

# SARS-CoV-2 (COVID-19): Beginning to Understand a New Virus

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## Abstract

Within the last two decades, several members of the Coronaviridae family demonstrated epidemic potential. In late 2019, an unnamed genetic relative, later named SARS-CoV-2 (COVID-19), erupted in the highly populous

neighborhoods of Wuhan, China. Unchecked, COVID-19 spread rapidly among interconnected communities and related households before containment measures could be enacted. At present, the mortality rate of COVID-19 infection worldwide is 6.6%. In order to mitigate the number of infections, restrictions or recommendations on the number of people that can gather in a given area have been employed by governments worldwide. For governments to confidently lift these restrictions as well as counter a potential secondary wave of infections, alternative medications and diagnostic strategies against COVID-19 are urgently required. This review has focused on these issues.

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## 1.1 Introduction

The Novel SARS-CoV-2 (COVID-19) is a zoonotic coronavirus belonging to Coronaviridae family of viruses, including those which caused severe respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome (MERS-CoV).



**Fig. 1.1** Centers for Diseases Control-confirmed COVID-19 cases globally. (Adapted from <https://www.cdc.gov/coronavirus/2019-ncov/global-covid-19/world-map.html>)

In late 2019, SARS-CoV-2 emerged in Wuhan province, China, infecting significant portions of the population. The highly infectious characteristics of SARS-CoV-2 permitted its rapid spread in Hubei province and other parts of the country [1, 2]. In the first weeks of January 2020, containment of this novel virus by local authorities was naturally fragmented due to the novelty and evolving diagnostic procedures, resulting in non-disclosure of the level of infection to the public and to the broader scientific community. Since this time, the virus has emerged on every habitable continent, infecting more than 4.9 million people and killing more than 320,000 individuals in 218 countries and territories (as of May 19, 2020, Johns Hopkins University website; <https://coronavirus.jhu.edu/map.html>) (Fig. 1.1).

COVID-19 remains a highly infectious disease even after incurring significant mutations in its genome during the last 5 months [3, 4], placing it front and center in the sights of the World Health Organization (WHO) as one of the most dangerous pandemics faced in the last 100 years. Hence, this perspective work aims to provide a timely overview of the COVID-19 outbreak and explore alternative avenues of testing and potential treatment approaches.

## 1.2 SARS-CoV-2 Phylogenetic Analysis

COVID-19 is caused by the SARS-CoV-2 RNA virus belonging to the Coronaviridae family. It contains a single-stranded positive-sense RNA, ranging from 26 to 32 kilobases (kb) in length. Coronaviruses are considered zoonotic, residing in many different animal species, such as camels, bats, mice, and dogs [5]. The SARS-CoV-2 viral genome includes 6–11 open reading frames (ORFs). The first ORF constitutes the largest part of the genome (67%) and encodes nonstructural proteins. The rest of the ORFs encode accessory and structural proteins. Four main structural proteins have been distinguished known as the spike (S), envelope (E), matrix (M), and nucleocapsid (N) proteins. The spike surface proteins are responsible for binding of the virus to host cell receptors and determine the host tropism and capacity of transmission [6].

Initial next-generation sequencing of the virus was performed on throat swabs and bronchoalveolar fluid collected from nine patients by Lu et al. [5]. An additional sequencing study was also performed by Zhou et al. on samples isolated from five patients [7]. Reverse transcription and full

annotation of the patient sample revealed that the viral genomes were 99.9% similar [5, 7]. Analysis of the SARS-CoV-2 genome revealed a 97–99% similarity to a SARS-like betacoronavirus (SL-CoVZC45) and another bat coronavirus (CoVZXC21). Genetically, SARS-CoV-2 demonstrated less genetic similarity with those causing SARS (~79%) and MERS (~50%) [5]. Phylogenetic analysis showed that SARS-CoV-2 belongs to subgenus Sarbecovirus of the betacoronavirus genus. This study also examined the spike protein sequence of SARS-CoV-2, which could be divided into S1 and S2 domains. These domains are thought to play a role in receptor-binding and membrane fusion. The SARS-CoV-2 S2 domain shared relatively high sequence homology with that of bat coronaviruses (93%), whereas the S1 domain showed only 68% homology. However, SARS-CoV-2 and SARS-CoV shared 50 conserved amino acids inside the S1 domain (located in C-terminal area), which were different in bat coronaviruses. Structural predictions revealed that the SARS-CoV-2 receptor-binding domain was similar to that of SARS-CoV, and previous studies suggested that angiotensin-converting enzyme 2 (ACE2) was a potential receptor for this virus [5]. Infectivity assays confirmed ACE2 as a receptor for SARS-CoV-2 [7]. In these studies, HeLa cells were infected with the virus, and cells which expressed the ACE2 receptor were more susceptible to infection. However, additional studies may be needed to determine or confirm the mechanism of transmission [8].

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### 1.3 Possible Origin of SARS-CoV2

Whether it is a product of natural evolution, a laboratory experiment, or otherwise engineered, the possible origins of SARS-CoV-2 have been debated since the beginning of the outbreak. A potential way to end the debate is to identify an intermediate host or hosts that can successfully transmit the virus to humans and vice versa [7]. Since the first cases of COVID-19 infections were thought to stem from the conjoined seafood and live animal market in Wuhan [1], the COVID-19 disease is generally considered a zoonotic dis-

ease (like SARS). Through the International Food Safety Authorities Network (INFOSAN), the national food safety authorities are seeking more information on the potential for persistence of COVID-19 in foods traded internationally as well as on the potential role of foods in the transmission of the virus. Currently, investigations are underway to evaluate the viability and survival time of SARS-CoV-2 [9–11]. As a general rule, the consumption of raw or undercooked animal products should be avoided. Raw meat, milk, or animal organs should be handled with care to avoid cross contamination with uncooked foods. However, human-to-human transmission has also been confirmed.

This novel virus responsible for the ongoing COVID-19 epidemic was initially thought to be a product of laboratory recombination as strongly claimed on the 3rd of February 2020 (<https://jameslyonsweiler.com/2020/02/02/moderately-strong-confirmation-of-a-laboratory-origin-of-2019-ncov/>) online by the chief executive officer of the Institute for Pure and Applied Knowledge. The claim stated that SARS-CoV-2 had a unique inserted sequence (1378 bp) located in the middle of its spike protein gene that did not match other coronaviruses but was similar to a sequence in the pShuttle-SN expression vector, commonly used in research laboratories. However, this hypothesis was rejected by Hao et al. [12]. These researchers aligned several coronavirus sequences and found that the “unique” sequence from SARS-CoV-2 was also found in these other viruses with a high sequence identity. This suggested that SARS-CoV-2 arose via a natural mechanism.

On the other hand, similar to the case for SARS-CoV and MERS-CoV [13], the bat is the most likely species of origin for COVID-19 due to the high degree of whole-genome identity with a bat coronavirus (BatCoV RaTG13) [7]. Notably, a SARS-like coronavirus was initially described close to when the COVID-19 outbreak occurred in lung samples from two dead Malayan pangolins [14], and these findings were confirmed by Zhang and colleagues [15]. This study suggests that the pangolin species may be a natural reservoir of coronaviruses. Other possible reservoirs include camels and civets (Fig. 1.2).

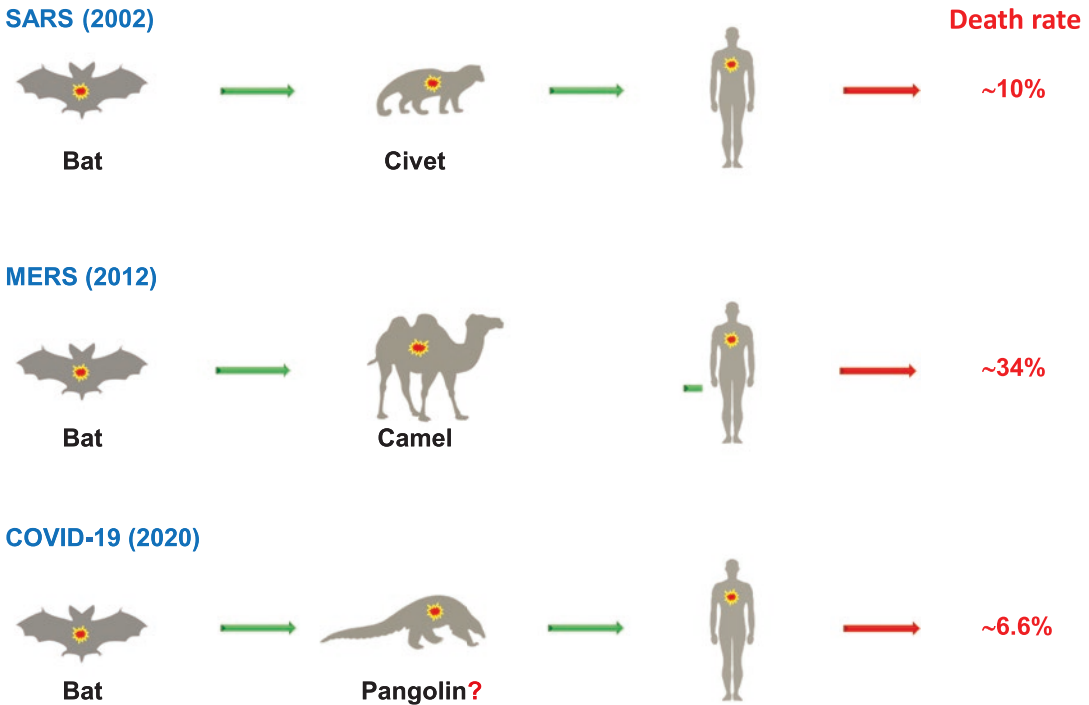


**Fig. 1.2** Possible origin of the coronaviruses including SARS, MERS, and COVID-19

#### 1.4 Main Properties of COVID-19 Infection

SARS-CoV-2 belongs to the betacoronaviridae family which usually displays a round/elliptic shape with a diameter of 60–140 nm. Similar to most coronaviruses, SARS-CoV-2 could be sensitive to heat, ultraviolet rays, and lipid solvents. Coronaviruses could infect several animal species (such as camels, cats, cattle, pangolins, and bats), and infection may be transmitted to humans [16]. The stability of SARS-CoV and SARS-CoV-2 may be similar, with capabilities of surviving for up to 3 h in aerosols, 4 h on copper, and up to 1 day on cardboard [17]. The virus has a longer viability on steel and plastic surfaces and could survive for approximately 2–3 days. Higher temperature and humidity may result in reduction in viral transmission not only in influenza and SARS but also in the case of COVID-19 [18].

The structure of SARS-CoV-2 is similar to that of other coronaviruses, which contain the proteins mentioned above (spike, envelope, membrane, and nucleocapsid proteins), along

with different enzymes, such as RNA polymerase, helicase, or papain [19]. The spike proteins on the viral surface play a significant role in target recognition and membrane fusion. These proteins also play a critical role in human-to-human transmission of the virus. The spike protein has two subunits. One of these contains the receptor-binding domain (RBD), which enhances the attachment between the virus and host cell, while the second subunit appears to be involved in membrane fusion [20]. Similar to SARS-CoV, the RBD is conserved, but there may be important differences between the two viral strains. In the RBD, there are five critical amino acid residues at positions 442, 472, 479, 487, and 491 which could be involved in the human-to-human transmission. These critical residues (apart from tyrosine 491) may not be conserved between SARS-CoV and SARS-CoV-2. Three-dimensional modeling has revealed that the spike protein of SARS-CoV-2 could interact with the ACE2 receptor [21], resulting in the virus-host interaction. The entry of the virus into the cell could then enhance the immune and inflamma-

tion response of the host [22]. The mechanism of entry involves fusion of the viral envelope with the host cell, allowing it to enter the endosomal system. Next, the virus is uncoated and releases its RNA into the host, and this is translated to the viral replicase polyproteins pp1a and pp1ab, which are then cleaved by viral proteinases. The polymerase then carries out discontinuous transcription to produce subgenomic mRNAs and these are translated into the viral proteins. Finally, the viral proteins and RNA genome are assembled into new virus particles in the endoplasmic reticulum, allowing these to be released from the cell via the secretory pathway [19].

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### 1.5 Typical Clinical Features of COVID-19 Cases

Several studies on the pneumonia outbreak caused by COVID-19 infection have been reported in the general population. In the first report, Huang et al. indicated that the infected patients had a history of exposure to the Huanan Seafood Wholesale Market [2]. Various symptoms such as fever, non-productive cough, dyspnea, myalgia, fatigue, normal or decreased leukocyte counts, and radiographic evidence of pneumonia could be seen in the infected cases, with organ dysfunction and death occurring in severe cases [2]. In the Wuhan region, 99 cases were confirmed to be infected with COVID-19 among groups of humans in close contact, with more severe effects seen in older people with comorbidities [23]. Case reports confirmed human-to-human transmission causing the novel coronavirus-infected pneumonia (NCIP) [11]. The clinical characteristics of COVID-19 in pregnancy and intrauterine vertical transmission potential have also been reported [24]. This study revealed that the symptoms of pregnant women with COVID-19 pneumonia were diverse, with the main ones being fever and cough but with no evidence for vertical transmission in late pregnancy [24]. Table 1.1 presents the typical clinical and imaging findings of COVID-19 as well as those of common cold, influenza, and other coronavirus diseases including SARS and MERS [11, 25–29].

Coronaviridae represent an important family of animal and human viruses that are in permanent circulation [32, 33]. The first SARS coronavirus had a low impact on global morbidity and mortality, with more than 8000 recognized cases and 774 deaths [34, 35]. In contrast, the MERS coronavirus remained localized in Saudi Arabia, with smaller numbers in other countries of the Middle East and South Korea [36]. Studies on both of these viral outbreaks highlighted the potential danger of nosocomial transmission to health-care personnel, which could be critical in such epidemics by incapacitating the normal caregivers [37]. A common feature of SARS, MERS, and COVID-19 infection is the presence of severe acute respiratory syndrome, although the current estimated fatality rate of COVID-19 (6.6%, as of May 19, 2020) is lower than that of SARS (~10%) and MERS (~36%) [33, 35]. However, COVID-19 has led to emergence of much larger numbers of infected patients (approximately five million, compared with approximately 11,000 for SARS and MERS combined, as of May 19, 2020). Similar to SARS-CoV, the entry of SARS-CoV-2 into host cells depends on the recognition and binding of the spike protein to the ACE2 receptor on host cells [39–45]. The high affinity of this protein to the ACE2 receptor likely contributes to the quick spreading of virus. This receptor is expressed at high levels in human organs, such as lung alveolar epithelial cells and enterocytes of the small intestine, which are potentially the targets of COVID-19 infection [46]. In addition, COVID-19 transmission is currently known to be similar to that of other coronaviruses (such as SARS and MERS) in which human-to-human transmission transfer occurs through droplets, contact, and fomites. Based on the transmission modes of SARS and MERS, avoiding close contact with people suffering from acute respiratory infections and frequent handwashing are recommended. In addition, SARS-CoV-2 particles have also been detected in stool samples of some patients, suggesting that a more possible fecal-oral transmission occurs [47]. This suggests that multiple shedding routes of SARS-CoV-2 might exist.

**Table 1.1** Comparison of typical clinical and imaging findings between common cold, influenza, SARS, MERS, and COVID-19

| Disease              | Respiratory symptom                                 | Constitutional syndromes                      | CT imaging findings                                                                                                                         | Diagnostic methods                                                                                                          | Refs.    |
|----------------------|-----------------------------------------------------|-----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|----------|
| Common cold          | Stuffy nose, runny nose, and sneezing               | No clear                                      | X-ray                                                                                                                                       | PCR and pan-viral DNA microarrays                                                                                           | [30, 31] |
| Influenza            | Stuffy nose, runny nose, sore throat, and dry cough | High fever, muscle ache, and malaise          | Small patch ground-glass opacity and consolidation with subpleural and/or peribronchial distribution                                        | PCR, RT-PCR, immunofluorescence, direct (DFA) or indirect (IFA) florescent antibody staining, and viral tissue cell culture | [25]     |
| SARS                 | Cough and dyspnea                                   | Fever, chill, malaise, headache, and diarrhea | Subpleural ground-glass opacity and consolidation, prominent lower lobe involved, and interlobular septal and intralobular septa thickening | RT-PCR, rRT-PCR, RT-LAMP, rRT-LAMP, and coronavirus detection kit                                                           | [26, 27] |
| MERS                 | Sore throat, dry cough, and dyspnea                 | Fever, chill, and rigor                       | Bilateral, basilar, and subpleural airspace, extensive ground-glass opacity, and occasional septal thickening and pleural effusions         | RT-PCR, rRT-PCR, RT-LAMP, rRT-LAMP, and coronavirus detection kit                                                           | [28, 29] |
| Mild SARS-COVID-19   | Possible cough and sore throat                      | Fever                                         | Multifocal patchy ground-glass opacity with subpleural distribution                                                                         | RT-PCR, rRT-PCR, RT-LAMP, rRT-LAMP, and coronavirus detection kit                                                           | [11]     |
| Severe SARS-COVID-19 | Breathless and respiratory failure                  | Fever, muscle ache, confusion, and headache   | Diffuse heterogeneous consolidation with ground-glass opacity                                                                               |                                                                                                                             | [11]     |

## 1.6 Immune Response to COVID-19 Infection

The innate immune response against viral infection typically involves production of the interferons, IFN $\alpha$  and IFN $\beta$ . In the case of MERS-CoV, the timing of interferon production appears to be critical in determining whether it protects against infection [48]. Interferon administration 1 day after infection was protective in a mouse model, whereas delayed interferon treatment did not block viral replication and actually increased lung inflammation, resulting in fatal pneumonia. Although the mechanisms of the immune response triggered by MERS infection and immune evasion strategies have not yet been fully studied, these findings indicate that the use of interferon-based therapies should be considered with caution [49]. A compromised immune

system may have been the cause of the high case-fatality rates in MERS-infected patients [50]. This is consistent with the findings of previous studies which indicate that older individuals with compromised immune systems are more susceptible to the effects of SARS infection [51, 52].

Coronaviruses have evolved multiple immune evasion mechanisms to limit the early induction of interferon. SARS-CoV encodes an enzyme that adds a 2' O-methyl group to the viral RNA, thereby evading detection by the viral sensor molecule, MDA5 [53, 54]. Similarly, coronaviruses encode proteases that antagonize the stimulator of interferon genes (STING) [55], which is thought to be the case for SARS-CoV-2. However, neutralizing antibodies that target the receptor-binding domain of the spike protein can competitively inhibit viral entry into host cells, as shown in the case of SARS-CoV [56]. Most initial efforts to generate vaccines against SARS-CoV-2 have focused on the corre-

sponding spike protein and its receptor-binding domain. Interestingly, the affinity of the SARS-CoV-2 spike protein for its receptor, ACE2, is approximately 15 nM, which is over 20 times greater compared to the SARS-CoV spike protein [41, 57]. Based on this, the development of neutralizing antibodies may prove to be more difficult for COVID-19 infection. However, some neutralizing antibodies developed for SARS-CoV do cross-react with SARS-CoV-2, and these bind to the protein core rather than the receptor-binding domain [58–60]. It is possible that some of these may be useful for vaccine development considering the sequence similarity of SARS-CoV-2 and SARS-CoV and the facts that they use the same ACE2 receptor for cellular entry and cause similar acute respiratory syndromes [61].

## 1.7 Diagnostic Strategies

The genome sequence of SARS-CoV-2 was released online in early January 2020 (GenBank accession number MN908947), which facilitated the development of nucleotide-based diagnostic methods. Real-time polymerase chain reaction (PCR) was verified as a routine method for detection [62]. Corman et al. developed a PCR method based on the similarity between SARS-CoV and SARS-CoV-2. The probes and primers targeted the receptor-binding gene (spike protein gene), as well as the E and N genes, which allowed successful discrimination between the SARS-CoV and SARS-CoV-2 viruses [63]. Chu et al. designed a quantitative real-time PCR test for the highly conserved ORF1b and N genes of the SARS-CoV-2 genome [38]. For this, SARS-CoV PCR products were also cloned into plasmids as references, and additional viral genomes were tested, such as those from MERS-CoV, avian influenza, human influenza, and parainfluenza. Patients with COVID-19 were positive for both SARS-CoV-2 genes and not those from the other viruses. The detection of the N gene appeared to be more sensitive, compared to ORF1b. Thus, N gene detection may be more effective in the case of SARS-CoV-2, and testing for the Orf1b gene could be used for confirmation.

Next-generation sequencing (NGS) has been regularly used for viral disease diagnosis and could detect all kinds of DNA/RNA fragments from viral infections. In addition, NGS approaches could play a role in the discovery of novel viruses. RNA-based NGS has also been suggested for rapid diagnosis of respiratory viruses. Compared to PCR methods, NGS may have higher specificity and could detect viruses even in the latency phase of infection [64]. Screening for ACE2 receptor expression in patients may also be helpful in disease risk assessment as this may help to identify the possible and main routes of infection. For this, bulk RNA sequencing and single-cell transcriptomic analysis was performed on patient samples taken from mucosa or the oral cavity [40]. This revealed that the ACE2 receptor was expressed in oral tissues, especially in the tongue. The finding that the expression of the ACE2 receptor is also elevated in the lungs lends further support that blocking its interaction with the virus may be a possible therapy against COVID-19 infection (see below) [39].

Currently, no proteomic biomarkers of COVID-19 infection have been validated, but several candidates are possible. Routine blood tests have revealed that procalcitonin and C-reactive protein (CRP) may be elevated in several disease cases but not in all patients [23, 65]. Cytokines may also be potential marker candidate of COVID-19 infections. Huang et al. analyzed the cytokine and chemokine levels in plasma of patients using a Human Cytokine Standard 27-Plex Assays panel and the Bio-Plex 200 device [2]. Several inflammatory markers showed elevated levels in affected patients, such as interleukin (IL-)1 $\beta$ , IL-1RA, IL-7, IL-8, IL-9, IL-10, fibroblast growth factor (FGF), tumor necrosis factor alpha (TNF $\alpha$ ), vascular endothelial growth factor VEGF, IFN $\gamma$ , and IFN $\gamma$ -inducible protein 10 (IP10). The same study also showed that patients who needed intensive care presented higher levels of plasma markers such as granulocyte colony-stimulating factor (GCSF), IP10, monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory protein 1A (MIP1A), and TNF $\alpha$ , compared to patients who

did not need critical care. These data revealed that elevated concentrations of cytokines may correlate with disease severity.

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### 1.8 Potential of SARS-CoV-2 Diagnostic Testing in Male Semen

Coronaviruses are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as MERS and SARS [66–68]. The SARS-CoV-2 virus was originally discovered when viral metagenomic analysis was carried out on three bronchoalveolar lavage specimens from Chinese adult patients with unexplained severe pneumonia [1]. Currently, SARS-CoV-2 can be detected in human clinical specimens including saliva, nasal fluid, blood, feces, and urine by NGS, real-time PCR, cell culture, and electron microscopy [1]. The Centers for Disease Control (CDC) has recommended that clinical virology laboratories should not attempt viral isolation from specimens collected from COVID-19 patients under investigation (<https://www.cdc.gov/coronavirus/nCoV-2019/guidelines-clinical-specimens.html>). Even though several countries are currently producing the test kit for the coronavirus after the availability of the SARS-CoV-2 genome, diagnostic testing for this virus should be carried out according to CDC guidelines.

Currently, to determine if someone has been infected with SARS-CoV-2, samples from the throat, coughed-up sputum, or lung fluid are used. Importantly, recent studies have indicated that semen from males infected with viruses could be considered as a primary route of human transmission [69–71]. In addition, the hypothesis that the ACE2 receptor could serve as a mediator for endocytosis of the SARS-CoV coronavirus [72] has led to the suggestion that SARS-CoV-2 may enter host cells and tissues through a similar mechanism (Fig. 1.3). From the protein expression atlas ([www.proteinatlas.org](http://www.proteinatlas.org)), high levels of the ACE2 precursor are found in kidney, intestines, and testis (Fig. 1.3) and not in female reproductive system. This latter finding could be

the possible explanation for the higher rate of death in males than females [23]. Hence, SARS-CoV-2 isolation from the semen or reproductive tracts of infected men may be considered as the only direct and definitive approach for proving infectivity or their potential function as reservoir. A study which carried out semen testing found that 6 patients (15.8%) had positive results for COVID-19, including 4 of 15 patients (26.7%) who were at the acute stage of infection and 2 of 23 patients (8.7%) who were recovering [73]. This latter finding is particularly noteworthy as it suggests that sexual transmission is possible. Therefore, it is important to determine if the viral infection is sexually transmitted to determine the risk, especially in the case of males who have recovered and may still harbor the virus.

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### 1.9 ACE-2: The SARS-CoV-2 Receptor Identified

As stated above, the coronavirus spike protein receptor-binding domain appears to be responsible for the interaction between the virus and the host cells, and one receptor for this is ACE2 [44, 74]. ACE2 is a transmembrane protein enzyme with its active site on the extracellular surface of cells. ACE2 plays a role in conversion of angiotensin II to angiotensin 1–7, which may play a role in regulation of cardiac function and blood pressure. In addition, overexpression of ACE2 may be involved in diseases such as cardiac dysfunction or diabetes [75]. Hence, many studies have been carried out by targeting ACE2 for treatment of circulatory diseases. In animal models of hypertension, reduced ACE2 expression or activity has been reported. Angiotensin 1–7 production via ACE2 cleavage could have several protective roles, including vasodilatory, antiproliferative, or anti-oxidative stress effects. Since hypertension is a major health issue in diseases such as diabetes, heart failure, liver failure, and pulmonary injury [76, 77], ACE2 and angiotensin 1–7 have been suggested as potential therapeutic targets. In addition, higher expressions of ACE2 have been found in renal and liver cancer and could therefore have prognostic value. On the

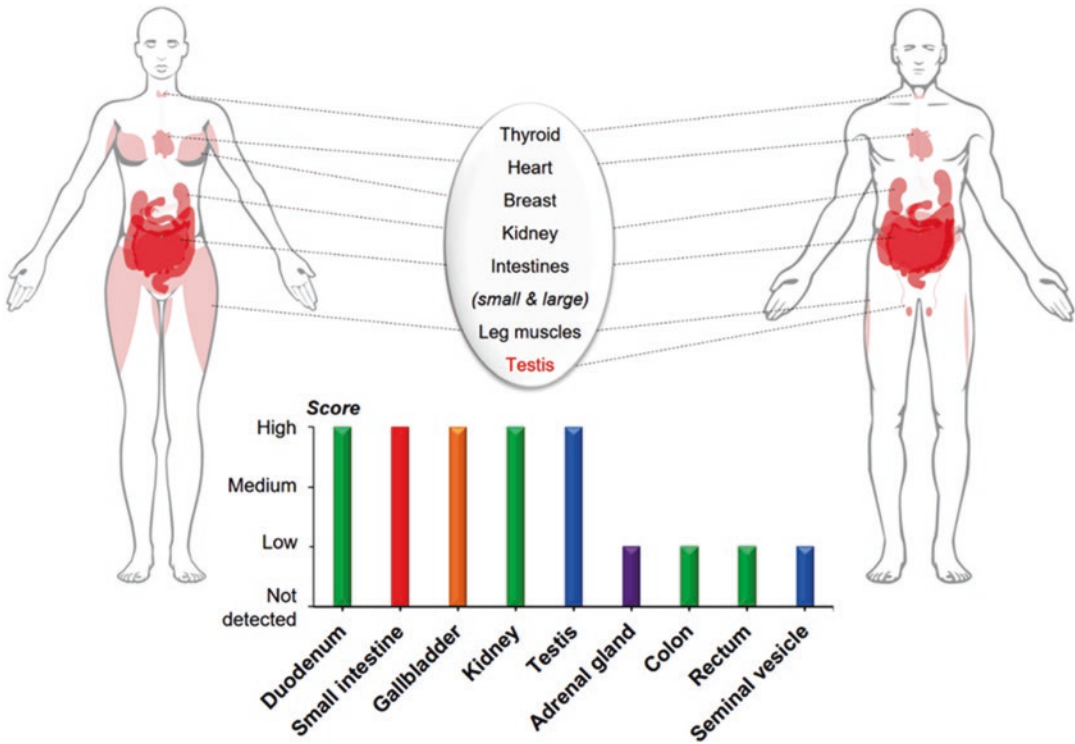


**Fig. 1.3** Selective ACE2 expression in thyroid, heart, breast, kidney, intestines, leg muscles, and testis and schematic overview of the ACE2 expression are shown for each of the nine tissues. Color-coding is based on tissue groups, and no protein expression data was observed in cerebral cortex, cerebellum, hippocampus, caudate, thyroid gland, parathyroid gland, nasopharynx, bronchus, lung, oral mucosa, salivary gland, esophagus, stomach, liver, pancreas, urinary bladder, epididymis, prostate,

vagina, ovary, fallopian tube, endometrium, cervix, uterine, placenta, breast heart muscle, smooth muscle, skeletal muscle, soft tissue, adipose tissue, skin, appendix, spleen, lymph node, tonsil, and bone marrow tissues. The proteomic analysis is combined with RNA-Seq on the organ, tissue, and cellular level, and all data are freely accessible on the Human Protein Atlas web portal, [www.proteinatlas.org](http://www.proteinatlas.org)

other hand, overexpression of ACE2 may be protective against oxidative stress [78].

The possibility that ACE2 expression may increase susceptibility to SARS-CoV-2 infection may be linked to the finding that older individuals show greater effects of viral infection [79]. This is due to the fact that ACE2 expression may be increased in adults in their 40s–50s since it may be associated with several protective mechanisms against age-related diseases. In addition, ACE2 is an important regulator of Ras signaling, which has been suggested as a modulator of the aging process. These findings suggest that children are less susceptible to the effects of SARS-CoV-2 infection due to lower expression of ACE2 [80, 81]. In addition, elderly individuals are

known to have reduced innate immune functions, resulting in a higher mortality rate [82, 83].

A study of approximately 40 patients with SARS-CoV-2 infections in early January 2020 found that the majority of these were male with underlying diseases, such as diabetes, hypertension, or cardiovascular disorders [2]. This was supported by a follow-up study which showed that over two-thirds of the patients were male with an average age of 55.5 years [23]. Together, these findings suggest that males with underlying comorbidities have a greater risk of infection and death from COVID-19.

The initial infection site for COVID-19 infection is the upper respiratory system where ACE2 sites are abundant, followed by the lower respira-

tory tract where numbers of these receptors are lower, and this can lead to bronchiolitis, secondary pneumonia, or both [43]. In addition, the reported symptoms of COVID-19 infection in the early phase of disease progression can include indigestion, nausea, diarrhea, or vomiting [84]. Coincidentally, ACE2 expression is high in several cells of the gastrointestinal system, including epithelial cells in the esophagus, enterocytes, colon, and ileum [85]. In addition, high levels of ACE2 are also found in the kidneys [42]. Receptor binding of SARS-CoV-2 was recently modelled, and this revealed that the receptor-binding domain has a stronger interaction with ACE2 due to a unique phenylalanine residue in the flexible loop of the spike protein [86].

Reports of finding RNA of the respiratory syncytial virus in urine have suggested the possibility that COVID-19 is also located in the urinary tract [87]. A study confirmed that urinary tract infection was present in 10% of children with acute bronchiolitis or other respiratory viruses, such as influenza virus or enterovirus, and coronavirus was detected in 7% of the cases [87]. Since high levels of ACE2 expressions have been reported in kidney, ACE2 could be involved in controlling the different renal functions, suggesting a potential route of SARS-CoV-2 infection or clearance [42].

ACE2 has been reported to have high expression levels in testis and seminal vesicles, especially in the Leydig and Sertoli cells [88]. Rat experiments showed that ACE2 may be a constitutive product of mature Leydig cells and may play a role in testicular functions, such as spermatogenesis [88]. Even though no evidence has been found regarding the potential involvement of COVID-19 in the testicular system, high ACE2 levels in testis and seminal vesicles may provide an additional route of infection or reservoir. Several viruses have been detected in the seminal fluid with long survival times, which has suggested that they may be sexually transmitted [69–71]. As an example, the Ebola virus was found to be sexually transmittable since a Liberian male survivor of Ebola infected his female partner with the virus. This study also revealed that infectious Ebola virus may be present at least for 179 days in

survivors after disease onset [89]. Additional male survivors were investigated, confirming the presence of virus in their semen [90, 91]. The researchers involved in this study suggested that the virus may hide in the testes and thereby escape immune system detection, leading to a longer incubation period [90]. In a larger study of 220 Ebola survivors, the viral RNA could be detected in semen from several months to more than 2 years after recovery [92, 93].

Currently, we do not know whether seminal fluid can act as transmissible vector for the SARS-CoV-2 virus in symptomatic and asymptomatic male patients [2, 23]. Investigations have revealed that since ACE2 is predominantly expressed in intestines, testis, and kidney, fecal-oral and other routes of transmission are possible [86] (Fig. 1.4). Remarkably, there have not been many reports regarding the presence of SARS-CoV-2 in vaginal secretions. However, a neonate with elevated IgM antibodies to SARS-CoV-2 born to a mother with a COVID-19 infection has been reported [10]. Therefore, there is still a controversy regarding whether or not COVID-19 can be transmitted in utero from an infected mother to her infant before birth.

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## 1.10 Current Treatment Strategies

Currently, there are no useful therapies available against COVID-19 infection although intensive efforts are underway to develop drugs and vaccines. Studies on the therapeutics used against SARS and MERS could also provide useful leads in developing new treatments against COVID-19. In addition to targeting the interaction between the ACE2 receptor and the viral spike protein, other possible therapeutic targets could be the two proteases involved in proteolysis and virion packaging: the coronavirus main proteinase (3CLpro) and the papain-like protease (PLpro) [93]. Traditional Chinese medicines may also be helpful by maintaining body health, and research is ongoing regarding the efficacy of this approach in treating COVID-19. Some Chinese medicines have been suggested to have some impact on disease by either preventing mild disease symptoms

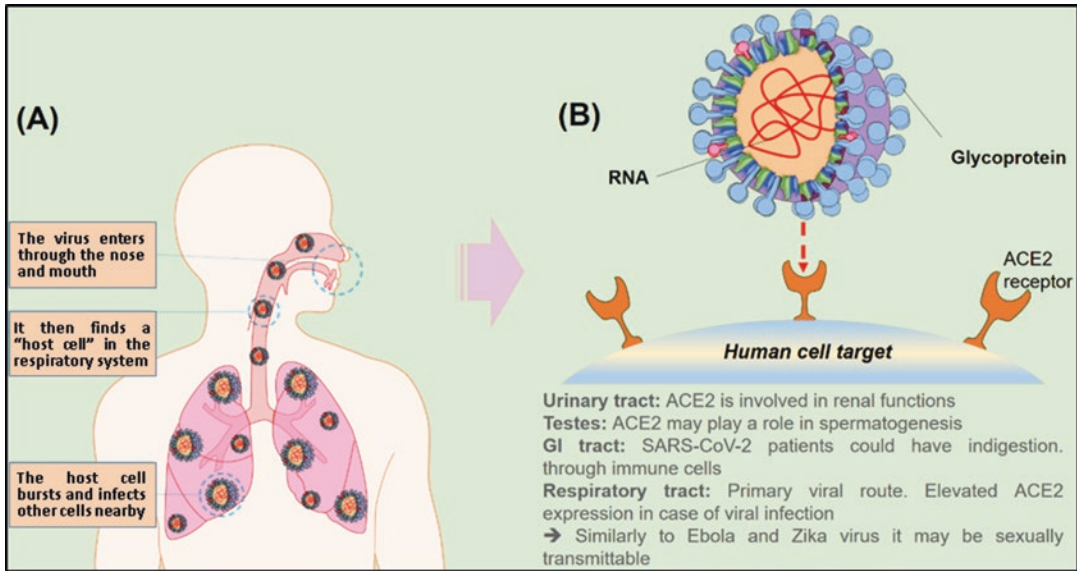


**Fig. 1.4** (a) Transmission of the coronavirus disease and (b) potential routes of SARS-COV-2 infection in terms of ACE2 expression

progressing to more severe ones or speeding up recovery from infection. However, such potential effects have been questioned in the case of COVID-19, and more scientific studies are needed to prove or disprove efficacy against the virus [94].

Currently, antibodies and vaccines are under development, which target the spike protein. Tian et al. discovered a monoclonal antibody, CR3022, which could bind to the receptor-binding domain of SARS-CoV-2 although it was initially used as SARS-CoV antibody [59]. In the case of SARS-CoV-2, CR3022 may recognize an epitope that does not overlap with the ACE2 binding site. Instead, CR3022 may be a neutralizing antibody and could therefore offer some protection against the disease via another mechanism.

Given the likely role of ACE2 in SARS-CoV-2 entry into cells, inhibitors of this molecule could play a significant role in the prevention or therapy against COVID-19 infection. There are several active compounds such as scutellarin, hesperetin, nicotianamine, and glycyrrhizin that may reduce ACE2 activity/expression or inhibit ACE2 and spike protein binding [95]. Chloroquine phosphate is an old drug used for the treatment of malaria or rheumatoid arthritis and may also be

useful against COVID-19 infection. This drug could protect against the virus-cell fusion, enhance endosomal functions, or interfere with glycosylation of coronavirus receptors. In addition, it may also have anti-inflammatory effects. Another antiparasitic drug called diminazene aceturate (DIZE) is known to exert vasodilatory effects in experimental models of hypertension, myocardial infarction, diabetes, and atherosclerosis, and it has mild antiviral properties [96]. Remdesivir is an adenosine analogue that can be used to inhibit viral gene expression. It was successfully used against different viral infections, including SARS and MERS [97]. Additional treatment strategies may inhibit the coronavirus proteases, prevent viral replication, and enhance the immune protection of the host [98]. The 3C protease and pyridoxal 5'-phosphate (PLP) inhibitors have also been used with some success against SARS and MERS. Ivermectin is a Food and Drug Administration (FDA)-approved antiparasitic drug, which could be used against different kinds of viruses such as HIV and Zika. Studies on Vero/hSLAM cells infected with virus suggested that ivermectin may significantly reduce viral RNA levels in less than 48 h [99]. In addition, melatonin is an anti-oxidative and anti-

inflammatory molecule that may protect against pathogens causing acute lung injury and respiratory diseases [100]. Chloroquine and hydroxychloroquine could also be protective by binding to sialic acids of respiratory systems, which may inhibit virus interactions with target cells [101, 102]. Teicoplanin is an antibiotic that has been used against respiratory viruses and gram-positive bacteria and may prevent viral RNA release and inhibit the viral cycle inside the cells [103]. Finally, remdesivir (GS-5734) is a nucleotide analogue of viral RNAs and appears to work by causing premature termination of viral replication [104]. It is currently considered to be among the best candidates for a drug against COVID-19 infection [105] (Fig. 1.5). In addition, potential natural/synthetic compounds for preventing COVID-19 infection are presented in Table 1.2.

### 1.11 Future Perspectives

Compared with the SARS and MERS viral outbreaks, the COVID-19 pandemic has thus far shown a lower mortality rate but with a drastically higher infection capability. In just a few months, this pandemic has resulted in a global challenge for disease prevention and control due

to its high rate of human-to-human transmission and the lack of availability of effective treatments. This has led to many countries implementing control strategies such as social distancing, isolation, and institution-wide lockdowns as early as possible in the outbreak. At present, the COVID-19 cases have dropped sharply in South Korea, while Europe and the United States are currently the main epicenters. Amid these dire trends, South Korea has emerged as a model to emulate with the most expansive and well-organized testing program in the world, combined with its extensive program to isolate infected people and trace and quarantine their contacts. As the genomics, phylogeny, antigenic structure, and various outcomes of infection have become increasingly understood, the development of diagnostic strategies, new therapeutic approaches, and vaccines against COVID-19 has become a worldwide effort. Perhaps one positive outcome of these efforts is that this collective mobilization has led to the scientific, technical, and financial support to combat this current pandemic at a speed that has never been seen before. It is hoped that this will help to lay the foundations to control this current outbreak and provide a strong base to tackle future pandemics.

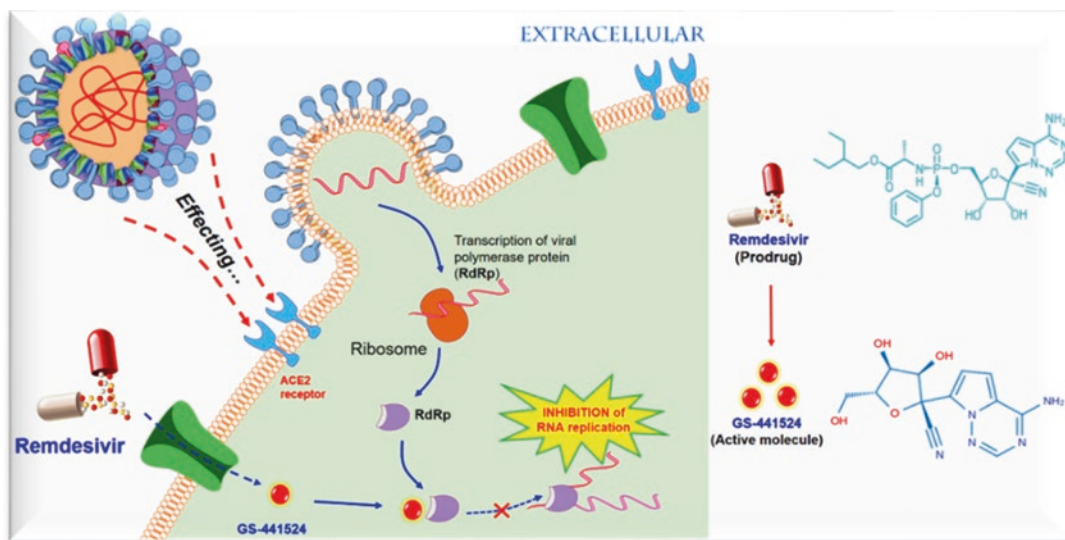


**Fig. 1.5** Potential repurposed remdesivir drug candidate for treatment of COVID-19

**Table 1.2** Suggested potential drug candidates against SARS-COV-2

| Drug candidate                                | Description                                                                                      | Possible role in COVID-19                                                           | Refs.    |
|-----------------------------------------------|--------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------|
| CR3022                                        | Monoclonal antibody                                                                              | May bind spike protein epitope                                                      | [59]     |
| Chloroquine phosphate                         | Old drug against malaria and rheumatoid arthritis                                                | May prevent virus-cell fusion and anti-inflammatory effects                         | [96]     |
| Remdesivir                                    | Adenosine analogue; results in premature termination of viral transcription                      | Useful against RNA viruses (SARS, MERS); may inhibit COVID-19 infection             | [98]     |
| Baicalin                                      | Natural compound from <i>Scutellaria baicalensis</i> Georgi; could have anti-inflammatory effect | Binds to ACE2; antiviral effect against SARS-COV. Possible effects against COVID-19 | [59, 86] |
| Scutellarin                                   | Natural compounds from <i>Erigeron breviscapus</i> ; could have anti-inflammatory effect         | Could bind to ACE2; may prevent COVID-19 binding                                    |          |
| Hesperetin                                    | Bioflavonoid from citrus; inhibits 3C-like protease                                              | Possible ACE2 inhibitor                                                             |          |
| Nicotianamine                                 | Can be isolated from soybean                                                                     | May block COVID-19 through inhibiting ACE2                                          |          |
| Glycyrrhizin                                  | Isolated from licorice root; protective against SARS virus                                       | Interacts with ACE2; further studies are needed on COVID-19                         | [100]    |
| Ivermectin                                    | Popular antiviral drug, HIV inhibitor, and promising candidate against SARS-COV-2                | May inhibit transport of viral proteins through IMP $\alpha$ / $\beta$ 1 pathway    |          |
| Melatonin                                     | Safe drug against several respiratory diseases                                                   | May reduce vessel permeability and improve life quality of patients with SARS-COV-2 | [101]    |
| Chloroquine (CQ) and hydroxychloroquine (HCQ) | Effective against several RNA viruses                                                            | Prevention of ACE2-virus interactions                                               | [102]    |
| Remdesivir (GS-5734)                          | Nucleotide analogue of viral RNA                                                                 | May result in premature viral transcription                                         | [105]    |
| Teicoplanin                                   | Glycopeptide antibiotic                                                                          | Prevention of the viral RNA release and viral cycle inside cells                    | [104]    |

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