks further transmission and increases the patient care ratio. Though there are detection kits and methods, the early diagnosis solely depends on the clinical symptoms ([Symptoms of COVID-19, 2021](#); [Weissleder et al., 2020](#)). Here, we discuss all the detection methods from early clinical signs to viral load detection in later stages of the infection.

### 19.3.1 Diagnosis dependent on symptoms

The initial signs of COVID-19 infection develop gradually over the week, which ranges from mild to chronic and can progress to dyspnea and shock. The initial signs are fever, cough, fatigue, headache, and body ache. Several patient data were analyzed to conclude that fever is the most common, followed by a cough. However, gastrointestinal symptoms were also variable depending on age and comorbidities ([Symptoms of COVID-19, 2021](#)). A few patients reported a loss of smell and taste. But clinical signs were inconsistent among individuals, starting from asymptomatic cases to severe cases like septic shock, acidosis, clotting dysfunction, and acute pneumonia, which makes further grounds for being tested ([Coronavirus: Symptoms, 2021](#)). Usually, samples are taken from the upper and lower respiratory tracts as the viral load is not found in the urine or blood of the infected ([Chen et al., 2021](#)).

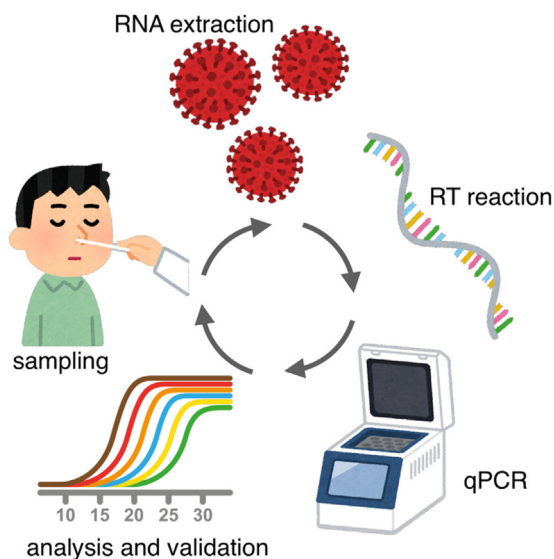
### 19.3.2 Reverse transcription-polymerase chain reaction technique for identification of virus

This viral identification technique has been the major benchmark for SARS-CoV-2 detection globally due to its direct detection of the viral genes rather than the secondary parameters like antibodies and antigens. The primers for RT-PCR were reported by the Malaysian Institute for Medical Research in January 2020 after the whole genome sequence of the virus was disclosed by China ([Table 19.2](#)). Later, other countries like India, England, South Korea, etc. declared their own clinically approved RT-PCR kits. The samples are collected using swabs from the upper and lower respiratory tracts from the nose and throat. The throat samples are sometimes misleading as the virus slowly moves down from the nasal passage to the lower respiratory tract after the first week of infection. Thus a precise collection of samples from deeper parts of the respiratory tract is usually advisable. The components of RT-PCR kits consist of reverse transcription enzymes and buffers, primers, and probes for the specific viral genome multiplication, along with reagents for positive, negative, and internal controls ([Bustin & Nolan, 2004](#); [Nolan et al., 2006](#)). It amplifies the viral ORF1ab, E (Envelope) gene and N (Nucleocapsid) genes like N1, N2, and N3, while RNase P is used as an internal control. The synthetic double-stranded DNA g-blocks of the SARS-CoV-2 gene fragment act as the positive control. Different kits usually have the RdRp and N genes for detection from samples collected like in the Abbott kit, which relies on buffer controls and even extraction mismanagement control, while the Roche company

**TABLE 19.2** Primers used for detection of Coronavirus disease-2019 in reverse transcription-polymerase chain reaction.

Gene name	Primers	Sequences
RdRP	Forward primer	GTGARATGGTCATGTGTGGCGG
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
	RdRp_SARSr-P1	FAM-CCAGGTGGWACRITCATCMGGTGATGC-BBQ
	Reverse primer	CARATGTAAASACACTATTAGCATA
E protein	Forward primer	ACAGGTACGTAAATAGTTAATAGCGT
	Reverse primer	ATATTGCAGCAGTACGCACACA
N protein	Forward primer	CACATTGGCACCCGCAATC
	Reverse primer	GAGGAACGAGAAGAGGCTTG
ORF1ab	Forward primer	CCCTGTGGGTTTTACACTTAA
	Reverse primer	ACGATTGTGCATCAGCTGA
N1	Forward primer	GACCCCAAATCAGCGAAAT
	Reverse primer	TCTGGTACTGCCAGTTGAATCTG
N2	Forward primer	TTACAAACATTGGCGCAA
	Reverse primer	GCGCGACATTCCGAAGAA
N3	Forward primer	GGGAGCCTTGAATACACCAAAA
	Reverse primer	TGTAGCACGATTGCAGCATTG
ORF1b	Forward primer	TGGGGYTTTACRGGTAACCT
	Reverse primer	AACRCGCTTAACAAAGCACTC

detects the ORF1a and E genes with pan-Sarbecovirus detection. Several studies from different groups have claimed the probes to be highly effective and sensitive for the detection of the virus with no cross-reactivity to other respiratory viruses (Hoshina et al., 2020). Home collection of nasal samples is also being approved for “Pixel by LabCorp COVID-19 home collection kit” following a COVID-9 questionnaire by healthcare personnel. The kit consists of a collection container, specimen collection swab, pre-labeled return envelope, insulated specimen pouch, dry ice gel pack, specimen biohazard bag, and the user guidelines for carrying out the test. After collection, the samples are placed in the saline tube, packed with the cooling ice, and sent back to the lab for further processing. The kit is currently available only at certain locations and numbers and not available on a global scale, which puts pressure on labs, doctors, and other frontline workers Fig. 19.3. Usually, if the results for all viral genes are negative, the test is termed as “invalid,” intermediate if a few genes get amplified, and positive if all the genes have amplification. Further, there are high chances of false negatives due to inadequate specimen quality, early or late detection of specimens, genetic mutation of the virus, presence of PCR inhibitors, and antiviral drug



**FIGURE 19.3** Schematic diagram of the workflow of an in-house RT-qPCR-based assay for detecting SARS-CoV-2, showing each step from patient sampling to reporting of results. A sample is collected from the patient's nasopharynx, saliva, or other tissues. RNA is extracted from the sample and then transcribed into cDNA (RT reaction). DNA polymerase amplifies the cDNA, degrading fluorescent probes which results in increased fluorescence intensity (qPCR). If the intensity reaches a certain threshold, the sample is classed as positive. Source: From Hoshina, T., Aonuma, H., Ote, M., Sakurai, T., Saiki, E., Kinjo, Y., Kondo, K., Okabe, M., & Kanuka, H. (2020). *Intensive diagnostic management of coronavirus disease 2019 (COVID-19) in academic settings in Japan: Challenge and future*. *Inflammation and Regeneration*, 40(1), 38. <https://doi.org/10.1186/s41232-020-00147-2>.

administration prior to testing (Garg et al., 2021; Hur et al., 2020; What Tests Could Potentially Be Used for the Screening, Diagnosis and Monitoring of COVID-19 and What Are Their Advantages and Disadvantages?, 2021). Although RT-PCR is the most reliable test, the number of tests that can be done on a single day is limited and can't cover the whole population on a relevant day. Another shortcoming of this is that it does not provide data on patients who have recovered from the infection as the viral load gets cleared off after full recovery and also shows no signs to patients on their first days of infection due to inadequate virus load. Hence, other methods have been developed to get rid of these shortcomings (Bustin & Nolan, 2004; Ko et al., 1998).

### 19.3.3 Antibody detection

Antibodies are the body's immunological response to antigens usually found on pathogenic organisms and substances. The immune system gets triggered each time it's invaded by a pathogen and puts up its defense by producing antibodies as one of the mechanisms. Antibodies are very specific and bind only to certain portions of a particular antigen to neutralize it and finally remove it from the body. Once the COVID-19 infection has occurred in the body, the immune system produces antibodies against the viral proteins like the spike protein or nucleocapsid, which can be detected post or during infection and hence used as a diagnostic approach as well. The variation of viral load among patients makes it difficult to detect viral antigens consistently, but antibodies against the virus provide a large window for diagnosis. This test was undertaken by a group where they focused on IgG and IgM immunoglobulins from the serum samples of patients and analyzed them using the ELISA kit. The Rp3 nucleocapsid is used as the antigen as it has 90% amino acid similarity to that of coronaviruses. It was found that in recovered patients, the IgM values increased from 50% to 81% and IgG increased from 81% to 100%.

SARS-CoV-2's recombinant nucleocapsid protein is the antigen that is immobilized on a nitrocellulose strip with IgG coupled to colloidal gold particles. An IgG antibody binds to the SARS-CoV-2 antigen to form a red-colored Ab–Ag complex, while in a few kits, a secondary antibody with horseshoe reddish peroxidase is bound to the antigen for the identification of a positive signal. This helps overcome the shortcomings of RT-PCR to detect the infection post-recovery and get an accurate number of SARS-CoV-2 infections. The disadvantage with this method is that antibodies may not be developed at a detectable level during the early stages of infection, which may result in false-negative cases. Therefore, the FDA has suggested that this should not be the sole basis for confirming a COVID positive/negative patient [[Antibody \(Serology\) testing for COVID-19: Information for patients and consumers, 2021](#); [Using Antibody Tests for COVID-19, 2021](#); [West et al., 2021](#)].

### 19.3.4 Hematological methods

The physio-pathological parameters like hematology and histology of tissues, organs, and blood show a change whenever an infection occurs in the body. These changes include an increase in leukocytes, neutrophils, monocytes, and other immune cells. Their number increases or they penetrate the tissues of infection to evoke a local inflammation. Hence, these parameters can act as measures for SARS-CoV-2 virus detection. There are studies suggesting that SARS-CoV-2 infected persons show clinical manifestations of systemic inflammation. All these can act as parameters for alternative early diagnosis of the infection once a standard has been finalized and set. The clinical manifested parameters also put an in on the severity of the disease in each patient and can act as a prognostic marker for early intervention of treatment too. Further, these parameters also help in the selection of an appropriate treatment strategy for moderately ill and critically ill patients ([Liao et al., 2020](#); [Usul et al., 2020](#)).

### 19.3.5 Radiological tests

The SARS-CoV-2 infection affects the lungs and several patients with different severities have shown various chest abnormalities due to it. Computed tomography (CT) is hence found its application to diagnose the disease as this viral infection shows unique abnormalities from other viral infections like poliomyelitis, SARS, MERS, and adenovirus. Though the results are very variable among the patient samples, which highlight the different stages of the infection, the tests can also help in providing insights about pneumonia. The usual abnormalities observed are patchy lesions which are nodular to irregularly shaped having ground-glass opacity which is consolidated in the subpleural region of the central lung lobes. Additionally, distinct reversed halo signs and pulmonary nodules with halo signs are also seen. With more follow-up tests, a migratory pattern of lesions with the absorbance of the primary lesions to emerge into new lesions in the pleural and subpleural regions was also noted. Such variability among patients in the CXR and CT scans makes it difficult to predict the actual pathogenic mechanism, but early detection of characterized abnormalities in both asymptomatic and symptomatic groups makes it a very good candidate for early diagnosis. Further, the time consumption is less compared to other methods

and has fewer chances of having false positive/negative (Ai et al., 2020; Martínez Chamorro et al., 2021).

### 19.3.6 Rapid testing kits

Rapid antigen kits were developed for easy and rapid diagnosis at home or any other convenient location. They are based on the principle of rapid chromatographic immunoassay which qualitatively detects the SARS-CoV-2 antigen. The presence of SARS-CoV-2 antigen is detected by its reaction with anti-SARS-CoV-2 antibody conjugated with color particles. This Ag-Ab-colored particle complex also produces a color whose intensity depends on the amount of virus present. There is no color production if the virus is absent in the sample. The regulatory authorities of South Korea gave conditional approval for such kits from SK Biosensor and Humasis Co. Ltd (COVID-19 Ag Home Test, 2021; COVID-19 Ag Test, 2021).

The USFDA approved the first COVID-19 self-testing home kit marketed by Lucira Health Inc. This kit utilizes RT-LAMP technology for N gene of SARS-CoV-2, wherein it amplifies the gene within 30 minutes (Ganguli et al., 2020). First, the RNA is converted into DNA by reverse transcriptase, followed by amplification of the DNA by DNA polymerase enzyme. A successful reaction results in a pH change of the testing mixture, resulting in a color change, which is detected using optical and electronic elements contained within the Test Unit. A microprocessor chip analyzes the color change and computes the amount of RNA present in the sample. The result is digitally displayed as positive, negative, or invalid within 10–15 minutes and it persists for at least an hour (Lucira™ COVID-19 All-In-One Test Kit, 2021).

## 19.4 Concluding remarks and future prospects

The COVID-19 pandemic is by far the major health, economic and humanitarian crisis which has affected people from all walks of life globally. It has brought scientists, governments, industrialists, and policymakers from all over the world to collaborate together to get rid of this horrifying catastrophe. The foremost priority is to find an effective antiviral drug to combat this unforeseen crisis that has never affected people to this magnitude in the past 10 decades. The barrier to the discovery of a novel antiviral molecule against COVID-19 is the limited time available as the pandemic is spreading rapidly in an erratic manner due to the generation of many mutant strains. Repurposing drugs with previous knowledge and artificial intelligence has helped a lot in fastening the drug discovery process against this dreadful virus. A combination of therapies has been successful in containing the disease to a certain extent. The treatment strategies keep changing with the arrival of better drugs, adverse and/or no significant outcome of the existing approved drug. In this review, we discussed all such drugs that have been approved across the globe. We expect more effective drug molecules from the existing clinical trials that are going on the world over. A more coordinated effort among the people involved in the drug discovery process is the need of the hour. We suggest a global drug discovery consortium of all

countries that will work more effectively to hasten the process of finding an effective treatment for COVID-19. Additionally, the WHO should ensure timely availability of drugs to undeveloped and developing countries. From a diagnostic point of view, a fast test is the need of the hour. The self-testing home kit marketed by Lucira Health Inc. The United States is the most successful one in this aspect. The availability of the same to undeveloped and developing countries is also a major concern. For example, in India, people in some places need to wait for 4–5 days to get a test report, during which a patient's condition may change from moderate to severe (Acharjee, 2021). In summary, the world should more unitedly fight to end this pandemic at the earliest.

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