

# CHAPTER 2

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## VIROLOGICAL CHARACTERISTICS OF THE SARS-CoV-2

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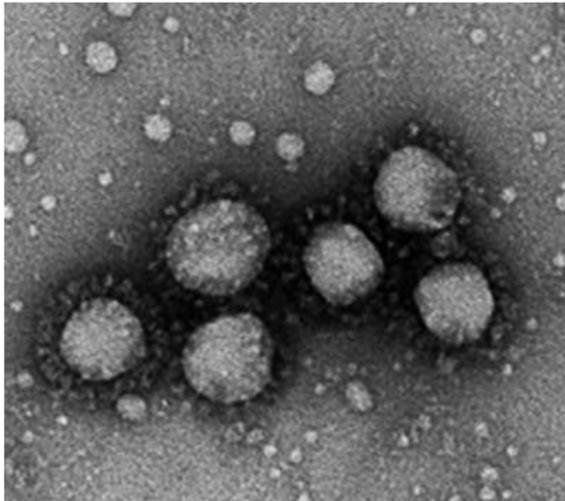
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### INTRODUCTION

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Coronaviruses (CoV), which are members of the *Coronaviridae* family, are enveloped, single-stranded viruses with the longest known RNA genome (26-32 kilobases). This virus family takes its name from the crown appearance of the protrusions on the round viral surface (crown, corona in Latin) (**Figure 1**).

The viral genome encodes non-structural proteins (nsp) and structural proteins (sp): *spike* (S protein) forming the protrusions on the surface, *envelope* (E protein), and *nucleocapsid* (nucleocapsid protein; N). The family is divided into four groups as alpha- (group 1), beta- (group 2), gamma- (group 3), and delta-CoV (group 4). In the 1970s, coronaviruses were detected in many animals (such as dogs, chicks, mice, rats) that cause various diseases, especially the respiratory and gastrointestinal systems. In humans, they mainly cause respiratory and gastrointestinal system complaints, usually mild and rarely more serious diseases such as bronchitis and pneumonia. Currently, six CoVs that cause disease in humans have been identified. While HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 cause mild respiratory infections, especially in children, SARS-CoV-1 and MERS-CoV cause more serious



**Figure 1.** Image of SARS-CoV-2 by electron microscopy (Copyright © 2020 National Institute for Viral Disease Control and Prevention, China CDC).

and fatal diseases. SARS-CoV-1 (Severe Acute Respiratory Syndrome-CoV-1), a beta-CoV, is responsible for the epidemics emerging in China between 2002-2003 and remained partially localized, with a mortality rate of approximately 10%. With a total of 8,273 cases and 775 deaths, this first serious CoV epidemic has reached a mortality rate of 50%, especially in the elderly. MERS-CoV (Middle East Respiratory Syndrome-CoV) was first isolated in Saudi Arabia in 2012, and it causes an infection with a mortality rate of approximately 30%. MERS-CoV-2 is considered one of the most dangerous viruses known since it has been detected in 2494 cases and caused 858 deaths (case/death rate: 34.4%).

## **SARS-COV-2**

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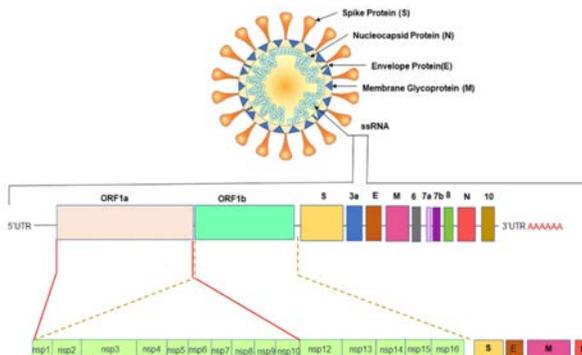
On December 31, 2019, 27 cases of pneumonia with none of the known infectious agents responsible were reported in Wuhan city, China. It has been found that almost all of the cases clinically characterized by dry cough, fever, respiratory distress, and bilateral lung findings are associated with the seafood market in Wuhan. On January 7, 2020, a new CoV was detected in throat swab samples taken from patients at the Chinese Center for Disease Control and Prevention. It was named 'Severe Acute Respiratory Syndrome Coronavirus 2', briefly SARS-CoV-2. The World Health Organization (WHO) named the

disease caused by this virus as COVID-19. As the epidemic tends to spread to different countries in a short time, WHO declared on January 30, 2020, that the problem is an international public health concern and declared that it poses a serious threat to the health systems of countries. This important health problem was declared as a pandemic on March 11, 2020, due to its global spread continuing with all its intensity.

SARS-CoV-2, a Beta-CoV subfamily member, is a spherical RNA virus 60-140 nm in diameter. The first 2/3 of the genome that forms its genetic structure consists of a polyprotein encoding 15 non-structural proteins (nsp) that play a role in the replication of the virus. The remaining 1/3 is responsible for encoding four major structural proteins (sp): S, E, M, and N proteins. Of these four structural proteins, the S (spike) protein is used for the attachment of the virus to the target cell. The E (envelope) protein enables the assembly of new viral particles and leaving the host cell. The M (matrix) protein plays a role in establishing the structural integrity of the virus. The N (nucleocapsid) protein, on the other hand, is responsible for protecting the structural features of the virus and providing its relationship with the immune response (**Figure 2**).

## ORIGIN OF CORONAVIRUSES AND THEIR SOURCE IN HUMANS

HCoV-229E, HCoV-NL63, SARS-CoV and MERS-CoV viruses originate from bats, while HCoV-OC43 and HCoV-HKU1 originate from rodents. Nowadays, it is accepted that bats carry many viruses (reservoir), but they do



**Figure 2.** Schematic presentation of the SARS-CoV-2 and its genome. (source: <https://respirator.research.biomedcentral.com/articles/10.1186/s12931-020-01581-z/figures/2>)

not directly transmit these microbes to humans, except for rabies. Generally, viral agents pass from bats to other animals (intermediate hosts) and from there to humans. Indeed, it has been proven that the intermediate host of MERS-CoV is camels, and the intermediate host of SARS-CoV is felines. Genetic analysis of viruses isolated from animals has enabled us to trace coronaviruses detected in different living species. By following this path, it was possible to determine the similarities, differences, and sources of viruses in different living organisms.

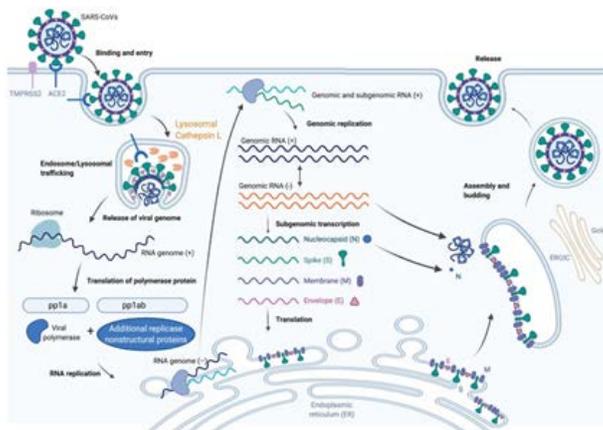
In the studies carried out in more than 19,000 animals (bats, rodents, and primates) in different geographies, 98% of the isolated coronaviruses were detected in bats. One or more CoVs were found in 8.6% of the 12,333 bats examined. Phylogenetic studies of the viruses isolated from nine patients in China in the early days of the COVID-19 pandemic have shown a similarity between human and bat coronaviruses and suggested that the virus would be transmitted to humans from bats in the first days of the epidemic. However, research has shown that although the origin of the virus is bats, the transmission does not occur directly from this animal. As a matter of fact, it has been determined that bats, which are mammals, hibernate in December and that bats are not sold in the winter period in the Huanan seafood market where the epidemic occurred. Also, since the genetic similarity is lower than 90%, it has been suggested that the possibility of transmission from bats is low, and even though the reservoir of the agent is bats, the virus was transmitted most likely from an intermediate host as in the MERS and SARS-CoV outbreaks. After that, it was investigated which animal was responsible for the transmission. The opinion that it could be pangolins (scaly mammals from the order Pholidota; anteater-like animals) was accepted. It is known that pangolins are used as food in Far Eastern countries. These animals caught, especially in the African continent, are brought to China illegally and sold in markets.

During the pandemic, several conspiracy theories on the subject have been put forward, and the opinion that the virus was produced in laboratories and spread to the public, either deliberately or accidentally, was frequently voiced. However, it has been scientifically demonstrated by genetic analysis of the isolated viruses that it is impossible to produce SARS-CoV-2 in the laboratory. Based on the fact that 75% of infectious diseases are zoonoses today, it is a scientific fact that SARS-CoV-2 originates from bats, is transmitted to humans through pangolins. It is one of many viruses that spread to humans by jumping species due to close contact with animals.

## MECHANISM OF SARS-COV-2 INFECTION

For the infection to begin, the causative virus must enter sensitive cells, and to do so; it must bind to the receptor on the cell surface. It has been shown that SARS-CoV-2 infects epithelial cells in the nose and bronchi at the first stage and binds to a receptor on these cells, called ACE-2 (angiotensin-converting enzyme 2), via the S protein on its surface. After this attachment, the contribution of the enzyme called TMPRSS2 (type 2 transmembrane serine protease), which is also found on the host cell membrane, is required for the virus to be taken into the cell. With the help of this enzyme, the virus attached to the cell is taken inside. Thereafter, the viral RNA passes into the cytoplasm of the infected cell, and the replication process of the virus begins. As a result, numerous new SARS-CoV-2 production takes place inside the cell, which is like a virus factory, and these viruses spread around and infect new cells with the destruction of the host cell (**Figure 3**).

Interestingly, unlike viruses that cause other respiratory infections such as influenza, ACE2, the receptor specific for SARS-CoV-2, is not only found on respiratory tract cells. The presence of this receptor on almost all tissues and organs in the body, such as the digestive, urogenital and nervous systems, outside of the respiratory system, causes SARS-CoV-2 to infect the whole body. The virus can bind to other receptors besides ACE2. It has been suggested that binding to the CD147 (basigin = EMMPRIN) receptor, which is among these and is also found in cells involved in the immune response, may trigger an abnormal immune response. In addition, the Neuropilin-1 receptor



**Figure 3.** Mechanism of SARS-CoV-2 infection (source: [https://www.cell.com/trends/immunology/fulltext/S1471-4906\(20\)30233-7](https://www.cell.com/trends/immunology/fulltext/S1471-4906(20)30233-7)).

has also been shown to facilitate the entry of SARS-CoV-2 into cells, especially in the nasal cavity.

## **SARS-COV-2 AND MUTATIONS**

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Since SARS-CoV-2 spread continues, mutations in the viral genome are inevitable. The evolutionary rate of SARS-CoV-2 is calculated as approximately  $6 \times 10^{-4}$  nucleotides/genome/year. This rate means that SARS-CoV-2, which has a genome of approximately 30 kb, will change approximately 20 nucleotides per year. Mutations in and around the receptor binding domain (RBD) can significantly alter the structure of the S protein. Thus, they should be constantly monitored in terms of their effect on the disease's transmission and severity. GISAID (Global initiative on sharing all influenza data; <http://gisaid.org/>) is continuously monitoring the new mutations of SARS-CoV-2 during the pandemic. Many of the variants appeared in late 2020 and early 2021 (Hodcroft E.M. 2021. CoVariants: SARS-CoV-2 Mutations and Variants of Interest. <https://covariants.org/>). However, mutations that cause altered function are particularly dangerous, as they increase transmissibility and disease severity. D614G is the most detected variant in all SARS-CoV-2 infections to date and is attributed to increasing transmission. The D614G variant is more infectious because the functional change in the structure of the S protein facilitates the binding of RBD to the receptor. Three new variants have recently emerged: 501Y.V1/B.1.1.7 from United Kingdom, 501Y.V2/B.1.351 from South Africa, and 501Y.V2/P.1 from Brazil. Lately, a new variant (B.1.429) suspected of causing a rapid increase in cases has been identified in the United States of America. The spread of these strains worldwide is of concern, as transmission may increase and the effect of vaccine/therapeutic neutralizing antibodies reduce. Variants B.1.1.7 and B.1.351 are partially resistant to antibody neutralization. In addition to being resistant to many monoclonal neutralizing antibodies, the Brazil variant P.1 is also more resistant to the plasma of convalescent and vaccinated individuals. Existing vaccines can achieve partial protection against the P1 variant. In studies conducted with Pfizer/BioNTech, Moderna, and Novavax vaccines, antibody titres did not change against B.1.1.7, but lower efficacy was observed against B.1.351 variant. The E484K mutation, found in both South African and Brazilian variants with reduced neutralization of some convalescent serum antibodies, is thought to contribute to resistance. Another variant found in the UK, South Africa, and

Brazil, N501Y, is thought to increase the transmission of SARS-CoV-2 by enhancing binding to the ACE2 receptor. These emerging variants have been shown to reduce the efficacy of existing antibody treatments used against COVID-19, in addition to vaccination.

## **TRANSMISSION CHARACTERISTICS OF SARS-COV-2**

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SARS-CoV-2 is transmitted from person to person through virus-containing particles scattered by coughing, sneezing, or even speech. At the beginning of the pandemic, only symptomatic patients were considered to spread the virus. However, it was later found out that asymptomatic individuals also disseminate the virus. It has been found that asymptomatic cases spread the virus for a long period of at least 19 days and the same amount as symptomatic patients. Some asymptomatic people start to show symptoms for a while, but a few (about 6.2%) continue to spread the virus without symptoms.

Classically, respiratory viruses are transmitted by droplets of infected individuals. Particles  $>5 \mu\text{m}$  in diameter fall to the ground quickly due to gravity's effect before they can go 1-2 m away. On the other hand, particles  $<5 \mu\text{m}$  in diameter can remain hanging in the air, and the virus is transmitted to humans from the air in environments that contain such particles. The main transmission route for SARS-CoV-2 is close and long-term contact in closed environments. As a matter of fact, the virus spreads rapidly in closed environments (cruise ships, restaurants, sports halls, religious ceremonies, choral work, celebrations, etc.). For this reason, it is necessary to comply with the physical distance rules besides using masks in preventing the spread. On the other hand, it is claimed that there are people defined as super spreaders in the transmission of infection in the community and spread a great number of viruses around. However, these individuals' characteristics, why, and how they spread viruses over a long time are not yet clear.

SARS-CoV-2 has also been detected in other body fluids such as feces, blood, and semen. However, the role of the isolation of the virus in these body fluids to the dynamics of the spread has not yet been clearly elucidated.

## **VIROLOGICAL DIAGNOSIS IN SARS-COV-2 INFECTIONS**

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Different laboratory methods are used at different stages in the diagnosis of virus infections. To detect acute cases in the early stages of SARS-CoV infec-

tions, virus propagation in cell culture, antigen detection, or nucleic acid detection methods is used. Specific antibodies are detected in the late period and especially in seroprevalence studies.

The most common method used in the early period is polymerase chain reaction (PCR). PCR detects the RNA of the virus. It should be kept in mind that not every PCR positivity necessarily indicates that the virus is replicated; that is, it is alive. The sensitivity limit for the detection of SARS-CoV-2 RNA was determined by the CDC as 4-10 RNA copies/ $\mu$ l. Primers targeting one or more genes of the RNA-dependent RNA polymerase (RdRp), N, and E genes of the virus are used. However, PCR, which has high sensitivity in diagnosing many infectious diseases such as viral hepatitis, influenza, or HIV infection, does not give similar results in detecting SARS-CoV-2 infections. The sensitivity of the PCR method varies between 58-96%, especially depending on the sample. In this context, where and how to get the sample is important. Bronchoalveolar lavage or sputum samples are particularly convenient for this virus that affects the lower respiratory tract. Depending on the dynamics of the virus, some unexpected results may occur in the detection of SARS-CoV-2 by PCR. For example, cases that give positive results in the convalescence phase or, as in asymptomatic cases, positivity in recovering cases. As a result, it should be kept in mind that a positive SARS-CoV-2 PCR test result is not always an indicator of active replication, and false negativity may be observed due to unsuitable sampling. Also, according to the tomography results, a large number of patients (on average 40%) who are thought to have COVID-19 patients but whose PCR tests are negative are encountered. Since SARS-CoV-2 infects other tissues and organs besides the respiratory system, the virus can also be detected in samples other than the respiratory tract. The virus can be detected in stool and serum for approximately 17 days. Viral shedding from the respiratory tract and stool may be prolonged. However, (except for immunosuppressive patients) no live virus was detected since the ninth day of the disease.

Many studies are carried out on the development of antigen detection tests. These tests, which are generally produced with "lateral flow" technology, can be used as Point-of-Care tests when they have high sensitivity and specificity. However, the most important problem with these tests is that they can't reach sufficient sensitivity. Therefore, antigen detection tests may not detect infected cases, especially with low viral load.

Serological tests detect SARS-CoV-2 specific antibodies as an indirect indicator of contact with the virus. However, antibody kinetics specific to COVID-19 are in contradiction. Antibodies do not become positive in all cases. It is not known whether they will cross-react with other coronaviruses and how long they will be found. Antibody levels are different in mild and severe cases.

## **HOST IMMUNE RESPONSE IN SARS-COV-2 INFECTION**

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As in other infectious diseases, in COVID-19, the immune system steps in, and a virus-specific response occurs. It is expected that the virus is inactivated in the immune response. However, it is seen that the immune response creates problems rather than benefits, especially in severe COVID-19 patients.

SARS-CoV-2 prevents the effect of interferon by disrupting or downregulating its synthesis. On the other hand, interferons that should work for the benefit of the host increase the number of ACE2 in some cases and allow more viruses to enter the cells. Furthermore, the p53 gene stimulated by interferons suppresses the differentiation of epithelial cells, leading to a more severe course of the disease and susceptibility to bacterial superinfections.

In some severe cases of COVID-19, the so-called cytokine storm is encountered. The activation of a large number of immune system cells causes an exaggerated cytokine production by these cells. This resulting abundance of cytokines causes damage through inflammation and an even more severe disease course. Exaggerated activation of cells resulting in excessive production of cytokines such as IL-1, TNF- $\alpha$ , IL-6 intensely stimulates new cells. Thus, it causes tissue damage, vascular destruction, multiple organ failure, and the clinical picture's aggravation.

Another feature of the immune system observed in COVID-19 cases is the numerical and functional decrease, especially in T cells. Since T cells play an important role in the immune response's formation and functioning, lymphopenia disrupts the entire system.

Specific antibodies are synthesized during SARS-CoV-2 infection. However, it has been suggested that these antibodies will only be useful when they are neutralizing the virus. Otherwise, they may be harmful by taking a role in carrying the virus into some cells.

## CONCLUSION

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As a result, the different parameters of the immune response that exist to protect the body are disrupted by SARS-CoV-2; multiple organ deterioration, vascular damage, coagulation disorder, and respiratory distress occurs.

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