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Review

# Novel severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) infection: Microbiologic perspectives and anatomic considerations for sanctuary sites

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ABSTRACT

*Introduction:* A significant chunk of global life – the economy, sports, aviation, academic, and entertainment activities – has significantly been affected by the ravaging outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) with devastating consequences on morbidity and mortality in many countries of the world.
*Methods:* This review utilized search engines such as google scholar, PubMed, ResearchGate, and web of science to retrieve articles and information using keywords like "Coronavirus", "SARS-CoV-2", "COVID-19", "Origin of coronavirus and SARS-CoV-2", "microbiology of coronavirus", "microbiology of SARS-CoV-2", COVID-19", "Coronavirus reservoir sites", "Anatomic sanctuary sites and SARS-CoV-2", biological barriers and coronavirus", biological barrier and SARS-CoV-2".
*Results:* While this pandemic has caught the global scientific community at its lowest level of preparedness, it has inadvertently created a unified and wholesome approach towards developing potential vaccine (s) candidates by escalating clinical trial protocols in many countries of Europe, China and the United States. Interestingly, viral pathobiology continues to be an evolving aspect that potentially shows that the management of the current outbreak may largely depend on the discovery of a vaccine as the administration of known antiviral drugs are proving to offer some respite. Unfortunately, discontinuation and longtime administration of these drugs have been implicated in endocrine, reproductive and neurological disorders owing to the development of pathological lesions at anatomical sanctuary sites such as the brain and testis, as well as the presence of complex biological barriers that permit the entry of viruses but selective to the entrance of chemical substances and drugs.
*Conclusion:* This review focuses on the microbiologic perspectives and importance of anatomical sanctuary sites in the possible viral rebound or reinfection into the system and their implications in viral re-entry and development of reproductive and neurological disorders in COVID-19 patients.


**Contents**



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## Introductory overview to novel coronavirus

The current global shutdown resulting from the "2019 novel coronavirus" (COVID-19) pandemic is significantly affecting all facets of our lives. The world economy, sports, entertainment, tourism, aviation, and academic activities have greatly suffered from the current Coronavirus pandemic. In addition, an increase in hospital bed admissions and the death of affected persons have made COVID-19 one of the greatest public health disasters in recent times.

On February 11, 2020, the International Committee on Taxonomy of Viruses categorized this novel coronavirus outbreak as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The committee described it as the seventh member in the family of Coronavirus that can infect humans following the two previous outbreaks; the Severe Acute Respiratory Syndrome (SARS-CoV) and the Middle East respiratory syndrome (MERS-CoV) [1]. The Coronaviruses (CoV) represent positive-sense ribonucleic acid (RNA) viruses of crown-like spikes that belong to the family of Coronaviridae, in the subfamily of Coronavirinae and order Nidovirates. The Coronavirus is sub-grouped into four genera, alpha, beta, gamma, and delta, based on the genomic composition. The subgroups that cause respiratory symptoms in the human population are the alpha-coronaviruses and beta-coronaviruses, which are also responsible for gastroenteritis in other vertebrates [2,3]. The cause of the current Coronavirus outbreak SARS-CoV-2 has been associated with a member of the beta coronavirus subgroup. This subgroup shares the same genus with known SARS-CoV and Bat coronaviruses, presented in a different clade. This novel Coronavirus surfaced toward the end of 2019, as it was presented as a cluster of pneumonia infections in a city called Wuhan in an Asian country, China. It started spreading throughout the cities of China, and subsequently, increasing numbers of cases are recorded throughout the nations of the world [4,5]. Toward the end of January 2020, the World Health Organization (WHO) described the COVID-19 outbreak as a public health issue of international concern and to demystify the magnitude of the outbreak. It was characterized as pandemic [6].

Presently, over one hundred and seventy-seven million confirmed cases of SARS-CoV-2 globally in people had been tested so far, while the total number of SARS-CoV-2 cases continue to rise owing to the second and third waves. The death rate tolls over three million and eighty-two thousand and more incidence as the disease progresses. The epicentres of the current SARS-CoV-2 outbreak are primarily in developed countries, such as China, the United States of America, and European countries [7]. Also, there have been reports in the media of people who had recovered and tested positive for SAR-CoV-2 due to reinfection. This phenomenon of reinfection continues to be a mystery to health officials and researchers. It focuses on viral 'sanctuary sites' as seen in other infectious viral diseases like the HIV/AIDS pandemic. Whilst the understanding of the pathobiology of SARS-CoV-2 continues to evolve daily, and we examine the possibility (however remote) of viral sequestration in specific organs in the body (like the testis, thymus, brain) that provide some form of sanctuary to viral particles based on particle size and specific morphological configurations inherent in these micro-anatomical structures. This review, therefore, focuses on the known facts about the novel SARS-CoV-2 outbreak and the special consideration that should be given to the anatomical sanctuary sites in the body as possible contributors to viral reinfection in recovered COVID-19 patients.

## Genomic and skeletal structures of SARS-COV-2

Seven known coronaviruses can infect humans: HcoV-229E, HCoV-0C43, SARs-CoV, HCoV-NL63, HKU1, MERS-CoV, and SARS-CoV-2. These coronaviruses have four genera (Alpha, Beta, Gamma, and Delta). Four (4) of out the seven (7), including HCoV-0C43, SARs-CoV, HKU1, and MERS-CoV and SARS-CoV-2, are beta while HcoV-229E and HCoV-NL63 are alpha [3,8,9]. The coronavirus genome has approximately 27–32 kb positive-sense single-stranded RNA and its encoding for three broad protein classes. The virion is about 90−120 nm in diameter, surrounded by a lipid bilayer that enveloped the helical nucleocapsid structure and protected the genome [10]. The intact virion encodes several structural proteins such as 180/90-kDa spike (S) protein, a ∼50- to 60-kDa nucleocapsid (N) protein, an 8-kDa envelope (E) protein, and the ∼23-kDa membrane (M) protein and also the group-specific proteins or accessory protein (Fig. 1). These proteins are unique and specific to a strain of Coronavirus. The primary functions of these proteins are still unknown but are believed to influence the pathogenesis, interaction of the virus, and host cells and support viral replication [11]. The first successful Isolation of SARS-CoV-2 by inoculating human airway epithelial cells was done with a bronchoalveolar-lavage fluid sample from a patient presented with pneumonia [12]. The whole-genome sequencing of SARS-CoV-2 was reported in 2019 after a sample was taken from a patient with pneumonia in South Korea. The Isolation was done by cell culture, and the isolated SARS-CoV-2 was named Beta-Cov/Korea/KCDC03/2020 [12]. Kim et al. [12] further reported the morphology of SARS-CoV-2 from electron micrographic analysis, and the virus has a particle size of about 70−90 nm with a wide range of intracellular organelles within the vesicles. Kim et al. [12] concluded the current diagnosis of SARS-CoV-2 is based on RT-PCR

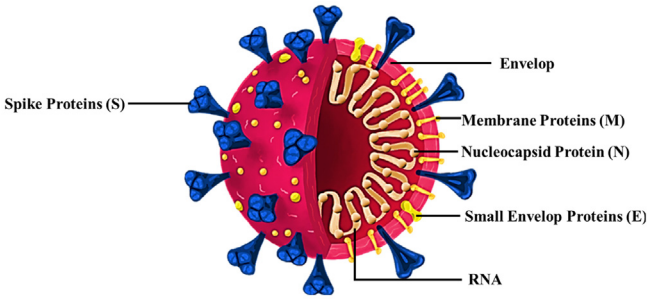


**Fig. 1.** Skeletal structure of novel SARS-CoV-2 showing all the E, S, M and N proteins as well as the RNA and envelop. Adapted from an image by Desiree Ho for the Innovative Genomics Institute.

for the detection of the gene. Besides, causative agent isolation will enhance serological test development and rapid diagnostic tests.

## Routes of transmission of novel SARS-CoV-2

The route of transmission of pathogens remains an essential step in assessing, curtailing, and managing infectious diseases. Presently, the transmission route of SARS-CoV-2 has not been fully established, although some routes of transmission have been recently documented [13]. The novel outbreak, SARS-CoV-2, spreads through the respiratory tract as the dominant means, which is similar to the transmission route in SARS-CoV and MERS-CoV, however, with more severe infectivity. It has been confirmed that people contract this infectious outbreak through droplet transmission; other routes of transmission asides respiratory system are emerging. Recently, transmission through aerosol has been suspected, but more evidence is needed to establish this finding [14]. More so, evidence of contracting SARS-CoV-2 on inanimate objects has been reported [14,15]. The presence of this infectious disease was discovered on the cell phones, items, and door handle of infected persons for a few hours to several days, which aggravates the possibility of infections in individuals that touches their eyes, nose, or mouth after an encounter with these materials [16]. This finding corroborates with the previous study that reported different time maintenance of infection of SARS-CoV and MERS-CoV on inanimate surfaces [17].

Some studies have confirmed the presence of SARS-CoV-2 RNA in the faecal samples of infected patients [18,19], which is an indication of active replication of the virus in patients' gastrointestinal tract and possible suggestion of faecal-oral transmission route of this infectious disease. Consequently, Munster et al. suggested that testing of SARS-CoV-2 should be extended to faecal samples [20]. Transmission through the ocular surface has been reported, but more studies are required to substantiate this finding [21]. It is worth noting that, no evidence of vertical transmission of SARS-CoV-2. However, the latest report suggested that vertical transmission should not be ignored owing to confirmation of two newborns tested positive for SARS-CoV-2 in Wuhan [22,23]. Furthermore, there was no evidence of SARS-CoV-2 in the breast milk of infected patients, which possibly rule out the risk of transmission from breastfeeding [24,25]. The ultimate path of transmission of SARS-CoV-2 remains the respiratory tract through a droplet means, though other routes and means that have been stated above are also essential and should not be ignored.

## Mechanism of infection of SARS-CoV-2

The previous outbreaks of SARS, MERS, four common coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1) and newly outbreak of SARS-CoV-2 shows the necessity to understand the mechanism(s) used by these viruses to attack the host immune system, involuntary orienting response, discover effective antiviral agents and development of a human vaccine [26]. The membrane ectopeptidases is the general protein family to which all the known cellular receptors are grouped into. The viral entry does not require the catalytic activity of these peptidases, but the activation of human Coronavirus (HCoVs) spike proteins requires co-expression of other host peptidases [27,28]. Previous studies documented that cleavage and activation of SARS-CoV, MERS-CoV, and HCoV-229E spike proteins employed human transmembrane serine proteases for viral entry [29,30].

The receptor of HCoV-229E is human aminopeptidase N (hAPN), and it represents only CoV that infects non-ciliated cells because of its high expression in non-ciliated cells of the human bronchus [31]. The SARS-CoV and HCoV-NL63 employed Angiotensin-Converting Enzyme 2 (ACE2) for binding with cells [32,33]. High expression of ACE2 in alveolar type I, alveolar type II, endothelial, and ciliated bronchial cells have been previously reported [34]. The receptor of MERS-CoV is dipeptidyl-peptidase 4 (DPP4), a different receptor with high expression in the epithelial tissues in the human body as well as the endothelial cells [35,36]. Presently, only the receptor determinants for HCoV-HKU1, O-Acetylated Sialic Acid, and HCoV-OC43, the N-acetyl-9-o-acetylneuraminic acid are known, but their surface receptors remain unknown [37,38]. The spike glycoprotein of SARS-CoV-2 has been described as the combination of known bat SARS-CoV and unknown Beta-CoV [39]. More importantly, it was evident from the latest fluorescent study that SARS-CoV-2 utilized the ACE2 cell receptors and employed the exact SARS-CoV mechanism of viral entry to the host cell [40]. Based on tropism, SARS-CoV and MERS-CoV exert different involuntary orienting response. The profuse replication of MERS-CoV takes place in both alveolar and bronchial tissues, while replication of SARS-CoV only occurs in alveolar tissue. Consequently, MERS-CoV's wide involuntary orienting response has been attributed to the severity of the disease and the high rate of death. In contrast, restriction in SARS-CoV replication has been linked to pneumonia that it usually presents [41].

## SARS-CoV-2 and vulnerability; an emerging field in infectious disease

### *Evolutionary trends and zoonotic importance of human coronaviruses*

Coronaviruses (CoV) are a group of disease-causing viruses that has been a subject of intense research since the 1960s. This virus infects humans and other vertebrates with clinical manifestations such as severe lower respiratory infections (pneumonia) and common cold in humans; consequently, respiratory tract and gastrointestinal systems are affected [42]. The evolutionary trends of alpha and beta CoV have been linked to discovering a wide range of CoV in bats. More so, evidence of intermediate host and animal reservoir has been found in other animal species. Notably, there were outbreaks of zoonotic coronaviruses such as Severe Acute Respiratory Syndrome (SARS-CoV) and Middle East Respiratory Syndrome (MERS-CoV) in 2003 and 2012, respectively. At the onset, the outbreak of SARS-CoV-2 disease was attributed to a live animal market, where the source has been traced to either bat (Origin) and civet cat or pangolin as intermediate hosts (Fig. 2). Moreover, it has been established that this novel SARS-CoV-2 is zoonotic, which is an indication that it can spread from humans to animals in some conditions, but to this present moment, no evidence that pets can spread this novel virus to humans [43,44].

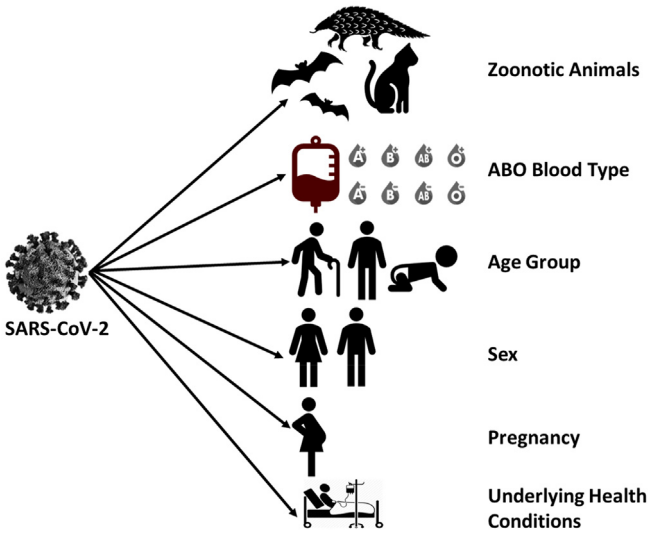


**Fig. 2.** Schematic illustration of the known vulnerable groups associated with SARS-CoV-2 infection. These groups include the (i) exposure to zoonotic animals; (ii) ABO blood group; (iii) different age groups; (iv) sex or gender of exposed individual; (v) pregnancy in women; and (vi) people with underlying health conditions.

*Sex-related vulnerability to COVID-19*

The magnitude, different reactions, and manifestations of disease in males and females are essential tools for understanding the impacts of an outbreak on the general populace, which would also help develop sex-based precision modalities for the prevention and treatment of the disease [45]. The sex-based disaggregating data revealed an equal number of SARS-CoV-2 cases in males and females; however, the latest reports show different patterns in susceptibility and mortality rates.

The data so far on the mortality rates have confirmed that male populations have a higher death rate than females. In a recent cohort study from Italy and China, it was established that males constitute a more significant percentage of the reported death tolls compared to females [46,47]. However, the clinical and scientific basis for disparity in mortality rates between each gender remains unknown. Still, a study has linked it to sex-based immunological differences [48] and a higher percentage of smoking in men relative to women [49]. In another study conducted on the plasma of 331 confirmed cases of CoV, females were shown to produce more antibodies against SARS-CoV-2 in their plasma than males [50]. Besides, the antiviral activities of estrogenic compounds have been previously described [51], and since estrogen is more concentrated in females, this may probably be another piece of evidence. Moreover, literature has documented that the gene controls the immune system is on the X-chromosome [52], and double X-chromosomes are found in the female, which gives an edge at combating the virus and exposes female to autoimmunity than male. Other studies have linked a higher level of haemoglobin in the blood of men compared to women as a possible reason why men are more likely to be susceptible to SARS-CoV-2 [53,54]. All these reports are emerging pieces of evidence, and further investigations are needed to corroborate their findings (Fig. 2).

*Age-related vulnerability to COVID-19*

More so, data emanating from epicentres in different countries such as Italy and China have revealed that older people aged ≥ 65 years are at higher risk of severe SARS-CoV-2 [55]. Another data emanated from China shows a substantial upsurge in the death rate in older people and low fatality cases in people under the age of 20 years [56]. Though preliminary data from China indicates that older people are more vulnerable, the latest data from the U.S. Center for Disease Control and Prevention (CDC) has shown that young people between the ages of 20–40 years old are also hit with SARS-CoV-2 outbreak. This finding is contrary to speculations at the onset of the SARS-CoV-2 pandemic. Furthermore, in another study on 56 patients, higher fatality cases were found in older patients than the young and middle-aged patients [57]. Thus, environmental or lifestyle factors may be contributing to the different age-related outcomes between both countries (Fig. 2).

*Vulnerability to COVID-19 as a result of underlying health conditions*

Based on the preliminary reports, SARS-CoV-2 was more severe in patients with underlying medical conditions such as diabetes mellitus, hypertension, coronary artery diseases, chronic renal failure, cerebrovascular diseases, chronic obstructive pulmonary diseases, chronic lung diseases, and smoking habit [45,55]. Recently published research that considered 355 patients revealed that just 3 out of 355 patients who died due to SARS-CoV-2 in Italy had no comorbidities [47]. This evidence suggests that patients with comorbidities constitute a more significant percentage of the dying population. According to the CDC, three significant comorbidities have been described as the most common underlying disease conditions that predispose to severe SARS-CoV-2. They include diabetes mellitus, chronic lung disease, and cardiovascular disease (Fig. 2). Among the three, diabetes mellitus is the most common underlying clinical condition in SARS-CoV-2 patients [45,58], followed by chronic lung disease, while the least is cardiovascular disease. Also, recent data from cases in the United States of America indicates that patients with respiratory diseases are at a higher risk of developing severe SARS-CoV-2 than those without manifestation of respiratory infections [45].

*Pregnancy-related vulnerability to COVID-19*

The gestational period in humans is marked by physiological adaptive changes and a compromised immune system that predisposes pregnant individuals to become vulnerable to pathogenic diseases such as SARS-CoV-2 (Fig. 2). The information on the susceptibility of pregnant women, obstetrics, and neonatal outcomes is essential as the SARS-CoV-2 outbreak continues to spread among the general populace. Now, information on the assessment and treatment of SARS-CoV-2 infected pregnant women is scanty. This report makes it difficult to ascertain the potential risk of vertical transmission to the foetus via the placenta circulation. Similar to SARS-CoV-2 infected non-pregnant women, good obstetrics and neonatal outcomes were reported by Luo and Yin in seven SARS-CoV-2 infected pregnant women [59,60]. However, these studies are not enough to substantiate the safety of antiviral therapies in pregnant women infected with SARS-CoV-2, the obstetrics outcomes, and neonatal outcomes. SARS during pregnancy has been previously linked to adverse neonatal outcomes such as neonatal death, preterm birth, intrauterine death, and intrauterine growth restriction [61]. In another study by Zhu et al. [62] that examined the neonatal outcomes of 10 pregnant women infected with SARS-CoV-2, 6 cases of premature delivery were observed. More so, possible adverse neonatal outcomes such as premature labour, respiratory distress, abnormal liver function, fetal distress, and thrombocytopenia have been described [62]. Therefore, more studies are needed to establish safe treatment modalities, obstetrics, and neonatal outcomes of pregnant women infected with SARS-CoV-2.

*Vulnerability to COVID-19 based on solid organ transplant*

As one of the advancements in contemporary medicine, solid organ transplantation has been successfully employed to replace failed organs caused by infection or diseases. This procedure has served as the most effective treatment of irreversible or termi-

nal organ failure. More so, organ transplantation has been used to improve the quality of life in patients with final stage disease [63].

The liver, kidney, pancreas, intestine, lung, and heart are the major organs that are customarily employed in organ transplantation [64,65]. Unfortunately, despite the improvement in the quality of life of patients with end-stage organ failure, organ transplants have many complications, ranges from neurological, renal, gastrointestinal, hepatic, and cardiovascular, with disastrous morbidity and mortality [66]. These complications, therefore, make patient with organ transplants susceptible to multiple infections or diseases such as COVID-19. The emerging report revealed that early organ post-transplant infection of COVID-19 might lead to devasted morbidity and mortality rates and an increase in hospital admissions, which may pose a significant threat to the healthcare system [67]. Imperatively, patients that receive solid organ transplant are known to have impaired T-cell regulated immune response [68]; therefore, they are susceptible to respiratory viral infections like influenza [69]. In addition, the Centers for Disease Control and Prevention have classified solid organ transplant recipients as people at high risk of severe morbidity from severe acute respiratory syndrome coronavirus (SARS-CoV-2) [70]. A recent systematic review and meta-analysis conducted between January and October 2020 reveal a surging number of hospital admission of solid organ transplant's patients infected with COVID-19 [71].

*Vulnerability to COVID-19 based on ABO blood group type*

A recent study has demonstrated the link between the ABO blood group antigen expression and their roles in many infections by serving as receptors or co-receptors for pathogenic microorganisms such as viruses and parasites [72]. This report is an indication that there is a precise interplay between disease transmission and ABO blood groups [73]. In a study of the SARS-CoV outbreak in a Hong Kong hospital, individuals with blood group O were shown to have very low odds of infection compared with individuals with blood groups A, B, and A.B. This finding indicates the resistance of blood group O to SARS-CoV infection [74]. The possible explanation for this has been described concerning the similarity between the coronavirus envelope and that of HIV, which targets host cells through a viral adhesion glycoprotein (gp). SAR-CoV has been previously described as a virus with 210–230 kDa glycoprotein that possesses 23 *N*-glycosylation sites and a wide range of structures for glycan analysis with 2–4 antennae that can support ABH epitopes.

Moreover, since the respiratory and gastrointestinal epithelium is the target of the SARS-CoV, there is a possibility that expression of ABH antigens on the S protein and host envelope glycosphingolipids are most common in human isolates. Also, human anti-A and monoclonal anti-A can block S protein-expressing A-antigen, such as the case of the envelope protein [74,75]. The latter study emanated from China and has provided us with information on how certain blood groups may be susceptible to novel SARS-CoV-2 than the other. Another study compared the ABO blood group in 2173 SARS-CoV-2 infected patients with non-infected people from the same geographical area. A higher risk of SARS infection was found in blood group A of the infected individual compared with non-infected blood group A. In the same study, the lower risk of SARS-CoV-2 infection was observed in blood group O of the infected patients compared to the blood group O of non-infected individuals [76]. This study has given us more direction about the association between the blood groups and coronavirus susceptibility (Fig. 2). Further studies are needed to validate this evidence.

## Anatomical sanctuary sites in SARS-COV-2 pathophysiology

While anatomical' sanctuary sites' could become viral reservoirs wherein the virus may become sequestered with the tendency of causing viral rebounds or reinfection after remission, there are still many aspects of this new pathogen-causing SARS-CoV-2 that is yet to be fully unravelled. As research intensifies, it will become more evident to researchers why the binding processes of the viral skeleton trigger the immune responses that ultimately shut down the functionality of many organs and systems in the body as well as why some specific categories of individuals and sex respond aggressively to the viral infection than others. The anatomical sanctuary sites are common extra lymphatic organs, such as the lung, testis, kidney, brain, genital tract, lymph nodes, and blood [77–79], that could encourage viral escape. They are the sources of drug-resistant, which makes virus eradication and cure difficult such that viral rebound inevitably occurs once antiviral is stopped [80]. The biological barriers that exist between the blood and different organs of the body serve as a defense mechanism against the spreading of toxins and infection to different organs [81].

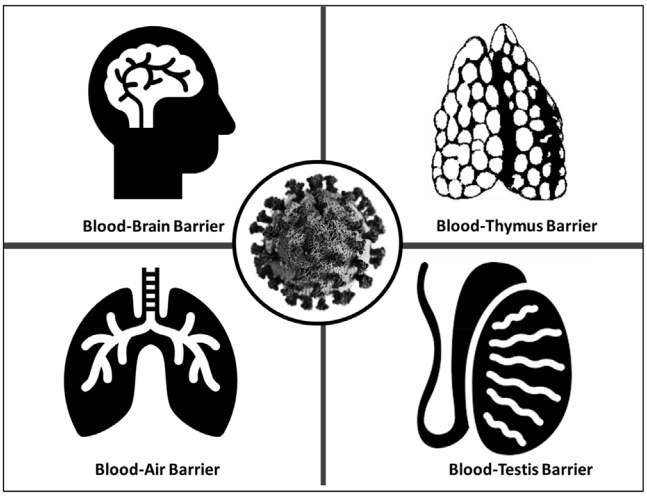


**Fig. 3.** Schematic illustration showing the potential relationship between different blood-organ barriers and SARS-CoV-2 infection. Many of these barriers are located in anatomical satellite sites. They include the Blood-Brain Barrier (BBB), Blood-Thymus Barrier (BThB), Blood-Air Barrier (BAB), and Blood-Testis Barrier (BTB).

Testis, as an anatomical sanctuary organ, presents a biological barrier known as the Blood-Testis Barrier (BTB), a unique barrier with complex structural configuration (Fig. 3). This barrier comprises adherent junction, tight junction, desmosome, tubulobulbar complex, ectoplasmic specialization, gap junction, and hemidesmosome [82]. The BTB forms demarcation that protects the functional parts of the seminiferous tubule from infectious disease, toxins, foreign bodies, and hormonal imbalance that may disrupt the reproductive function [83]. A prior study has shown the importance of the drug transporters and receptors at the junction of these barriers as determinants of the level of chemicals and drugs that can enter the testis both in disease and healthy situations [84].

The brain is another anatomical sanctuary organ that also has a Blood-Brain Barrier (BBB). This biological barrier functions to protect the brain tissue from harmful substances, pathogens, and toxic substances [85]. BBB permits the passage of viruses but restricts the penetration of certain drugs [86]. Specific receptors and carrier proteins have been described as determining factors for the entry of essential nutrients and substances through BBB [87].

Another biological barrier is the Blood-Thymus Barrier (BThB), a physiological and selective barrier that separates T-lymphocytes from the blood and the cortical capillaries inside the cortex of the thymus [88]. This barrier is formed by many layers of cells that primarily serve to impede the movement of macromolecules from the vascular environment to the trabeculae of the cortex.

**Table 1**
Tissue distribution of Angiotensin-Converting Enzyme II receptors (ACE2) in tissues/organs and currently reported the detection of severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 in anatomic satellite organs.

| S/N | Tissue expression of ACE2 | References | Detection of SARS-CoV | References | Detection of SARS-CoV-2 | References |
|---|---|---|---|---|---|---|
| 1. | Lung (Alveolar epithelial cells) | [34,97] | Presence of CoV-like structures | [114–117] | Presence of SARS-CoV-2 | [118] |
| 2. | Small intestine (enterocytes) | [34,119] | Presence of SARS-CoV | [120,121] | | |
| 3. | Kidney | [101,122,123] | Presence of SARS-CoV | [115,121] | | |
| 4 | Spleen | [34] | Presence of SARS-CoV monocytes | [121] | Presence of SARS-CoV-2 antigen | [124] |
| 5. | Lymph node | [34] | Presence of SARS-CoV | [121] | | |
| 6. | Pancreas | [125,126] | Presence of SARS-CoV | [115,121] | | |
| 7. | Liver | [126,127] | Presence of SARS-CoV | [121] | | |
| 8. | Heart | [46,122,128] | Presence of SARS-CoV | [129] | | |
| 9. | Testis | [100,101,122,130] | Presence of SARS-CoV in testicular epithelial cells | [131] | | |
| 10. | CNS (Brain, CSF) | [103,105,132] | Presence of SARS-CoV | [105,107,133,134] | Presence of SARS-CoV-2 | [104] |

The vascular endothelium covered by the pericytes forms the first layer of this barrier. The next layer superficial to the endothelial cells and pericytes in the squamous thymic epithelial cells (STEC). The squamous thymic epithelial cells are reticular and epithelial cells that are connected by occluding junctions and desmosomes [81]. Thymic epithelial cells do not permit the transport of macromolecules and foreign antigens, but it allows the exchange of tiny molecules through their high density of intercellular junctions and the membrane transporters [89]. Various conditions and factors such as sex hormones, adrenocorticotropic hormone, stress, and glucocorticoids can compromise the integrity of the BThB. More so, they can pave the way for the entry of foreign antigens into the cortex of the thymus, consequently, lead to impaired immunity [88]. The thymus has been described as a target for various infections that may affect the actions of the peripheral T-lymphocytes [90]. The expression of the ACE2 has been discovered in the thymus, which could make the thymus susceptible to the tropism of the novel SARS-CoV-2.

The Blood-Air Barrier (BAB) is a functional barrier formed by the two alveolar epithelial cells of the peripheral lung [91]. The alveolar epithelial type I (ATI) cells account for 90–95% of the alveolar surface with large size, while alveolar type II (ATII) cells are cuboidal in shape, smaller in size, and represent 5% of the alveolar surface [92]. The alveolar type I cell create a large surface area for the exchange of air and involved in the transport of protein and ion. In contrast, alveolar type II participated in the production of surfactant and are progenitor cells for ATI cell regeneration [93,94]. The BAB is of clinical significance in the drug delivery system [95]. There was a report that aerosol drug administration to the respiratory system allows the delivery of both macro and micro molecules. Moreover, it aids the rapid absorption of drugs and avoids the first-pass effect [91]. High expression of ACE2 has been widely documented in the

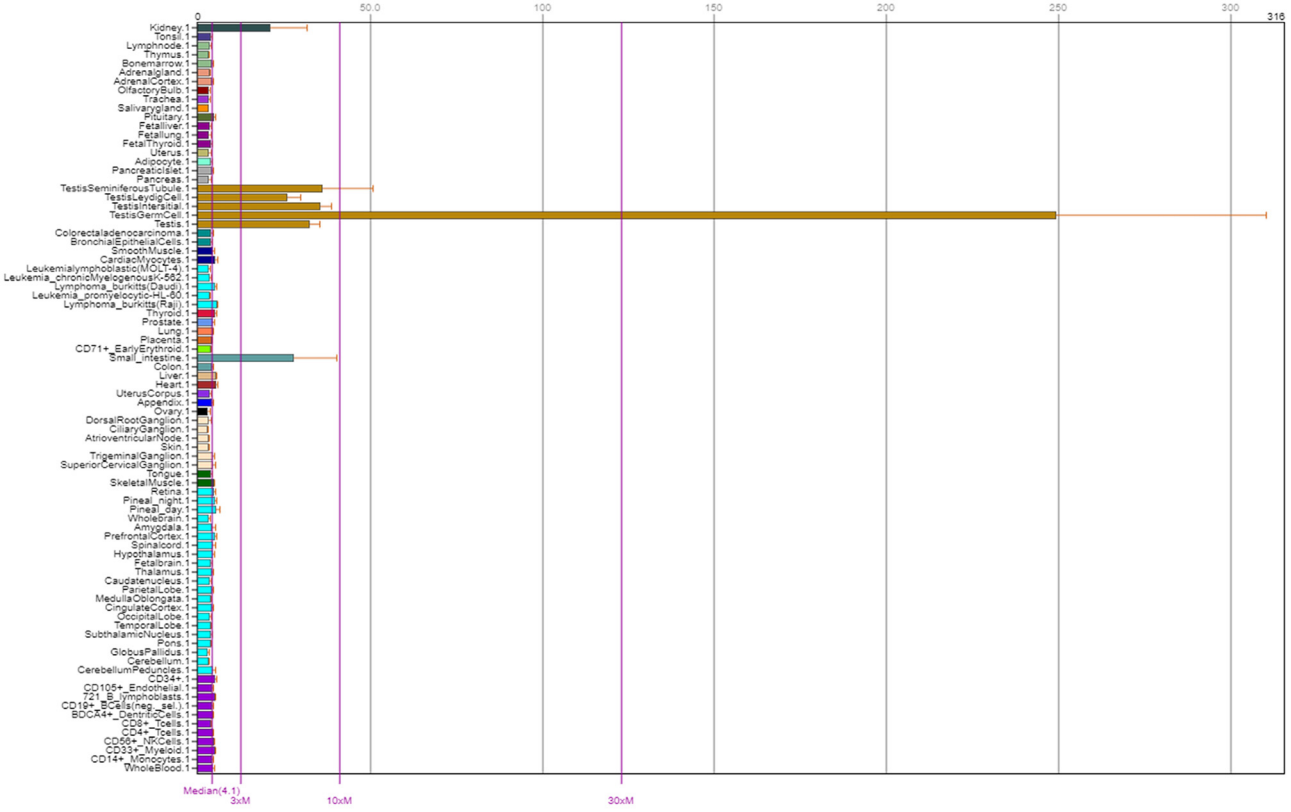


**Fig. 4.** Bar chart showing the tissue-enriched gene expression profile of ACE2, the receptor for SARS-CoV-2 in normal 79 human tissues (176 distinct tissue/cell samples). ACE2 is found to be highly expressed in the testis, small intestine, and the kidney while other cells have medium to low expression of ACE2. Adapted from the BioGPS website: http://ds.biogps.org/?dataset=GSE1133&gene=59272.

human airways and lung parenchyma [34,96,97]. This expression implicates BAB in SARS-CoV-2 infectivity as long as the required quantity of ACE2 receptors is available.

The importance of anatomical sanctuary sites in disease pathogenesis cannot be overemphasized. Anatomical sanctuary organs can bring about the reactivation of pathogens, including viruses, during immunocompromised conditions, such as pregnancy, chemotherapy, organ transplants, and stress, and consequently complicate virus elimination [98]. Emerging reports from the latest published fluorescent studies show that SARS-CoV-2 uses Angiotensin-Converting Enzyme II (ACE2) receptors as the mechanism of viral entry and infect the host cells [40,99]. ACE2 is expressed at the RNA and protein levels in many tissues and organs in the human body (Table 1). Other studies have reported high expression of ACE2 in the alveolar type I and type II epithelial cells. Also, in the Leydig cells and spermatogenic cells of the seminiferous tubules of the testis. This evidence may lead to lung and testicular tissue damage after SARS-CoV-2 infection [100,101]. Since ACE2 has been confirmed to be the significant structural and functional receptor that mediates the entry of SARS-CoV-2 into the human cells, there is a high possibility that most ACE2 expressing tissues in the body may be potential reservoir sites for SARS-CoV-2 infection and reactivation. Interestingly, a newly published study on thirty SARS-CoV-2 infected patients has reported SARS-CoV-2 in the semen of four infected patients and the urine of seven patients [102]. This study has shown that urogenital tracts harbours SARS-CoV-2 and may serve as a viral reservoir site.

The latest finding documented the expression of ACE2 receptor in the brain tissue to neuronal pathogenicity of SARS-CoV-2 [103]. The presence of SARS-CoV2 was detected in the cerebrospinal fluid of SARS-CoV-2 patients [104]. The expression of ACE2 receptors has been previously reported in the neurons and neuroglia. This evidence shows the susceptibility of the brain tissue to SARS-CoV-2 (Fig. 4). Similarly, neuronal death has been attributed to the SARS-CoV presence in the brain tissues, which may have escaped into the brain through olfactory epithelial cells of the nasal cavity [105]. The pieces of evidence mentioned above implicate the anatomical sanctuary sites/organs in the pathogenesis and possible reinfection of SARS-CoV-2 in recovered patients. Also, the multi-tissue expression of ACE2 receptors, as seen in the testis, small intestine, kidney, among others, may provide a hiding site for SARS-CoV-2 in the body and aid the prolonged pathogenicity and viral re-entry into the systemic circulation (Table 1). This evidence may be responsible for the multiple waves of cytokine storms observed in SARS-CoV-2 patients, as well as multi-organ failures, including reproductive, digestive, urogenital, and neurological disorders.

In addition to the ACE 2 receptors, the latest findings have shown that SARS-CoV-2 can use Transmembrane serine protein 2 (TMPRSS 2) and Cathepsin (CatB/L) for cell entry [106]. Furthermore, recent reports have underscored the expressions of ACE 2 and TMPRSS 2 [107] and the cathepsin L in different regions of the brain [108,109].

A recent preliminary study has shown high expression of TMPRSS 2 was observed in the lung, a target tissue for SARS-CoV-2, the testis, prostate gland, kidney, colon, intestine, thyroid gland, and the breast. Therefore, the transmembrane protein expression could make these tissues susceptible to the entry and tropism of the novel SARS-CoV-2 [110].

Cathepsin L is involved in many diseases' conditions such as viral infection, metabolic disorders, cancer, inflammatory conditions, musculoskeletal disorders, and renal diseases. Expressions of the cathepsin L receptors in the human lung epithelial cells have been reported [111]. This lysosomal enzyme's expression is up-regulated in the chronic inflammatory process and participates in lowering the extracellular matrix, an essential activity for the entry of SARS-CoV-2 into the host cells [112].

## Conclusion

This review employed the available literate to explore the microbiologic perspectives. Also, it suggests that for the management of SARS-CoV-2 infection involving antiviral drugs, careful consideration of the anatomical sanctuary sites as hiding places for SARS-CoV-2 and the presence of pathologic lesions must be taken seriously. This is as a result of emerging reports on COVID-19 points to the possibility of reinfection or viral reactivation. Currently, coronaviruses have neither been reported to encode an integrase like enzyme nor have such mechanisms previously described for HIV. However, HIV infection has been proven to encode an integrase enzyme that facilitates the integration of the viral genome into the host cellular genome. This mechanism is responsible for evading the host's immune system and rebound when antiretroviral drugs are withdrawn or when favourable conditions arrive. Therefore, future research should look toward encoding enzyme and mechanism that supports SARS-CoV-2 replication and rebound at the withdrawal of antiviral drugs currently used to manage the chronic SARS-CoV-2 infection.

Careful diagnostic and prognostic modalities should be put in place to check for viral localization in anatomical satellite sites before discharging recovered or asymptomatic patients. In addition, long-term effects of viral tropism and the pathophysiology of SARS-CoV-2 in anatomical satellite organs/sites need to be studied to understand this disease better and eradicate or manage it. This understanding would be beneficial to prevent viral reactivation and viral complications, such as reproductive dysfunction, neurological disorders, endocrine disorders, kidney problems, and liver disorders.

## Possible strategies to circumvent ace receptor-mediated virus compartmentalization as future prospect for covid-19 control

At the onset of SARS-CoV-2, many therapeutic modalities have been employed. Currently, some vaccines have been approved and in use while others are in the pipeline, though there are no approved drugs to treat the SARS-CoV-2 virus. Since ACE 2, TMPRSS 2, and Cathepsin are essential to the entry and tropism of virus, any drugs expressing the ability of their inhibition could act as a potential SARS-CoV-2 therapeutic modality. More so, as one of the ways to get around the ACE receptor-mediated virus compartmentalization, we hereby suggest the list of examples of brand and generic drug for ACE inhibitors such as benazepril (Lotensin), captopril (Capoten- discontinued brand), enalapril (Vasotec, Epaned, [Lexxel- discontinued brand]), fosinopril (Monopril- Discontinued brand), lisinopril (Prinivil, Zestril, Qbrelis), moexipril (Univasc-Discontinued brand), perindopril (Aceon), quinapril (Accupril), ramipril (Altace), trandolapril(https://www.medicinenet.com/ace_inhibitors/article.htm#why_are_ace_inhibitors_prescribed_uses); Transmembrane protease, serine 2 (TMPRSS2) inhibitors drugs like, Nafamostat mesylate, Camostat mesylate, Aprotinin, Rimantadine (https://www.rndsystems.com/target/tmprss2-inhibitors) and Cathepsin inhibitors [113]. These drugs could be used synergistically with other drugs to prevent viral invasion. Hence, it could serve as a prospect for SARS-CoV-2 control.

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All authors have read and accepted responsibility for the content of the manuscript

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## References

[1] Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. Int J Infect Dis 2020;10 (10.1016).
[2] Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, et al. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. Nature 2018;556(7700):255–8.
[3] Cui J, Li F, Shi Z-L. Origin, and Evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17(3):181–92.
[4] McIntosh K, Hirsch MS, Bloom A. Coronavirus disease 2019 (COVID-19), 30; 2020. Available from: https://www.UpToDate.com/contents/coronavirus-disease-2019-COVID-19-epidemiology-virology-clinical-features-diagnosis-and-prevention. [Accessed 2020].
[5] Yang Y, Peng F, Wang R, Guan K, Jiang T, Xu G, et al. The deadly coronaviruses: the 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. J Autoimmun 2020;1:109, 102434.
[6] WHO, March 19th, 2020 Considerations for quarantine of individuals in the context of containment for coronavirus disease (COVID-19): interim guidance. World Health Organization; 2020.
[7] Liu Q, Liu Z, Zhu J, Zhu Y, Li D, Gao Z, et al. Assessing the global tendency of COVID-19 outbreak. medRxiv 2020.
[8] Monchatre-Leroy E, Boué F, Boucher JM, Renault C, Moutou F, Ar Gouilh M, et al. Identification of alpha and beta coronavirus in wildlife species in France: bats, rodents, rabbits, and hedgehogs. Viruses 2017;9(12):364.
[9] Peng X, Xu X, Li Y, Cheng L, Zhou X, Ren B. Transmission routes of 2019-nCoV and controls in dental practice. Int J Oral Sci 2020;12(1):1–6.
[10] Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YS, et al. The genome sequence of the SARS-associated Coronavirus. Science 2003;300(5624):1399–404.
[11] De Haan CA, Masters PS, Shen X, Weiss S, Rottier PJ. The group-specific murine coronavirus genes are not essential, but their deletion, by reverse genetics, is attenuating in the natural host. Virology 2002;296(1):177–89.
[12] Kim JM, Chung YS, Jo HJ, Lee NJ, Kim MS, Woo SH, et al. Identification of coronavirus isolated from a patient in Korea with COVID-19. Osong Public Health Res Perspect 2020;11(1):3.
[13] Han Q, Lin Q, Ni Z, You L. Uncertainties about the transmission routes of 2019 novel coronavirus. Influenza Other Respir Viruses 2020;14(4):470.
[14] Ather A, Patel B, Ruparel NB, Diogenes A, Hargreaves KM, et al. Coronavirus Disease 19 (COVID-19): implications for clinical dental care. J Endod 2020;46(5):584–95.
[15] Perisetti A, Gajendran M, Boregowda U, Bansal P, Goyal H. COVID-19, and gastrointestinal endoscopies: current insights and emergent strategies. Dig Endosc 2020;32(5):715–22.
[16] Khanna RC, Honavar SG. All eyes on coronavirus—what do we need to know as ophthalmologists. Indian J Ophthalmol 2020;68(4):549.
[17] Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect 2020;104(3):246–51.
[18] Wu Y, Guo C, Tang L, Hong Z, Zhou J, Dong X, et al. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol Hepatol 2020;5(5):434–5.
[19] Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, et al. The presence of SARS-CoV-2 RNA in feces of COVID-19 patients. J Med Virol 2020;92(7):833–40.
[20] Munster VJ, Koopmans M, van Doremalen N, van Riel D, de Wit E. A novel Coronavirus emerging in China—key questions for impact assessment. N Engl J Med 2020;382(8):692–4.
[21] Lu CW, Liu XF, Jia ZF. 2019-nCoV transmission through the ocular surface must not be ignored. Lancet (London, England) 2020;395(10224):e39.
[22] Lu Qi, Shi Y. Coronavirus disease (COVID-19) and neonate: what neonatologist need to know. J Med Virol 2020;92(6):564–7.
[23] Jiatong S, Wenjun L. COVID-19 epidemic: disease characteristics in children. J Med Virol 2020.
[24] Davanzo R, Moro G, Sandri F, Agosti M, Moretti C, Mosca F. Breastfeeding, and coronavirus Disease-2019. Ad interim indications of the italian society of neonatology endorsed by the union of european neonatal & perinatal societies. Matern Child Nutr 2020;16(3):e13010.
[25] Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 2020;395(10226):809–15.
[26] Jonsdottir HR, Dijkman R. Coronaviruses, and the human airway: a universal system for virus-host interaction studies. Virol J 2016;13(1):24.
[27] Bosch BJ, Smits SL, Haagmans BL. Membrane ectopeptidases targeted by human coronaviruses. Curr Opin Virol 2014;6:55–60.
[28] Heald-Sargent T, Gallagher T. Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence. Viruses 2012;4(4):557–80.
[29] Bertram S, Dijkman R, Habjan M, Heurich A, Gierer S, Glowacka I, et al. TMPRSS2 activates the human coronavirus 229E for cathepsin-independent host cell entry and is expressed in viral target cells in the respiratory epithelium. J Virol 2013;87(11):6150–60.
[30] Glowacka I, Bertram S, Müller MA, Allen P, Soilleux E, Pfefferle S, et al. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. J Virol 2011;85(9):4122–34.
[31] Yeager CL, Ashmun RA, Williams RK, Cardellichio CB, Shapiro LH, Look AT, et al. Human aminopeptidase N is a receptor for human coronavirus 229E. Nature 1992;357(6377):420–2.
[32] Tseng CT, Huang C, Newman P, Wang N, Narayanan K, Watts DM, et al. Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human Angiotensin-converting enzyme two virus receptor. J Virol 2007;81(3):1162–73.
[33] Hofmann H, Pyrc K, Van Der Hoek L, Geier M, Berkhout B, Pöhlmann S. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. Proc Natl Acad Sci U S A 2005;102(22):7988–93.
[34] Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. The first step in understanding SARS pathogenesis. J Pathol 2004;203:631–7.
[35] Lambeir AM, Durinx C, Scharpé S, De Meester I. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. Crit Rev Clin Lab Sci 2003;40(3):209–94.
[36] Raj VS, Mou H, Smits SL, Dekkers DH, Müller MA, Dijkman R, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 2013;495(7440):251–4.
[37] Krempl C, Schultze B, Herrler G. Analysis of cellular receptors for human coronavirus OC43. In: Corona-and Related Viruses. Springer; 1995. p. 371–4.
[38] Huang X, Dong W, Milewska A, Golda A, Qi Y, Zhu QK, et al. Human coronavirus HKU1 spike protein uses *O*-acetylated sialic acid as an attachment receptor determinant and employs hemagglutinin-esterase protein as a receptor-destroying enzyme. J Virol 2015;89(14):7202–13.
[39] Li B, Si HR, Zhu Y, Yang XL, Anderson DE, Shi ZL, et al. Discovery of bat coronaviruses through surveillance and probe capture-based next-generation sequencing. Msphere 2020;5(1), e00807-19.
[40] Gralinski LE, Menachery VD. Return of the Coronavirus: 2019-nCoV. Viruses 2020;12(2):135.
[41] Chan RW, Chan MC, Agnihothram S, Chan LL, Kuok DI, Fong JH, et al. Tropism of and innate immune responses to the novel human betacoronavirus lineage C virus in human ex vivo respiratory organ cultures. J Virol 2013;87(12):6604–14.
[42] Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Seminars in immunopathology, 39. Berlin Heidelberg: Springer; 2017. p. 529–39.
[43] Ahmad T, Khan M, Haroon TH, Nasir S, Hui J, Bonilla-Aldana DK, et al. COVID-19: zoonotic aspects. Travel Med Infect Dis 2020;36:101607.
[44] Singhal T. A review of coronavirus disease-2019 (COVID-19). Indian J Pediatr 2020:1–6.
[45] CDC. Severe outcomes among patients with coronavirus disease 2019 (COVID-19)-United States, February 12–March 16, 2020. MMWR Morb Mortal Wkly Rep 2020;69:343–6.
[46] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395(10223):507–13.
[47] Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. JAMA 2020.
[48] Iyer SP, Ensor J, Anand K, Hwu P, Subbiah V, Flowers C, et al. Higher mortality in men from COVID19 infection-understanding the factors that drive the differences between the biological sexes. medRxiv 2020.
[49] Liu S, Zhang M, Yang L, Li Y, Wang L, Huang Z, et al. Prevalence and patterns of tobacco smoking among Chinese adult men and women: findings of the 2010 national smoking survey. J Epidemiol Community Health 2017;71(2):154–61.
[50] Zeng F, Dai C, Cai P, Wang J, Xu L, Li J, et al. A comparison study of SARS-CoV-2 IgG antibody between male and female COVID-19 patients: a possible reason underlying different outcomes between gender. J Med Virol 2020;92(10):2050–4.
[51] Peretz J, Pekosz A, Lane AP, Klein SL. Estrogenic compounds reduce influenza A virus replication in primary human nasal epithelial cells derived from female, but not male, donors. Am J Physiol Lung Cell Mol Physiol 2016;310(5):L415–25.
[52] Libert C, Dejager L, Pinheiro I. The X chromosome in immune functions: when a chromosome makes the difference. Nat Rev Immunol 2010;10(8):594–604.
[53] Read R. Flawed methods. In: COVID-19: attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism; 2020.

[54] Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, et al. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res 2020;30(4):343–55.

[55] Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, et al. Comorbidity and its impact on 1590 patients with Covid-19 in China: a Nationwide Analysis. Eur Respir J 2020;55(5).

[56] Bi Q, Bi Q, Wu Y, Mei S, Ye C, Zou X, Zhang Z, et al. Epidemiology and Transmission of COVID-19 in Shenzhen China: analysis of 391 cases and 1,286 of their close contacts. MedRxiv 2020.

[57] Liu K, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: a comparison with young and middle-aged patients. J Infect 2020;80(6):e14-8.

[58] Halpin DM, Faner R, Sibila O, Badia JR, Agusti A. Do chronic respiratory diseases or their treatment affect the risk of SARS-CoV-2 infection? Lancet Respir Med 2020;8(5):436–8.

[59] Luo Y, Yin K. Management of pregnant women infected with COVID-19. Lancet Infect Dis 2020.

[60] Yu N, Li W, Kang Q, Xiong Z, Wang S, Lin X, et al. Clinical features and obstetric and neonatal outcomes of pregnant patients with COVID-19 in Wuhan, China: a retrospective, single-centre, descriptive study. Lancet Infect Dis 2020;20(5):559–64.

[61] Lam CM, Wong SF, Leung TN, Chow KM, Yu WC, Wong TY, et al. A case-controlled study comparing clinical course and outcomes of pregnant and non-pregnant women with severe acute respiratory syndrome. BJOG Int J Obstet Gynaecol 2004;111(8):771–4.

[62] Zhu H, Wang L, Fang C, Peng S, Zhang L, Chang G, et al. Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. Transl Pediatr 2020;9(1):51.

[63] Grinyó JM. Why is organ transplantation clinically important? Cold Spring Harb Perspect Med 2013;3(6):a014985, http://dx.doi.org/10.1101/cshperspect.a014985.

[64] Bezinover D, Saner F. Organ transplantation in the modern era. BMC Anesthesiol 2019;19:32, http://dx.doi.org/10.1186/s12871-019-0704-z.

[65] Beyar R. Challenges in organ transplantation. Rambam Maimonides Med J 2011;2(2):e0049, http://dx.doi.org/10.5041/RMMJ.10049.

[66] Sen A, Callisen H, Libricz S, Patel B. Complications of solid organ transplantation: cardiovascular, neurologic, renal, and gastrointestinal. Crit Care Clin 2019;35(1):169–86, http://dx.doi.org/10.1016/j.ccc.2018.08.011. Epub 2018 October 25. PMID: 30447778.

[67] Chadban SJ, McDonald M, Wyburn K, Opdam H, Barry L, Coates PT. Significant impact of COVID-19 on organ donation and transplantation in a low-prevalence country: australia. Kidney Int 2020;98(6):1616, http://dx.doi.org/10.1016/j.kint.2020.10.007.

[68] L'Huillier AG, Ferreira VH, Hirzel C, Nellimarla S, Ku T, Natori Y. T-cell responses following natural influenza infection or vaccination in solid organ transplant recipients. Sci Rep 2020;10:10104.

[69] Kumar D, Ferreira VH, Blumberg E, Silveira F, Cordero E, Perez-Romero P. A 5-year prospective multicenter evaluation of influenza infection in transplant recipients. Clin Infect Dis 2018;67:1322–9.

[70] Coronavirus Disease. People of any age with underlying medical conditions. Centers for Disease Control and Prevention; 2019.

[71] Raja MA, Mendoza MA, Villavicencio A, Anjan S, Reynolds JM, Kittipibul V, et al. COVID-19 in solid organ transplant recipients: a systematic review and meta-analysis of current literature. Transplant Rev (Orlando) 2020;35(1):100588, http://dx.doi.org/10.1016/j.trre.2020.100588. Advance online publication.

[72] Cooling L. Blood groups in infection and host susceptibility. Clin Microbiol Rev 2015;28(3):801–70.

[73] Cheng Y, Cheng G, Chui CH, Lau FY, Chan PK, Ng MH, et al. ABO blood group and susceptibility to the severe acute respiratory syndrome. JAMA 2005;293(12):1447–51.

[74] Guillon P, Clément M, Sébille V, Rivain JG, Chou CF, Ruvoën-Clouet N, et al. Inhibition of the interaction between the SARS-CoV spike protein and its cellular receptor by anti-histo-blood group antibodies. Glycobiology 2008;18(12):1085–93.

[75] Ritchie G, Harvey DJ, Feldmann F, Stroeher U, Feldmann H, Royle L, et al. Identification of *N*-linked carbohydrates from severe acute respiratory syndrome (SARS) spike glycoprotein. Virology 2010;399(2):257–69.

[76] Zhao J, Yang Y, Huang H, Li D, Gu D, Lu X, et al. Relationship between the ABO blood group and the COVID-19 susceptibility. medRxiv 2021;73(2):328–31.

[77] Azu OO. Highly active antiretroviral therapy (HAART) and testicular morphology: current status and a case for a stereological approach. J Androl 2012;33(6):1130–42.

[78] Bruggeman LA, Ross MD, Tanji N, Cara A, Dikman S, Gordon RE, et al. Renal epithelium is a previously unrecognized site of HIV-1 infection. J Am Soc Nephrol 2000;11(11):2079–87.

[79] Stewart JP, Usherwood EJ, Ross A, Dyson H, Nash T. Lung epithelial cells are a major site of murine gammaherpesvirus persistence. J Exp Med 1998;187(12):1941–51.

[80] Dahl V, Josefsson L, Palmer S. HIV reservoirs, latency, and reactivation: prospects for eradication. Antiviral Res 2010;85(1):286–94.

[81] Bruss DM, Ely S. Anatomy, head and neck, blood thymus barrier. In: StatPearls [Internet]. StatPearls Publishing; 2019.

[82] Cheng, Mruk DD. Regulation of spermiogenesis, spermiation and blood–testis barrier dynamics: novel insights from studies on Eps8 and Arp3. Biochem J 2011;435(3):553–62.

[83] Cheng CY, Mruk DD. Cell junction dynamics in the testis: sertoli-germ cell interactions and male contraceptive development. Physiol Rev 2002;82(4):825–74.

[84] Kis O, Robillard K, Chan GN, Bendayan R. The complexities of antiretroviral drug–drug interactions: role of ABC and SLC transporters. Trends Pharmacol Sci 2010;31(1):22–35.

[85] Keaney J, Campbell M. The dynamic blood-brain barrier. FEBS J 2015;282(21):4067–79.

[86] Jolliet-Riant P, Tillement JP. Drug transfer across the blood-brain barrier and improvement of brain delivery. Fundam Clin Pharmacol 1999;13(1):16–26.

[87] Ghalamfarsa G, Hojjat-Farsangi M, Mohammadnia-Afrouzi M, Anvari E, Farhadi S, Yousefi M, et al. Application of nanomedicine for crossing the blood-brain barrier: theranostic opportunities in multiple sclerosis. J Immunotoxicol 2016;13(5):603–19.

[88] Ribatti D. The discovery of the blood–thymus barrier. Immunol Lett 2015;168(2):325–8.

[89] Palmer E. Negative selection—clearing out the bad apples from the T-cell repertoire. Nat Rev Immunol 2003;3(5):383–91.

[90] Savino W. The thymus is a common target organ in infectious diseases. PLoS Pathog 2006;2(6).

[91] Daum N, Kuehn A, Hein S, Schaefer UF, Huwer H, Lehr CM. Isolation, cultivation, and application of human alveolar epithelial cells. In: Human cell culture protocols. Springer; 2012. p. 31–42.

[92] Crapo JD, Barry BE, Gehr P, Bachofen M, Weibel ER. Cell number, and cell characteristics of the normal human lung. Am Rev Respir Dis 1982;126(2):332–7.

[93] Fehrenbach H. Alveolar epithelial type II cell: defender of the alveolus revisited. Respir Res 2001;2(1):33.

[94] Williams MC. Alveolar type I cells: molecular phenotype and development. Annu Rev Physiol 2003;65(1):669–95.

[95] Grainger CI, Greenwell LL, Lockley DJ, Martin GP, Forbes B. Culture of Calu-3 cells at the air interface provides a representative model of the airway epithelial barrier. Pharm Res 2006;23(7):1482–90.

[96] Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, et al. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on the differentiation of human airway epithelia. J Virol 2005;79(23):14614–21.

[97] Zou X, Chen K, Zou J, Han P, Hao J, Han Z, et al. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med (Lausanne) 2020;12:1–8.

[98] Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation. N Engl J Med 2006;354(21):2235–49.

[99] Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the Novel Coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci 2020;63(3):457–60.

[100] Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem 2000;275(43):33238–43.

[101] Fan C, Lu W, Li K, Ding Y, Wang J. ACE2 expression in kidney and testis may cause kidney and testis damage after 2019-nCoV infection. MedRxiv 2021;7:1045.

[102] Saylam B, Uguz M, Yarpuzlu M, Efesoy O, Akbay E, Çayan S. The presence of SARS-CoV-2 virus in semen samples of patients with COVID-19 pneumonia. Andrologia 2021;11:e14145.

[103] Baig AM, Khaleeq A, Ali U, Syeda H. Evidence of the COVID-19 virus targeting the CNS: tissue distribution, host-virus interaction, and proposed neurotropic mechanisms. ACS Chem Neurosci 2020;11(7):995–8.

[104] Toljan K. Letter to the editor regarding the viewpoint Ëvidence of the COVID-19 virus targeting the CNS: tissue distribution, host–virus interaction, and proposed neurotropic mechanism. ACS Chem Neurosci 2020;11(8):1192.

[105] Netland J, Meyerholz DK, Moore S, Cassell M, Perlman S. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. J Virol 2008;82(15):7264–75.

[106] Hoffmann Markus, Kleine-Weber Hannah, Schroeder Simon, Krüger Nadine, Herrler Tanja, Erichsen Sandra, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181(2):271–80.

[107] Qiao J, Li W, Bao J, Peng Q, Wen D, Wang J, et al. The expression of SARS-CoV-2 receptor ACE2 and CD147, and protease TMPRSS2 in human and mouse brain cells and mouse brain tissues. Biochem Biophys Res Commun 2020;533(4):867–71, http://dx.doi.org/10.1016/j.bbrc.2020.09.042.

[108] Gu YH, Kanazawa M, Hung SY, Wang X, Fukuda S, Koziol JA, et al. Cathepsin L acutely alters microvessel integrity within the neurovascular unit during focal cerebral ischemia. J Cereb Blood Flow Metab 2015;35(11):1888–900.

[109] Funkelstein L, Toneff T, Hwang SR, Reinheckel T, Peters C, Hook V. Cathepsin L participates in the production of neuropeptide Y in secretory vesicles, demonstrated by protease gene knockout and expression. J Neurochem 2008;106(1):384–91.

[110] Piva F, Sabanovic B, Cecati M, Giulietti M. Expression and co-expression analyses of TMPRSS2, a key element in COVID-19. Eur J Clin Microbiol Infect Dis 2020;40(2):451–5.

[111] Gerber A, Welte T, Ansorge S, Bühling F. Expression of Cathepsins B and L in Human Lung Epithelial Cells is Regulated by Cytokines. Adv Exp Med Biol 2000:287–92, http://dx.doi.org/10.1007/0-306-46826-3_31.

[112] Gomes CP, Fernandes DE, Casimiro F, da Mata GF, Passos MT, Varela P, et al. Cathepsin L in COVID-19: from pharmacological evidences to genetics. Front Cell Infect Microbiol 2020;10, http://dx.doi.org/10.3389/fcimb.2020.589505.
[113] Liu T, Luo S, Libby P, Shi GP. Cathepsin L-selective inhibitors: a potentially promising treatment for COVID-19 patients. Pharmacol Ther 2020;213:107587, http://dx.doi.org/10.1016/j.pharmthera.2020.107587.
[114] Ding Yanqing, He Li, Zhang Qingling, Huang Zhongxi, Che Xiaoyan, Hou Jinlin, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol 2004;203(2):622–30.
[115] Ding Y, Wang H, Shen H, Li Z, Geng J, Han H, et al. The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. J Pathol 2003;200(3):282–9.
[116] Nicholls JM, Poon LL, Lee KC, Ng WF, Lai ST, Leung CY, et al. Lung pathology of the fatal severe acute respiratory syndrome. Lancet 2003;361(9371):1773–8.
[117] Shieh WJ, Hsiao CH, Paddock CD, Guarner J, Goldsmith CS, Tatti K, et al. Immunohistochemical, in situ hybridization, and ultrastructural localization of SARS-associated Coronavirus in the lung of a fatal case of severe acute respiratory syndrome in Taiwan. Hum Pathol 2005;36(3):303–9.
[118] Liu R, Han H, Liu F, Lv Z, Wu K, Liu Y, et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. Clin Chim Acta 2020;505:172–5.
[119] Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature 2012;487(7408):477–81.
[120] Shi X, Gong E, Gao D, Zhang B, Zheng J, Gao Z, et al. Severe acute respiratory syndrome-associated Coronavirus is detected in intestinal tissues of fatal cases. Am J Gastroenterol 2005;100(1):169–76.
[121] Zhang QL, Ding YQ, Hou JL, He L, Huang ZX, Wang HJ, et al. Detection of severe acute respiratory syndrome (SARS)-associated coronavirus RNA in autopsy tissues with in situ hybridization. Di Yi Jun Yi Da Xue Xue Bao 2003;23(11):1125–7.
[122] Riordan James F. Angiotensin-I-converting enzyme and its relatives. Genome Biol 2003;4(8):225.
[123] Ye M, Wysocki J, William J, Soler MJ, Cokic I, Batlle D. Glomerular localization and expression of angiotensin-converting enzyme 2 and angiotensin-converting enzyme: implications for albuminuria in diabetes. J Am Soc Nephrol 2006;17(11):3067–75.
[124] Feng Z, Diao B, Wang R, Wang G, Wang C, Tan Y, et al. The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) directly decimates human spleens and lymph nodes. medRxiv 2020.
[125] Bindom SM, Hans CP, Xia H, Boulares AH, Lazartigues E. Angiotensin I–converting enzyme type 2 (ACE2) gene therapy improves glycemic control in diabetic mice. Diabetes 2010;59(10):2540–8.
[126] Roca-Ho H, Riera M, Palau V, Pascual J, Soler MJ. Characterization of ACE and ACE2 expression within different organs of the NOD mouse. Int J Mol Sci 2017;18(3):563.
[127] Cheng, Leung Po Sing. An update on the islet renin-angiotensin system. Peptides 2011;32(5):1087–95.
[128] Goulter AB, Goddard MJ, Allen JC, Clark KL. ACE2 gene expression is up-regulated in the human failing heart. BMC Med 2004;2(1):19.
[129] Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, et al. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. Eur J Clin Invest 2009;39(7):618–25.
[130] Wang Z, Xu X. scRNA-seq Profiling of human testes reveals the presence of the ACE2 receptor, a target for SARS-CoV-2 infection in spermatogonia, leydig, and sertoli cells. Cells 2020;9(4):920.
[131] Lang ZW, Zhang LJ, Zhang SJ, Meng X, Li JQ, Song CZ, et al. A clinicopathological study of three cases of severe acute respiratory syndrome (SARS). Pathology 2003;35(6):526–31.
[132] Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin-converting enzyme. FEBS Lett 2002;532(1–2):107–10.
[133] Gu J, Gong E, Zhang B, Zheng J, Gao Z, Zhong Y, et al. Multiple organ infection and the pathogenesis of SARS. J Exp Med 2005;202(3):415–24.
[134] Lau KK, Yu WC, Chu CM, Lau ST, Sheng B, Yuen KY. Possible central nervous system infection by SARS coronavirus. Emerging Infect Dis 2004;10(2):342.