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Archives of Physiology and Biochemistry
The Journal of Metabolic Diseases

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iarp20

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To cite this article: Heena Rehman & Md Iftekhar Ahmad (2020): COVID-19: a wreak havoc across the globe, Archives of Physiology and Biochemistry, DOI: 10.1080/13813455.2020.1797105

To link to this article: https://doi.org/10.1080/13813455.2020.1797105

ARCHIVES OF PHYSIOLOGY AND BIOCHEMISTRY
https://doi.org/10.1080/13813455.2020.1797105

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REVIEW ARTICLE

COVID-19: a wreak havoc across the globe

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ABSTRACT

Coronavirus disease (COVID-19) is an infectious airborne viral pneumonia caused by a novel virus belonging to the family *coronaviridae*. On February 11, 2019, the Internal Committee on Taxonomy of Virus (ICTV) announced the name of the novel virus as "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). One of the proteins present on its membrane i.e. the Spike protein is responsible for the attachment of the virus to the host. It spreads through the salivary droplets released when an infected person sneezes or coughs. The best way to slow down the disease is by protecting self by washing hands and using the disinfectant. Most of the infected people experience mild to moderate breathing issues. Serious illness might develop in people with underlying cardiovascular problems, diabetes and other immuno-compromised diseases. To date, there is no effective medicine available in the market which is effective in COVID-19. However, healthcare professionals are using ritonavir, flavipiravir, lopinavir, hydroxychloroquine and remdesivir. Along with the medicines, some countries are using convalescent plasma and mesenchymal stem cells for treatment. Till date, it has claimed millions of death worldwide. In this detailed review, we have discussed the structure of SARS-CoV-2, essential proteins, its lifecycle, transmission, symptoms, pathology, clinical features, diagnosis, prevention, treatment and epidemiology of the disease.

ARTICLE HISTORY
Received 21 May 2020
Accepted 6 July 2020
Published online 29 July 2020

KEYWORDS
COVID-19; spike protein; ACE2; acute respiratory distress syndrome; real time reverse transciptase; polymerase chain reaction; cellular and humoral immunity

Introduction

The shape of the coronavirus is variable from spherical, elliptical to pleomorphic form with the size of 125 nm as shown by cryo-electron tomography and cryo-electron microscopy (Neuman 2006, Bárcena *et al*. 2009). The characteristic feature of coronavirus is the presence of club or clove head-shaped spikes on the surface. The name of coronavirus is named after the presence of spikes (corona refers to the crown). Some of the common features of coronavirus are the presence of conserved genomic organisation with a large replicase gene preceded by structural and accessory genes; expression of non-structural genes by ribosomal frameshifting, several unique enzymatic activities encoded within replicase–transcriptase polyprotein, expression of the downstream gene by 3′ nested subgenomic messenger RNA (mRNA) (Strauss and Strauss 2008). The viral envelope is comprised of phospholipids, proteins and glycoproteins. The glycoprotein present on the surface of the envelope facilitates the identification and binding to the receptor. There are four proteins present on the surface of coronavirus, namely-spike (S), membrane (M), envelope (E), and haemagglutinin (HA) proteins (Figure 1).

Taxonomical classification

Coronaviruses are considered the largest group of RNA viruses belonging to the order Nidovirales. The order Nidovirales is named so because of the presence of 3′ nested subgenomic ribonucleic acid (RNA). In Latin, nidus refers to nest. The Nidovirales is comprised of four different families, named as Coronaviridae, Mesoniviridae, Arteriviridae and Roniviridae. The family Coronaviridae comprises of two subfamilies, namely Coronavirinae and Tonovirinae. The subfamily Coronavirinae is further classified into four groups, namely alpha, beta, gamma and delta virus based on the phylogenetic clustering. The SARS-CoV-2 belongs to the beta group of the subfamily Coronavirinae.

Genomic organisation

The genome of coronavirus is positive-sense, non-segmented, linear RNA of 28–32 kilobase (Lee *et al*. 1991, Bonilla *et al*. 1994, Drosten *et al*. 2003). The genome is helically coiled and is encapsulated by nucleocapsid (N) protein. The genome consists of 5′ cap structure and 3′ poly adenine (A) tail which facilitates in acting as mRNA for translation of polyproteins (Van Marle *et al*. 1995). The replicase gene comprises around 20 kilobase of the genome and encodes non-structural proteins. The 5′ end of RNA consists of leader sequence and untranslated region that contains multiple stem-loop structure. This multiple stem lop structure is required for replication and transcription. The 3′ (untranslated region) UTR is essential for the replication and synthesis of viral RNA. The gene in coronavirus is organised as 5′leader-UTR-replicase-S-E-M-N-3′UTR-poly(A) tail (Figure 2).

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The accessory proteins are not important for *in vitro* replication, but they play a significant role in the pathogenesis (Zhao *et al*. 2012).

Structure of virion

The coronavirus is an enveloped virus and beneath the envelope lays the nucleocapsid. The nucleocapsid of coronaviruses is helically symmetrical. The helically symmetric nucleocapsid is a common feature of negative-sense RNA virus, and it is rarely observed in positive-sense RNA viruses. The envelope of coronavirus consists of four principal structural proteins; all of these are encoded within the 3′ end of the viral genome.

Spike (S) protein

The spike (S) protein is a 180-kilodalton sized glycoprotein encoded by a gene of around 14,000 nucleotide bases. The S protein facilitates the attachment of the virion to the host receptor (Collins *et al*. 1982). Apart from the attachment, the S protein plays a significant role in cell tropism, host selection, and species specificity (Li *et al*. 2006b, Graham and Baric 2010, Li 2013). The structure of S protein was described by cryoelectron microscopy. The S protein is comprised of three segments: large ectodomain, single-pass transmembrane anchor and a short intracellular tail. In some of the coronaviruses, the ectodomain of S protein is cleaved by the host cell furin-like protease in two polypeptides, namely S1 and S2 (Luytjes *et al*. 1987, Abraham *et al*. 1990). The S1 forms the large receptor binding domain and the S2 forms the stalk of the spike protein (Horzinek *et al*. 1987). Both of them are non-covalently linked to each other. In SAR-CoV-2, the S protein binds to the angiotensin-converting enzyme 2 (ACE2) through S1 and S2 facilitates the fusion of viral and host membrane. It uses the serine protease TMPRSS2 for the priming of S protein (Matsuyama *et al*. 2010, Shulla *et al*. 2011, Glowacka *et al*. 2011).

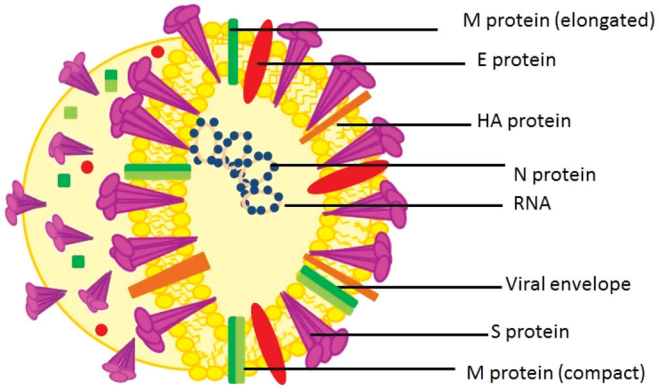

Figure 1. Structure of SARS-CoV-2.

The binding of the murine hepatitis virus (MHV) has been mapped to the amino (N)-terminal 330 amino acids of S1 (Taguchi 1995), and the membrane fusion function resides in S2 region (Yoo *et al*. 1991). The head of the S protein is dimeric (Vennema *et al*. 1990, Lewicki and Gallagher 2002) or trimeric (Delmas and Laude 1990, Beniac *et al*. 2006) and the stalk is trimeric (Beniac *et al*. 2006, Li *et al*. 2006a, Kirchdoerfer *et al*. 2016, Walls *et al*. 2016). The head of S protein sits on the top of S2 stalk and prevents it from undergoing any conformational transition. The S1 protein has two different domains, namely S1 carboxy-terminal domain (CTD) and S1 amino-terminal domain (NTD). The S1 CTD domain is located at the top of the spike and the S1 NTD domain is in direct contact with the structurally constrained S2 stalk. The S2 protein has two different domains, namely HR-N (96AA) and HR-C (39AA). The HR-N is formed of a helically coiled polypeptide, whereas, the polypeptide region of HR-C is poorly ordered. The HR-N region is closed to the fusion peptide and the HR-C region is closed to the transmembrane (TM) anchor region. Downstream from the N-terminus of S2, a polypeptide naming fusion peptide is located. As a consequence of the presence of fusion peptide between the S1 and S2, it is also referred to as internal fusion peptide. The fusion peptide is comprised of short helical structures and a loop in which the hydrophobic amino acid residues are buried inside the pre-fusion structure. The HR region forms a six-helix bundle that is comprised of homotrimeric coiled-coil of HR-N domain. The HR-C region is packed antiparallel to the HR-N region. There are two proteolysis sites present

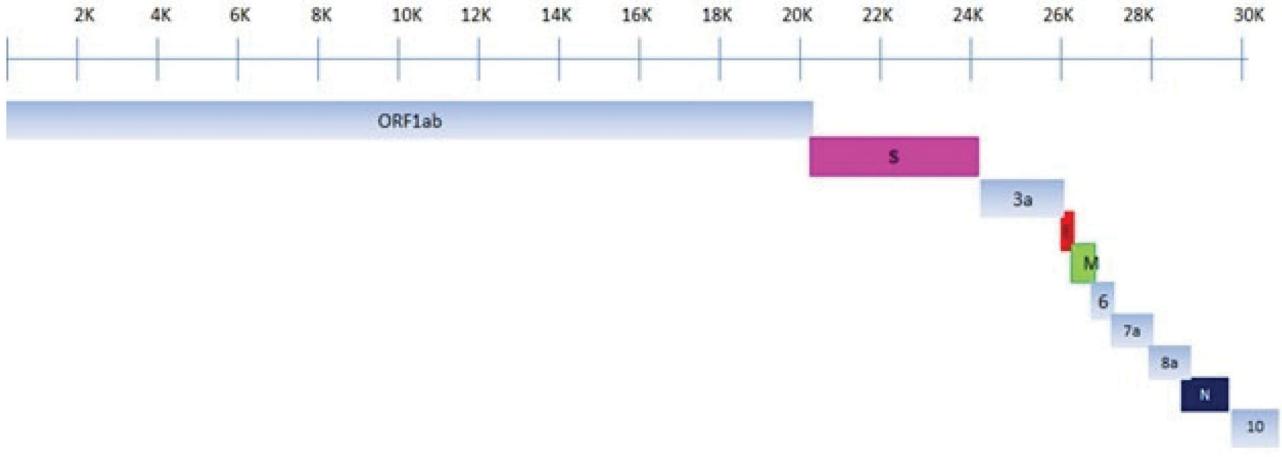

Figure 2. Organisation of genes in SARS-CoV-2 with their respective sizes in kilobase pair.

between the S1 and S2 which play a significant role in the conformational transition of S2. One of the proteolysis sites is present at S1/S2 boundary and the other is present at the N terminus of the internal fusion peptide. The S protein exists in two different conformations, namely- prefusion and postfusion. The transition from pre-fusion to post-fusion is trigger-dependent. The trigger can be proteolytic priming, trypsin cleavage, receptor binding, incubation with urea, low pH, and exposure to high temperature (White *et al*. 1981, Stein *et al*. 1987, Mothes *et al*. 2000).

Haemagglutinin protein (HA)

The HA protein is expressed as a single chain precursor. The pre-fusion structure is quite similar to the structure of S protein. Similar to the S protein, it is also a trimeric protein and is cleaved by host protease into HA1 and HA2 subunits. The HA1 subunit binds to the receptor, whereas, the HA2 protein is associated with the fusion of membranes. During the entry of virion into the cell, the HA1 binds to the sugar receptor and then dissociates after viral entry. The HA2 region undergoes a dramatic conformational change after the entry. In the pre-fusion state, the three hydrophobic regions of HA2 remain buried; however, they become exposed after fusion and insert into the target host membrane. Though the pre-fusion structure of HA protein is energetically more stable than the post-fusion structure, still it requires energy for conformational transformation. This activation energy is provided by proteolytic priming, receptor binding, low pH or combination of two (White *et al*. 1981, Stein *et al*. 1987, Mothes *et al*. 2000).

Membrane protein (M)

The M protein is associated with the assembly of the virus by helping during the formation of new viral particles. The M protein boosts the assembly of protein by interacting with the ribonucleoprotein of virus and S glycoprotein at the exit or the budding site (Nguyen and Hogue 1997, de Haan *et al*. 1999, Narayanan *et al*. 2000, Escor *et al*. 2001a, 2001b, Kuo and Masters 2002). It does so by interacting with them through the endodomain and the transmembrane domain. The M protein exists in two different conformations, namely compact and elongated. The compact M protein conformation is 6 nm in length and is associated with the low spike density and flexibility. The elongated conformation is 8 nm in length and is associated with the high spike density, rigidity and narrow curvature of the viral membrane.

Envelope (E) protein

The envelope protein is a transmembrane protein of 8–12 kilodalton in size. The envelope protein has three domains, namely the N terminal ectodomain, C terminal domain and ion channel activity. The ectodomain is heavily glycosylated. The ectodomain of N protein facilitates in the assembly and release of the virus.

Nucleocapsid (N) protein

The N protein has two separate domains, namely the N terminal domain and C terminal domain. The N protein in different viruses uses different mechanisms for binding to the RNA. The N terminal domain is heavily phosphorylated. It binds to the viral genome in beads on a string manner. There are basically two substrates of N protein. One of the substrates is the trinucleotide repeat sequence (TRSs) which binds to the C terminus and other substrate binds to the genomic packaging signal. The gene coding for N protein precedes the 3′UTR of the coronavirus genome (Rota *et al*. 2003). Nucleocapsid is involved in viral packaging, viral core formation, and vRNA synthesis (Hiscox *et al*. 2001). The N protein of novel coronavirus consist of a short lysine rich region (KTFPPTEPKKDKKKKTDEAQ) near the C terminal which is unique (Marra *et al*. 2003). It is speculated that this region acts as a nuclear localising region which allows N protein to enter the nucleus through passive diffusion (Rowland *et al*. 1999). It gets phosphorylated after translation. The phosphorylation allows it to enter the nucleus at specific stages of the cell cycle (Hiscox *et al*. 2001). During the interphase, the N protein gets an opportunity to interact with various transcription factors and regulatory complexes. N protein affects the signal transduction pathways resulting in inflammation, apoptosis, and several other cellular processes.

Life cycle

Attachment and entry

The primary attachment of the virion to the host cell begins with the interaction between the S protein and the receptors present on the surface of the host cell. The receptor-binding domain (RBD) of the coronavirus lies within the S1 region. However, the exact location varies depending on the virus. In some of the viruses such as MHV, the RBD is present at the N-terminus of S1 and in other such as SARS-CoV-2, the RBD is present at the C-terminus of S1 (Kubo *et al*. 1994, Cheng *et al*. 2004, Lan *et al*. 2020). The interaction between the S protein and the receptor on host cell plays a key determinant role in the infection. It also governs the tissue tropism. The viruses from the coronavirinae family use peptidases as the receptor, as such, there is no reason behind this, as the entry of virus remains unhindered even in the absence of enzymatic domain.

The entry of the coronavirus into the host cell is mediated by another protein named as cathepsin. Acid dependent proteolytic cleavage of the S protein by cathepsin follows the fusion of viral and host membrane. The S protein is cleaved at two different sites (Figure 3). With the first cleavage, the RBD and fusion domains get separated. The second cleavage at S2′ leads to the exposure of the fusion peptide.

After getting exposed, the fusion peptide inserts itself into the target membrane (Figure 4(B)). The insertion is followed by the joining of two heptad repeats in S2. This forms an antiparallel six-helix bundle (Figure 4(C)) (Bosch *et al*. 2003). The formation of antiparallel helix bundle facilitates the fusion of viral and cellular membranes (Figure 4(D))

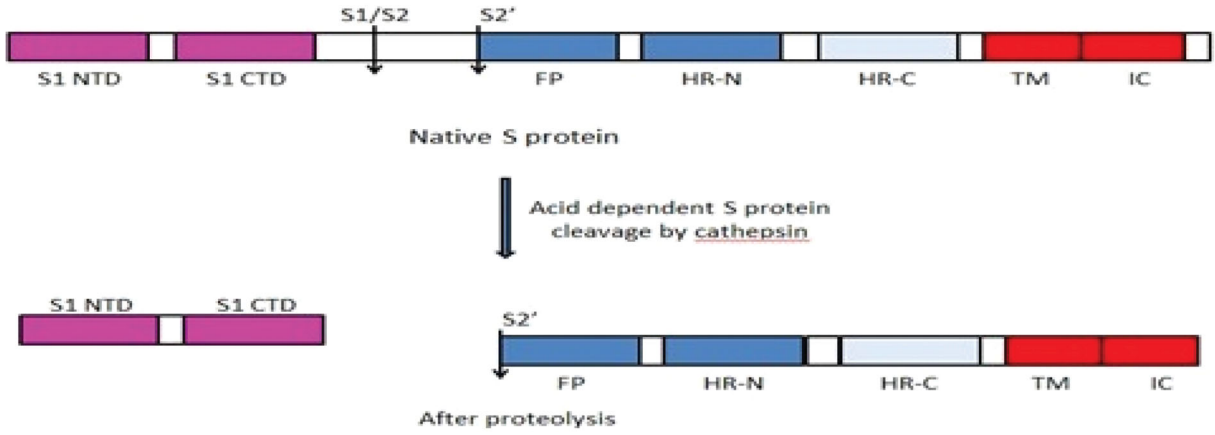

Figure 3. The structure of S protein polypeptide before and after proteolysis.

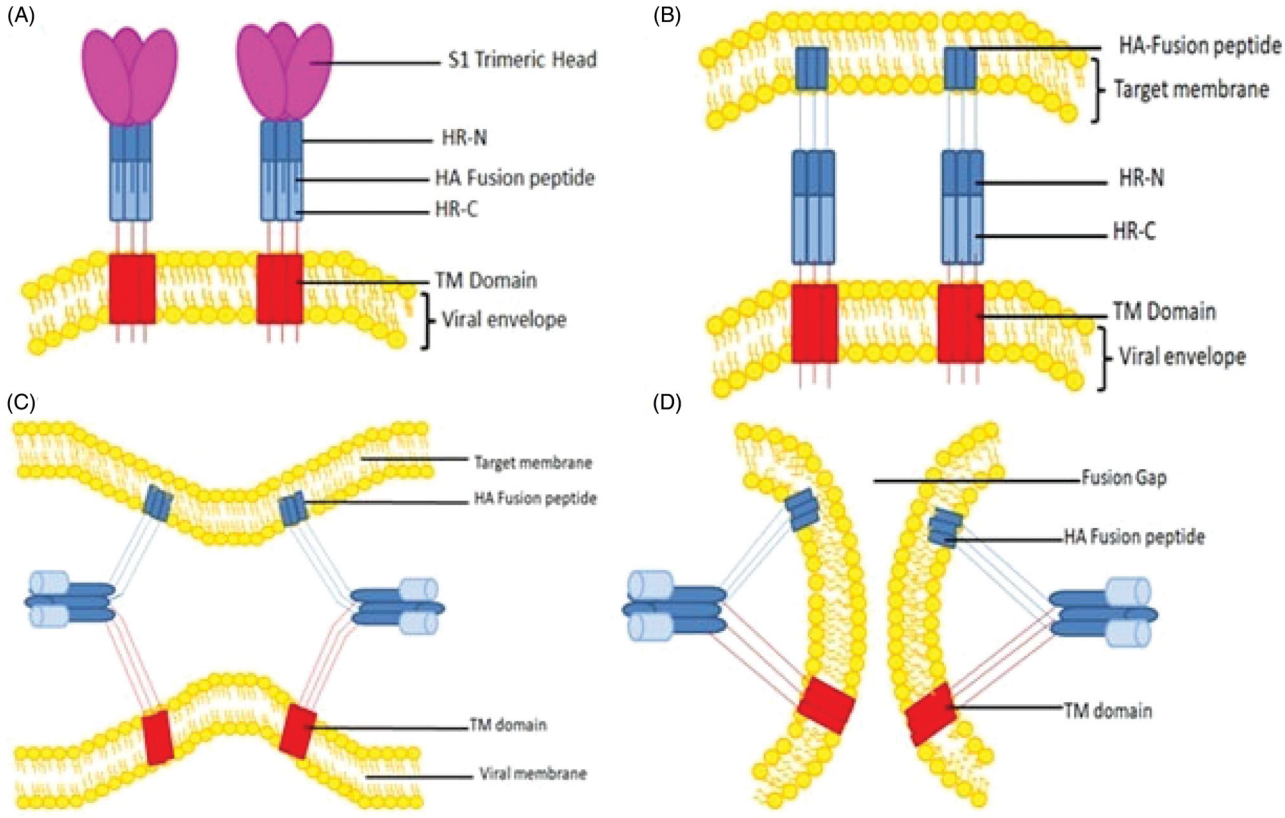

Figure 4. (A) The pre-fusion structure of S protein. (B) Fusion peptide inserted into the target membrane, (C) Formation of antiparallel six-helix bundle, (D) Fusion of viral and cellular membranes.

which results in the fusion of membranes and the release of viral genome inside the cell.

Replicase protein expression

After the fusion of membranes, the next step is the translation of replicase gene. The replicase gene encodes two open reading frames (ORFs), namely rep1a and rep1b. These two genes express two coterminal polyproteins, namely pp1a and pp1ab respectively. For expressing these two proteins, the viruses utilise two mechanisms. Firstly, the virus uses slippery sequence 5′-UUUAAAC-3′ and secondly, the virus utilises RNA pseudoknot that causes ribosomal frameshifting from rep1a to rep1b. Mostly, the ribosome unwinds the pseudoknot and carries on the translation until it comes across rep1a stop codon. However, many a time, pseudoknot hinders ribosome, resulting it to halt on the slippery sequence. This pause results in changing of the reading frame by moving back one nucleotide; this is known as −1 frameshift. This allows the ribosome to melt down the pseudoknot structure and continues translation into rep1b. This results in the translation of pp1ab protein (Brierley *et al.* 1989, Baranov *et al.* 2005). The exact reason behind this

mechanism is unknown, however, it is speculated that it facilitates in maintaining a specific ratio between rep1a:rep1b protein or to impede the production of rep1b until rep1a protein has created a suitable environment. The proteins pp1a and pp1ab are polypeptides and contain 1–11 non-structural proteins (nsps) and 1–16 nsps, respectively. In pp1ab, nsp 11 from pp1a becomes the nsp12. This extends pp1a to pp1ab. The coronavirus encodes two to three proteases which cleave the replicase polyproteins. The proteases produced by coronavirus are papain-like protease (PLpro) and serine-type protease (Mpro). The PLpro is encoded within the nsp3 and the Mpro is encoded within nsp5. The PLpro cleaves nsp1/2, 2/3 and 3/4, whereas, M pro cleaves rest of the eleven nsps. Several nsps congregate into replicase–transcriptase complex and create an environment appropriate for synthesis of RNA. This environment also facilitates the replication of RNA and transcription.

Replication and transcription

There are two types of RNA which are synthesised by the virus, namely genomic and subgenomic RNAs. The sub-genomic RNAs act as mRNA for the structural and accessory genes. These genes are present downstream of the replicase polyprotein. The genomic and subgenomic RNAs are produced from negative RNA strand intermediate. There are certain cis-acting sequences which play a significant role in the replication of RNA. One of the cis-acting sequences is present within the 5′ UTR region. A seven looped structure is present in 5′ UTR region, which may stretch into replicase 1a (Raman *et al*. 2003, Brown *et al*. 2007, Liu *et al*. 2009, Guan *et al*. 2011). Another cis-acting sequence is present in the 3′ UTR region which is a bulged stem-loop, hypervariable region and a pseudoknot (Hsue and Masters 1997, Williams *et al*. 1999, Liu *et al*. 2001, Goebel *et al*. 2007). However, the stem loop structure and pseudoknot in the 3′ UTR region overlap each other; hence, they cannot form together (Hsue and Masters 1997, Williams *et al*. 1999, Hsue *et al*. 2000, Goebel *et al*. 2007). All of these structures control alternate phases of synthesis of RNA. During the production of subgenomic RNAs, the body TRS and leader sequences fuse together. It is speculated that it occurs during the synthesis of positive strand of RNA (Sawicki *et al*. 2007). RNA dependent RNA polymerase (RdRp) halts at any of the TRS sequence. After this halt, the RdRp either continues elongation to next TRS or switches to multiply leader sequence at 5′ endguided by complementarity of TRS to leader TRS.

Assembly and release

After the replication and synthesis of subgenomic RNA; S, E and M are translated and inserted into the endoplasmic reticulum (ER). All of these proteins move into the ER-Golgi intermediate compartment (Tooze *et al*. 1984, Krijnse-Locker *et al*. 1994). In that compartment, the N proteins encapsulate the viral genome. The bud is formed which contains viral structural proteins; this forms mature virions (de Haan and Rottier 2005) (Figure 5). For the assembly of coronaviruses, protein–protein interactions are required which are directed by M protein interaction. Virus-like particles (VLPs) cannot be formed by M protein alone. But, if both M and E protein are expressed simultaneously, the coronavirus envelope is produced (Bos *et al*. 1996). This can be speculated as these function together. Formation of VLP is enhanced by the N protein. This suggests that the fusion of encapsulated genomes into Golgi complex enhances the viral envelope encapsulation (Siu *et al*. 2008). After the encapsulation of the virions, the S protein is incorporated; however, it is not required for assembly. It is important for the S protein to interact with the M protein inside the Golgi complex for its incorporation into the virion.

In comparison to the M protein, the E protein is present in small quantities; hence, for the maturation of envelope, M protein interaction is required. It is still unknown the way through which E protein facilitates M protein in assembly of virion. It has been indicated by some studies that E protein induces the membrane curvature (Fischer *et al*. 1998, Raamsman *et al*. 2000, Corse and Machamer 2000, Boscarino *et al*. 2008). One of the studies suggests that the M protein aggregation is prevented by E protein.

Transmission

The SARS-CoV-2 virus is an enveloped virus. As a consequence of the presence of envelope in coronaviruses, the virions are less stable in the environment. The SARS-CoV-2 is stable on the environmental surfaces; hence the transmission is associated with close contact. The stability of virus depends on the surface. Though the exact reason behind the infectivity of enveloped virion in presence of proteolytic enzymes and bile is not known; it is speculated that the infectivity is provided by the extensive glycosylation. A large number of people working in the wet animal market in Wuhan, China were found to be infected. It was initially speculated that the disease was spread from bats. Studies of COVID-19 have revealed that the reservoir for coronavirus could possibly be birds or mammals (Bassetti *et al*. 2020, Ji *et al*. 2020). Genomic sequence studies of COVID-19 revealed two bat derived coronaviruses (Lu *et al*. 2020, Wan *et al*. 2020). The principal source of transmission is from the COVID-19 patients; however, it can also transmit from asymptomatic patients (Xu *et al*. 2020, Bai *et al*. 2020). Main route of transmission is respiratory droplets and things contaminated with the droplets of virus. The nucleic acid of SARS-CoV-2 has been detected in faeces and urine of COVID-19 patients. This suggests that SAR-CoV may be transmitted through feco-oral route.

For the first time, the COVID-19 has been established in a tiger at Bronx Zoo, New York. Six other tigers and lions said to have developed symptoms. Some of the conservation experts think that this disease could also affect wild gorillas, chimps and orang-utans. The SARS-CoV-2 virus has close relatives in *Rhinolophus rouxii* (rufous horseshoe bats) (Mackenzie and Smith 2020), *Rhinolophus affinis* (intermediate horseshoe bats) (El-Duah *et al*. 2019) and *Manis pentadactyla* (Pangolin) [https://www.nature.com/articles/d41586-020-

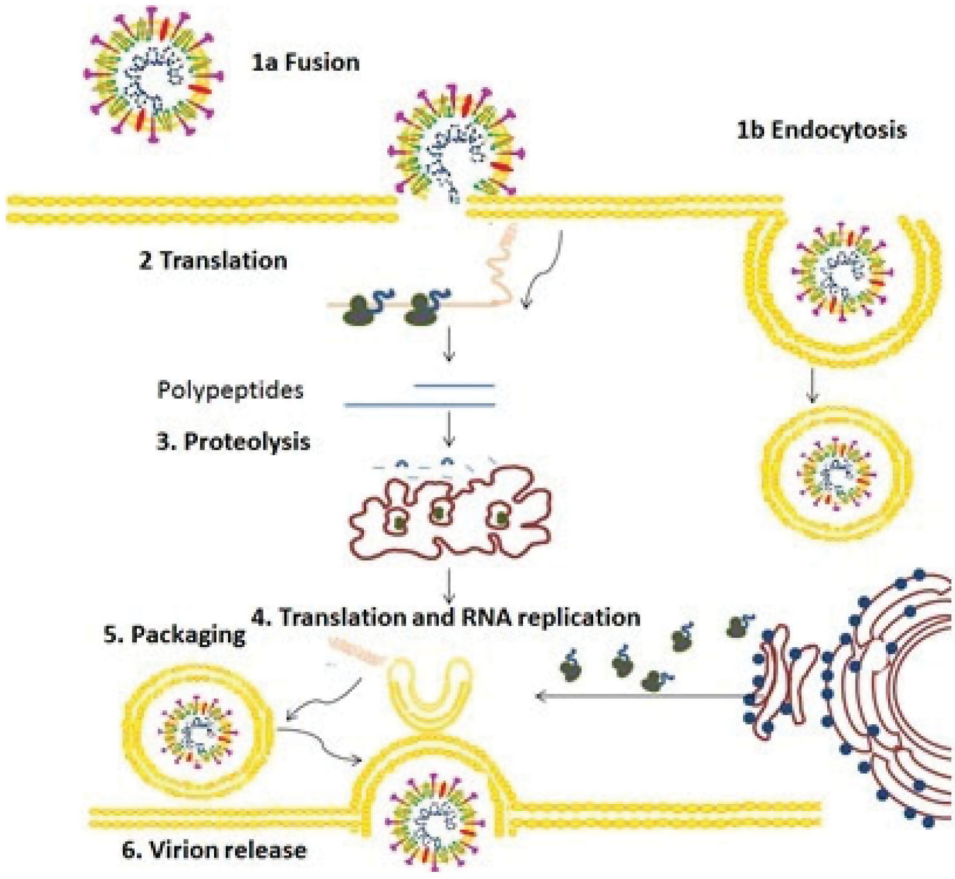

Figure 5. Assembly and release of SARS-CoV-2.

00548-w]. All of these are considered as delicacies in Chinese cuisine. Recently, two pet cats in New York have tested positive for COVID-19. These two have become the first domesticated animals to have COVID-19 [https://www.livescience.com/cats-test-positive-coronavirus-ny.html].

Symptoms

The incubation period of the disease is between 1 and 14 days (Wang W *et al*. 2020d) with peak reaching around 3–7 days. The approximate incubation period is 5.2 days (Li *et al*. 2020). The main symptoms of COVID-19 are fever, dry cough, fatigue, sore throat, runny nose, diarrhoea, production of sputum, haemoptysis (Ren *et al*. 2020, Huang *et al*. 2020, Carlos *et al*. 2020) and diffused alveolar damage (Poutanen *et al*. 2003). One of the chief clinical officer and leader symptom assessment providers to NHS stated that loss of appetite and smell also occurs in several patients (https://www.express.co.uk/life-style/health/1268457/coronavirus-update-uk-cases-news-symptoms-loss-of-appetite). Some patients have dyspnoea. Most of the patients exhibit mild to moderate symptoms, however, 15% progress to severe pneumonia and 5% may develop acute respiratory distress syndrome (ARDS) (Chang *et al*. 2020a, Holshue *et al*. 2020) septic shock, and multiple organ failure (Huang *et al*. 2020, Xu *et al*. 2020). The spectrum of symptoms varies from mild to critical. Most of the COVID-19 infections are not severe (Wang D *et al*. 2020b, Chan *et al*. 2020, Bajema *et al*. 2020, Huang *et al*. 2020, Chen *et al*. 2020b, Liu *et al*. 2020, Yang *et al*. 2020). In around 81% of the reported cases, patients had mild or no pneumonia. Severe infection was reported in 14% of the cases, where >50% of involvement of lungs on imaging was observed within 24–48 h. Only 5% of the reported cases were critically infection, where respiratory failure or multiple organ dysfunction was observed.

Pathology

The SARS-CoV-2 has developed strategies to counter innate immune response. It has been found out that the N protein and SARS-CoV-2 accessory protein ORF6 and ORF3b prevents the induction of interferons. The N protein also prevents the nuclear translocation of proteins which contains nuclear import signals including STAT1. The pathological findings have revealed the over activation of T cells and respiratory findings which are abnormal. This over-activation is a result of increase in Th17 and high cytotoxicity of CD8 T cells. This partially accounts for severe immune injury (Xu *et al*. 2020). The virus attacks the immune system and results in lymphopenia with decreased number of CD4+, CD8+, B cells and NK cells (Qin *et al*. 2020, Shi *et al*. 2020). The studies have revealed that the patients have developed leucopoenia. Along with these, there is a decrease in percentage of monocytes, eosinophils and basophils (Zhang *et al*. 2020a). An increased level of C-reactive protein was also found out. D-dimer and high sedimentation rate of erythrocytes was

found out in COVID19 patients (Lei *et al*. 2020). However, the efficacy of the innate immune system decides the extent of initial replication of virus. Inside the human body, the virus triggers series of cellular and humoral immune response. The viruses infect macrophages and dendritic cells; however, the replication is not completed in these cells. The infected dendritic cells express several proinflammatory cytokines IL2, IL10, 1L7, IP10 and GCSF and chemokines like IL-6, TNF-alpha, MCP-2, RANTES, MIP-1, MCP-1 and IP-10 (Huang *et al*. 2020). A high titre of SAR-CoV-2 specific cytotoxic T-lymphocytes and neutralising antibodies have been detected in patients. This suggests that both the cellular and humoral immune response is significant for viral clearance.

Risk factors for severe COVID-19 infections

Severe form of COVID-19 infection can occur in a healthy person, however, it occurs mostly in elderly people or people with medical comorbidities such as cardiovascular disease, obesity (body mass index >30), chronic kidney disease, cancer, chronic lung disease, hypertension and diabetes mellitus (Zhou *et al*. 2020, Wu and McGoogan 2020, Liang et al 2020, Lighter *et al*. 2020). The CDC also included liver diseases and immunocompromising conditions as risk factors (https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-at-higher-risk.html). The males have higher number of deaths from Italy and China (Onder *et al*. 2020, Chen *et al*. 2020c). As a consequence of the socioeconomic disparities, the Africans have a higher death rate (https://www.michigan.gov/coronavirus/0,9753,7-406-98163_9817300, https://www.dph.illinois.gov/covid19/covid19-statistics, http://ldh.la.gov/Coronavirus/) (Garg *et al*. 2020).

Many countries have employed lockdown to prevent the spread of COVID-19 further. Though, somehow it has been successfully employed and decreased the spread, however, other serious complications may arise due to lockdown. As a consequence of sedentary lifestyle during lockdown, there is a high risk of people getting affected with cardiovascular disease and obesity. The food habits are influenced by the availability, access to food and personal choices (Mattioli *et al*. 2020).

Clinical features and pathogenesis

Respiratory clinical features and pathogenesis

The coronaviruses replicate inside the ciliated (HCoV-OC43, NL63, HKU) and non-ciliated (HCoV-229E) epithelial cells of nasopharynx (Dijkman *et al*. 2013). The principal clinical manifestation of COVID-19 was severe pneumonia, presence of nucleic acid in serum (RNAemia) along with the ground glass opacities (Huang *et al*. 2020). A high level of pro-inflammatory cytokines might lead to respiratory failure. High levels also mediate different pulmonary pathology which leads to increased inflammation.

Cardiac clinical features and pathogenesis

Although the clinical symptoms of COVID 19 are overshadowed by respiratory symptoms, some of the patients develop severe cardiovascular injury (Huang *et al*. 2020). The patients with cardiovascular injury have high risk of death. The SAR-CoV2 binds to the ACE2 receptor in humans which is present in the cardiac cells and is involved in functioning of heart, development of hypertension and diabetes mellitus. ACE2 receptors are higher in number in lungs and heart cells (Turner *et al*. 2004). Studies have revealed that MERS-CoV can cause acute myocarditis and cardiac arrest (Alhogbani 2016). MER-CoV and SAR-CoV2 have similar pathogenicity. Five out of forty one patient initially admitted in Wuhan because of COVID-19 had myocardial injury. They also had an increased sensitivity for cardiac troponin I and electrocardiographic changes (Li *et al*. 2003, Harris *et al*. 2019). Some of the confirmed cases of SARS-CoV-2 infection first went to see doctor because of cardiovascular problems. The patients were facing heart palpitations and chest tightness.

Gastrointestinal clinical features and pathogenesis

The ACE2 receptors are also expressed in the stratified epithelial cells of the oesophagus and the absorptive enterocytes of colon and ileum (Zhang *et al*. 2020b). After the infection caused by virus, there occurs an increase in the permeability of the gastrointestinal wall. As a consequence of enterocytes malabsorption, enteric symptoms such as diarrhoea will occur. Theoretically, it is speculated that the digestive system might be susceptible to COVID-19.

Hepatic clinical features and pathogenesis

In patients suffering from COVID-19 mild to moderate liver damage involving hypoproteinemia, elevated aminotransferases, and prolongation of prothrombin time have been reported. Percutaneous liver biopsies of COVID-19 patients have revealed apoptosis and conspicuous mitoses. This is accompanied by unique features like acidophilic bodies, lobular activities without fibrin deposition and ballooning of hepatocytes (Chau *et al*. 2004). It is speculated that the SARS-associated hepatotoxicity is because of the viral hepatitis or the secondary effects of antiviral medicines or over reaction of immune system. Recent single cell RNA sequencing revealed that the ACE2 is expressed more in cholangiocytes. This reflects that the SARS-CoV-2 directly damages intrahepatic bile ducts (Chai *et al.* 2020).

Diagnosis

For regulating the global pandemic of COVID-19, it is important to identify the infected person. The reference standard for the diagnosis of COVID-19 is real-time reverse transcriptase polymerase chain reaction (real time RTPCR). However serological immunoassays and point-of-care technologies are also emerging. South Korea used unique and atypical testing efforts to slow down the pandemic (Sheridan 2020). In just

9 weeks after the first case, South Korea performed more than 300,000 tests (Sheridan 2020). On the other hand, Singapore used contact tracing and isolation (Lee *et al.* 2020). Management also screened patients with influenza or pneumonia like disease in hospitals and deaths (Lee *et al.* 2020). Almost similar approaches were used by Taiwan and Hong Kong (Wang CJ *et al.* 2020c). Different countries are using different approaches depending on the spread of virus, public health resources and testing capacity. The Centres for Disease Control and Prevention (CDC) recommended testing for three prior groups: hospitalised patients showing symptoms of COVID-19, symptomatic person at risk, and persons who had close contact with someone suspected or have a travel history in COVID-19 affected area [Centers for Disease Control and Prevention. Evaluating and Testing Persons for Coronavirus Disease 2019 (COVID-19) accessed at www.cdc.gov/coronavirus/2019-nCoV/hcp/clinical-criteria.html]. The CDC has recommended testing the presence of nucleic acid targets. The preferred choice for swab based testing is nasopharyngeal specimen. Apart from nasopharyngeal specimen, anterior nare samples, mid-turbinate and oropharyngeal samples are also acceptable. After the collection of specimen, the samples are used for RNA extraction which is followed by qualitative RT-PCR.

Point-of-care diagnostic methods are less complex and produce rapid results. The CLIA (Clinical laboratory improvement amendment waved point-of-care test include GeneXpert, cobas Liat, BioFire FilmArray, and Abbott ID NOW (Hogan *et al.* 2018). The Xpert Xpress test is performed on GeneXpert platform. The GeneXpert platform is already used for HIV testing and tuberculosis. The tools available for the detection of respiratory syncytial or influenza virus lack sensitivity to exclude disease (Chartrand *et al.* 2015, Merckx *et al.* 2017). Such types of tests have not received regulatory approval for detection of coronaviruses (Lau *et al.* 2004, Chen *et al.* 2015), however, they are under development (SARS-CoV-2 Diagnostic Pipeline. Accessed at www.finddx.org/covid-19/pipeline on 23 March 2020). The monoclonal antibodies have been generated against N protein of SARS-CoV-2. These antibodies might facilitate future antigen detection (Sheridan 2020). The serological tests are lesser complex than the molecular tests. Serological tests are being used to identify the antibodies such as IgG and IgM (Guo *et al.* 2020). But, the use of serological testing is limited around the onset time of symptoms. At the onset time, the transmission risk is highest (Zou *et al.* 2020). Cross-reactivity of antibody to proteins of coronavirus is a problem. Many of the healthcare facilities and hospitals have used chest imaging for the diagnosis of COVID-19 [Bai HX]. Bilateral pneumonia is more reported feature than the unilateral focus (Chung *et al.* 2020, Wang D *et al.* 2020b). More than radiography, computed tomography is sensitive. On computed tomography of chest, features such as vascular thickening, fine reticular opacities and peripheral distribution are reported with patients of COVID-19. Certain biomarkers have been found to be associated with COVID-19 such as lymphopenia, elevated lactate dehydrogenase, elevated C reactive protein, and decreased albumin. Some other biomarkers associated with COVID-19 patients are increased creatinine and bilirubin levels, leukocytosis, leukopoenia, elevated creatinine kinase levels, alanine aminotransferase, aspartate aminotransferease and erythrocyte sedimentation rates (Chen *et al.* 2020a, Chen *et al.* 2020b, Huang *et al.* 2020).

Prevention

The first case of coronavirus reported in Wuhan was the person who visited Chinese 'wet' food market. As a preventive measure, the Chinese authorities closed seafood market. However, strict control over those markets is recommended by scientist instead of just forbidding it. Another urgent need of the hour is providing protection to the healthcare professionals against nosocomical infections. Surgical masks are not sufficient for healthcare professional; rather they need personal protective equipment (PPE) including N95 masks, protective gowns and goggles (Chang *et al.* 2020b). For general public, avoiding handshakes, maintaining social distancing (at least 2 m), washing hands frequently, sneezing and coughing etiquette, avoiding touching of mouth, eyes and nose, and wearing masks are recommended (Wang C *et al.* 2020a). Cleaning and disinfecting the touched objects and surfaces frequently is also recommended. Other preventive measures such as limiting the movement of people to and fro from red zones of infections, isolation of patients, and avoiding consumption of meat play significant role in controlling the disease.

Treatment

Chinese physicians have been using triage algorithm based on the experience from Wuhan, where they are separating patients into two groups. First group includes those people who are receiving treatment at their homes and other group includes people receiving treatment for regular community-acquired pneumonia (Zhang *et al.* 2020c). The patients showing severe infection are given supplemental oxygen supply. As there is no vaccine available for SARS-CoV-2, the physicians are managing the symptoms and giving oxygen therapy with mechanical ventilation.

The viral pneumonia patients are treated with antiviral drug including Arbidol [www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2/files/b218cfeb1bc54639af227f9 22bf6b817.pdf], Remdesivir (nucleoside analogue) (Al-Tawfiq *et al.* 2020), lopinavir (antiretroviral proteinase inhibitor) (Dayer *et al.* 2017), and ritonavir (cytochrome P450 inhibitor). However, research has revealed that ritonavir/lopinavir and arbidol are not effective for the treatment of mild-moderate COVID-19. Different countries have used hydroxychloroquine for the treatment; however, several studies have revealed that it has no potential benefits. Rather, a higher death rate has been recorded in patients taking it (Magagnoli *et al.* 2020). Using pioneer molecular evolution technique, Professor Nick Brindle and Dr. Julian Sale at MRC lab of molecular biology are trying to create a decoy protein that binds to the SARS-CoV-2 and prevent it from infecting human cells (https://le.ac.uk/news/2020/april/17-decoy-

Figure 6. Confirmed cases of COVID-19 all over the world (circumference reflects morbidity).

protein-covid-19?fbclid=IwAR3IdmgBbxVMKcqjTUatGkY4Z9lmbuLAV7udANFjrMmvVp_JTubKDhmE_A). Several clinical trials are undergoing in China and other countries to test the efficacy of different compounds on SARS-CoV-2. Several countries are using convalescent plasma containing neutralising antibodies (Shen *et al*. 2020). The patients treated with the convalescent plasma have shown positive results. The SARS-CoV-2 specific neutralising antibodies are identified through high-throughput techniques such as large scale single cell RNA sequencing of B cells. Specific IgG and IgM are also being used in conjunction with RTPCR. Patients with severe COVID-19 infection have increased levels of IgG and high titre of antibodies (Zhang *et al*. 2020a, Zhao *et al*. 2020). Several countries are trying to find the vaccine that targets the virus or blocks the entry of virus into the cell. One of the stable indicators of poor health of severely infected patients is the elevated levels of IL-6. Clinical trial (ChiCTR2000029765) has been reported to control fever and bring improvement of respiratory function. This clinical trial is based on use of IL-6 receptor targeted monoclonal antibody. Another approach used by researchers is the use of mesenchymal stem cells which have anti-apoptotic and anti-inflammatory effects. These cells also have the ability to repair pulmonary epithelial cells and also increased the fluid clearance in alveoli. A Chinese medicine Lianhuaqingwen has demonstrated clinical efficacy (Runfeng *et al*. 2020). In vitro studies on this medicine have found that this drug inhibits the replication of SARS-CoV-2 and blocks the pro-inflammatory cytokine production. Recently, WHO has approved the use of dexamethasone (an anti-inflammatory drug) for the most critical COVID-19 patients.

Epidemiology

Globally around 8,766,037 confirmed cases have been reported by June 20, 2020 [https://www.worldometers.info/coronavirus/]. Since the first case of coronavirus infection from Wuhan, China, more than 82,000 cases with total death of more than 4,500 have been solely reported in China. WHO along with China-Fact-finding mission estimated that the peak of infection was between January and February [https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—24-february-2020]. However, new cases are now emerging in China again. So far, the world has observed more than 167,000 deaths. Cases of COVID-19 have been reported in all the continents, except Antarctica (Figure 6). The first case of human-to-human transfer of COVID-19 was first reported in the US [https://www.cdc.gov/media/releases/2020/p0130].

Conclusion

The family of coronaviridae comprises of several lethal viruses. The viruses have the tendency to mutate and evolve. It can be speculated that in near future also, they will continue to evolve and mutate and cause human and veterinary diseases. They have the ability to recombine, mutate, evolve

and infect multiple species and cell types. The SARS-CoV-2 uses the receptor ACE2 in humans. Future research on SARS-CoV-2 needs to be focussed on different aspects of viral replication, translation, and pathogenesis. The ability of the viruses to establish an infection in one species and jumping from one species to another needs to be further investigated to predict the occurrence of disease. It is also interesting and needs investigation of the way through which the bats avoid evident diseases even in the presence of the virus. Further studies are required to find out the functions of several accessory and non-structural proteins encoded by SARS-CoV-2 as these can be a target for vaccines. For designing suitable vaccines, researchers need to find out the mechanisms through which the virus causes disease. The transplantation of (ACE2 and TMPRSS negative) MSCs might be beneficial in COVID-19 as they have been proved to regulate the inflammatory response. The passive immunisation of convalescent plasma might also be beneficial. Inhibitors of JAK (Janus Kinase) and IL-6 inhibitors can also be helpful in treating COVID-19. Double filtration plasmapheresis has been proved beneficial in ribavirin and Peg-interferon resistant patients. This might also be proved beneficial in case of COVID-19, however, further clinical studies are required to prove their efficacy.

Acknowledgement

The authors thank James Keach for assistance in English language.

Disclosure statement

The authors declare that they do not have conflict of interest regarding article publication.

References

Zhao, L., *et al.*, 2012. Antagonism of the interferon-induced OAS-RNase L pathway by murine coronavirus ns2 protein is required for virus replication and liver pathology. *Cell host & microbes*, 11 (6), 607–616.

Abraham, S., *et al.*, 1990. Deduced sequence of the bovine coronavirus spike protein and identification of the internal proteolytic cleavage site. *Virology*, 176 (1), 296–301.

Alhogbani, T., 2016. Acute myocarditis associated with novel Middle East respiratory syndrome coronavirus. *Annals of Saudi medicine*, 36 (1), 78–80.

Al-Tawfiq, J.A., Al-Homoud, A.H., and Memish, Z.A., 2020. Remdesivir as a possible therapeutic option for the COVID-19. *Travel medicine and infectious disease*, 34, 101615.

Bai, H.X., et al., 2020. Performance of radiologists in differentiating COVID-19 from Non-COVID-19 viral pneumonia at chest CT. *Radiology*, 296 (2), E46–E54. doi:10.1148/radiol.2020200823

Bai, Y., *et al.*, 2020. Presumed asymptomatic carrier transmission of COVID-19. *JAMA*, 323 (14), 1406–1407.

Bajema, K.L., *et al.*,; 2019-CoV Persons Under Investigation Team. 2020. Persons evaluated for 2019 novel Coronavirus - United States, January 2020. *Morbidity and mortality weekly report*, 69 (6), 166–170.

Baranov, P.V., *et al.*, 2005. Programmed ribosomal frameshifting in decoding the SARS-CoV genome. *Virology*, 332 (2), 498–510.

Bárcena, M., *et al.*, 2009. Cryo-electron tomography of mouse hepatitis virus: insights into the structure of the coronavirion. *Proceedings of the national academy of sciences of the United States of America*, 106 (2), 582–587.

Bassetti, M., Vena, A., and Giacobbe, D.R., 2020. The novel Chinese coronavirus (2019-nCoV) infections: challenges for fighting the storm. *European journal of clinical investigation*, 50 (3), e13209.

Beniac, D.R., *et al.*, 2006. Architecture of the SARS coronavirus prefusion spike. *Nature structural & molecular biology*, 13 (8), 751–752.

Bonilla, P.J., Gorbalenya, A.E., and Weiss, S.R., 1994. Mouse hepatitis virus strain A59 RNA polymerase gene ORF 1a: heterogeneity among MHV strains. *Virology*, 198 (2), 736–740.

BOS, E.C., *et al.*, 1996. The production of recombinant infectious DI-particles of a murine coronavirus in the absence of helper virus. *Virology*, 218 (1), 52–60.

Boscarino, J.A., *et al.*, 2008. Envelope protein palmitoylations are crucial for murine coronavirus assembly. *Journal of virology*, 82 (6), 2989–2999.

Bosch, B.J., *et al.*, 2003. The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. *Journal of virology*, 77 (16), 8801–8811.

Brierley, I., Digard, P., and Inglis, S.C., 1989. Characterization of an efficient coronavirus ribosomal frameshifting signal: requirement for an RNA pseudoknot. *Cell*, 57 (4), 537–547.

Brown, C.G., *et al.*, 2007. An RNA stem-loop within the bovine coronavirus nsp1 coding region is a cis-acting element in defective interfering RNA replication. *Journal of virology*, 81 (14), 7716–7724.

Carlos, W.G., *et al.*, 2020. Novel Wuhan (2019-nCoV) coronavirus. *American journal of respiratory and critical care medicine*, 201 (4), P7–8.

Centers for Disease Control and Prevention. Evaluating and testing persons for coronavirus disease 2019(COVID-19). Available from: www.cdc.gov/coronavirus/2019-nCoV/hcp/clinical-criteria.html [Accessed 21 March 2020].

Chai, X., *et al.*, 2020. Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection. *biorxiv*. doi:10.1101/2020.02.03.931766

Chan, J.F., *et al.*, 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *The lancet*, 395 (10223), 514–523.

Chang, D., *et al.*, 2020a. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. *JAMA*, 323 (11), 1092–1093.

Chang, D., *et al.*, 2020b. Protecting health-care workers from subclinical coronavirus infection. *The lancet respiratory medicine*, 8 (3), e13.

Chartrand, C., *et al.*, 2015. Diagnostic accuracy of rapid antigen detection tests for respiratory syncytial virus infection: systematic review and meta-analysis. *Journal of clinical microbiology*, 53 (12), 3738–3749.

Chau, T.N., *et al.*, 2004. SARS-associated viral hepatitis caused by a novel coronavirus: report of three cases. *Hepatology (Baltimore, MD)*, 39 (2), 302–310.

Chen L., et al., 2020a. Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia. *Zhonghua jie he he hu xi za zhi= Zhonghua jiehe he huxi zazhi= Chinese journal of tuberculosis and respiratory diseases,* 43. doi:10.3760/cma.j.issn.1001-0939.2020.0005

Chen, N., *et al.*, 2020b. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The lancet*, 395 (10223), 507–513.

Chen, T., *et al.*, 2020c. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ*, 368. doi:10.1136/bmj.m1295

Chen, Y., *et al.*, 2015. A sensitive and specific antigen detection assay for Middle East respiratory syndrome coronavirus. *Emerging microbes & infections*, 4 (1), 1–5.

Cheng, P.K., *et al.*, 2004. Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. *The lancet*, 363 (9422), 1699–1700.

Chung, M., *et al.*, 2020. CT imaging features of 2019 novel coronavirus (2019-nCoV). *Radiology*, 295 (1), 202–207.

Collins, A.R., *et al.*, 1982. Monoclonal antibodies to murine hepatitis virus-4 (strain JHM) define the viral glycoprotein responsible for attachment and cell-cell fusion. *Virology*, 119 (2), 358–371.

Corse, E., and Machamer, C.E., 2000. Infectious bronchitis virus E protein is targeted to the Golgi complex and directs release of virus-like particles. *Journal of virology*, 74 (9), 4319–4326.

Dayer, M.R., Taleb-Gassabi, S., and Dayer, M.S., 2017. Lopinavir; a potent drug against coronavirus infection: insight from molecular docking study. *Archives of clinical infectious diseases*, 12 (4). doi:10.5812/archcid.13823

de Haan, C.A., *et al.*, 1999. Mapping of the coronavirus membrane protein domains involved in interaction with the spike protein. *Journal of virology*, 73 (9), 7441–7452.

de Haan, C.A., and Rottier, P.J., 2005. Molecular interactions in the assembly of coronaviruses. *Advances in virus research*, 64, 165–230.

Delmas, B.E., and Laude, H.U., 1990. Assembly of coronavirus spike protein into trimers and its role in epitope expression. *Journal of virology*, 64 (11), 5367–5375.

Dijkman, R., *et al.*, 2013. Isolation and characterization of current human coronavirus strains in primary human epithelial cell cultures reveal differences in target cell tropism. *Journal of virology*, 87 (11), 6081–6090.

Drosten, C., *et al.*, 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *The New England journal of medicine*, 348 (20), 1967–1976.

El-Duah, P., *et al.*, 2019. Potential intermediate hosts for coronavirus transmission: no evidence of Clade 2c coronaviruses in domestic livestock from Ghana. *Tropical medicine and infectious disease*, 4 (1), 34.

Escors, D., *et al.*, 2001a. Organization of two transmissible gastroenteritis coronavirus membrane protein topologies within the virion and core. *Journal of virology*, 75 (24), 12228–12240.

Escors, D., *et al.*, 2001b. The membrane M protein carboxy terminus binds to transmissible gastroenteritis coronavirus core and contributes to core stability. *Journal of virology*, 75 (3), 1312–1324.

SARS-CoV-2 Diagnostic Pipeline. Available from: www.finddx.org/covid-19/pipeline [Accessed 23 March 2020].

Fischer, F., *et al.*, 1998. Analysis of constructed E gene mutants of mouse hepatitis virus confirms a pivotal role for E protein in coronavirus assembly. *Journal of virology*, 72 (10), 7885–7894.

Garg, S., *et al.*, 2020. Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019 - COVID-NET, 14 States, March 1-30, 2020. *Morbidity and mortality weekly report*, 69 (15), 417–458.

Glowacka, I., *et al.*, 2011. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *Journal of virology*, 85 (9), 4122–4134.

Goebel, S.J., *et al.*, 2007. A hypervariable region within the 3′ cis-acting element of the murine coronavirus genome is nonessential for RNA synthesis but affects pathogenesis. *Journal of virology*, 81 (3), 1274–1287.

Graham, R.L., and Baric, R.S., 2010. Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. *Journal of virology*, 84 (7), 3134–3146.

Guan, B.J., Wu, H.Y., and Brian, D.A., 2011. An optimal cis-replication stem-loop IV in the 5′ untranslated region of the mouse coronavirus genome extends 16 nucleotides into open reading frame 1. *Journal of virology*, 85 (11), 5593–5605.

Guo, L., *et al.*, 2020. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). *Clinical infectious diseases*, 21. doi:10.1093/cid/ciaa310

Harris, J.E., *et al.*, 2019. Frequency of troponin elevations in patients with influenza infection during the 2017-2018 influenza season. *International journal of cardiology*, 22, 145–147.

Hiscox, J.A., *et al.*, 2001. The coronavirus infectious bronchitis virus nucleoprotein localizes to the nucleolus. *Journal of virology*, 75 (1), 506–512.

Hogan, C.A., Caya, C., and Papenburg, J., 2018. Rapid and simple molecular tests for the detection of respiratory syncytial virus: a review. *Expert review of molecular diagnostics*, 18 (7), 617–629.

Holshue, M.L., *et al.*, 2020. First case of 2019 novel coronavirus in the United States. *New England journal of medicine*, 382 (10), 929–936.

Horzinek, M.C., *et al.*, 1987. Evidence for a coiled-coil structure in the spike proteins of coronaviruses. *Journal of molecular biology*, 196 (4), 963–966.

Hsue, B., Hartshorne, T., and Masters, P.S., 2000. Characterization of an essential RNA secondary structure in the 3′ untranslated region of the murine coronavirus genome. *Journal of virology*, 74 (15), 6911–6921.

Hsue, B., and Masters, P.S., 1997. A bulged stem-loop structure in the 3′untranslated region of the genome of the coronavirus mouse hepatitis virus is essential for replication. *Journal of virology*, 71 (10), 7567–7578.

Huang, C., *et al.*, 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet*, 395 (10223), 497–506.

Ji, W., *et al.*, 2020. Homologous recombination within the spike glycoprotein of the newly identified coronavirus may boost cross-species transmission from snake to human. *Journal of medical virology*. doi:10.1002/jmv.25682

Kirchdoerfer, R.N., *et al.*, 2016. Pre-fusion structure of a human coronavirus spike protein. *Nature*, 531 (7592), 118–121.

Krijnse-Locker, J., *et al.*, 1994. Characterization of the budding compartment of mouse hepatitis virus: evidence that transport from the RER to the Golgi complex requires only one vesicular transport step. *The journal of cell biology*, 124 (1-2), 55–70.

Kubo, H., Yamada, Y.K., and Taguchi, F., 1994. Localization of neutralizing epitopes and the receptor-binding site within the amino-terminal 330 amino acids of the murine coronavirus spike protein. *Journal of virology*, 68 (9), 5403–5410.

Kuo, L., and Masters, P.S., 2002. Genetic evidence for a structural interaction between the carboxy termini of the membrane and nucleocapsid proteins of mouse hepatitis virus. *Journal of virology*, 76 (10), 4987–4999.

Lan, J., *et al.*, 2020. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, 581 (7807), 215–220.

Lau, S.K., *et al.*, 2004. Detection of severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in SARS patients by enzyme-linked immunosorbent assay. *Journal of clinical microbiology*, 42 (7), 2884–2889.

Lee, H.J., *et al.*, 1991. The complete sequence (22 kilobases) of murine coronavirus gene 1 encoding the putative proteases and RNA polymerase. *Virology*, 180 (2), 567–582.

Lee, V.J., Chiew, C.J., and Khong, W.X., 2020. Interrupting transmission of COVID-19: lessons from containment efforts in Singapore. *Journal of travel medicine*, 27 (3). doi:10.1093/jtm/taaa039

Lei, J., *et al.*, 2020. CT imaging of the 2019 novel coronavirus (2019-nCoV) pneumonia. *Radiology*, 295 (1), 18.

Lewicki, D.N., and Gallagher, T.M., 2002. Quaternary structure of coronavirus spikes in complex with carcinoembryonic antigen-related cell adhesion molecule cellular receptors. *Journal of biological chemistry*, 277 (22), 19727–19734.

Li, F., 2013. Receptor recognition and cross-species infections of SARS coronavirus. *Antiviral research*, 100 (1), 246–254.

Li, F., *et al.*, 2006a. Conformational states of the severe acute respiratory syndrome coronavirus spike protein ectodomain. *Journal of virology*, 80 (14), 6794–6800.

Li, Q., *et al.*, 2020. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. *New England journal of medicine*, 382 (13), 1199–1207. doi:10.1056/NEJMoa2001316

Li, S.S., *et al.*, 2003. Left ventricular performance in patients with severe acute respiratory syndrome: a 30-day echocardiographic follow-up study. *Circulation*, 108 (15), 1798–1803.

Li, W., *et al.*, 2006b. Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. *Journal of virology*, 80 (9), 4211–4219.

Liang, W., *et al.*, 2020. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. *The lancet*, 21 (3), 335–337.

Lighter, J., *et al.*, 2020. Obesity in patients younger than 60 years is a risk factor for Covid-19 hospital admission. *Clinical infectious diseases*. doi:10.1093/cid/ciaa415

Liu, K., *et al.*, 2020. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chinese medical journal*. 133 (9), 1025–1031. doi:10.1097/CM9.0000000000000744

Liu, P., *et al.*, 2009. Mouse hepatitis virus stem-loop 2 adopts a uYNMG(U)a-like tetraloop structure that is highly functionally tolerant of base substitutions . *Journal of virology*, 83 (23), 12084–12093.

Liu, Q., Johnson, R.F., and Leibowitz, J.L., 2001. Secondary structural elements within the 3′ untranslated region of mouse hepatitis virus strain JHM genomic RNA. *Journal of virology*, 75 (24), 12105–12113.

Lu, R., *et al.*, 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The lancet*, 395 (10224), 565–574.

Luytjes, W., *et al.*, 1987. Primary structure of the glycoprotein E2 of coronavirus MHV-A59 and identification of the trypsin cleavage site. *Virology*, 161 (2), 479–487.

Mackenzie, J.S., and Smith, D.W., 2020. COVID-19: a novel zoonotic disease caused by a coronavirus from China: what we know and what we don't. *Microbiology Australia*, 41 (1), 45–50.

Magagnoli, J., *et al.*, 2020. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with Covid-19. *Med*. doi:10.1016/j.medj.2020.06.001

Marra, M.A., *et al.*, 2003. The genome sequence of the SARS-associated coronavirus. *Science*, 300 (5624), 1399–1404.

Matsuyama, S., *et al.*, 2010. Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. *Journal of virology*, 84 (24), 12658–12664.

Mattioli, A.V., *et al.*, 2020. COVID-19 pandemic: the effects of quarantine on cardiovascular risk. *European journal of clinical nutrition*, 74 (6), 852–855.

Merckx, J., *et al.*, 2017. Diagnostic accuracy of novel and traditional rapid tests for influenza infection compared with reverse transcriptase polymerase chain reaction: a systematic review and meta-analysis. *Annals of internal medicine*, 167 (6), 394–409.

Mothes, W., *et al.*, 2000. Retroviral entry mediated by receptor priming and low pH triggering of an envelope glycoprotein. *Cell*, 103 (4), 679–689.

Narayanan, K., *et al.*, 2000. Characterization of the coronavirus M protein and nucleocapsid interaction in infected cells. *Journal of virology*, 74 (17), 8127–8134.

Neuman, B.W., *et al.*, 2006. Supramolecular architecture of severe acute respiratory syndrome coronavirus revealed by electron cryomicroscopy. *Journal of virology*, 80 (16), 7918–7928.

Nguyen, V.P., and Hogue, B.G., 1997. Protein interactions during coronavirus assembly. *Journal of virology*, 71 (12), 9278–9284.

Onder, G., Rezza, G., and Brusaferro, S., 2020. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. *JAMA*. doi:10.1001/jama.2020.4683

Poutanen, S.M., *et al.*,; Canadian Severe Acute Respiratory Syndrome Study Team. 2003. Identification of severe acute respiratory syndrome in Canada. *The New England journal of medicine*, 348 (20), 1995–2005.

Qin, C., *et al.*, 2020. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clinical infectious diseases*. doi:10.1093/cid/ciaa248

Raamsman, M.J., *et al.*, 2000. Characterization of the coronavirus mouse hepatitis virus strain A59 small membrane protein E. *Journal of virology*, 74 (5), 2333–2342.

Raman, S., *et al.*, 2003. Stem-loop III in the 5′ untranslated region is a cis-acting element in bovine coronavirus defective interfering RNA replication . *Journal of virology*, 77 (12), 6720–6730.

Ren, L.L., *et al.*, 2020. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. *Chinese medical journal*, 133 (9), 1015–1024.

Rota, P.A., *et al.*, 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science (New York, N.Y)*, 300 (5624), 1394–1399.

Rowland, R.R., *et al.*, 1999. The localization of porcine reproductive and respiratory syndrome virus nucleocapsid protein to the nucleolus of infected cells and identification of a potential nucleolar localization signal sequence. *Virus research*, 64 (1), 1–2.

Runfeng, L., *et al.*, 2020. Lianhuaqingwen exerts anti-viral and anti-inflammatory activity against novel coronavirus (SARS-CoV-2). *Pharmacological research*, 156, 104761.

Sawicki, S.G., Sawicki, D.L., and Siddell, S.G., 2007. A contemporary view of coronavirus transcription. *Journal of virology*, 81 (1), 20–29.

Shen, C., *et al.*, 2020. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. *JAMA*, 323 (16), 1582.

Sheridan, C., 2020. Fast, portable tests come online to curb coronavirus pandemic. *Nature biotechnology*, 38 (5), 515–518.

Shi, Y., *et al.*, 2020. Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. *Immunology,* 160 (3), 261–268. doi:10.1111/imm.13223

Shulla, A., *et al.*, 2011. A transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry. *Journal of virology*, 85 (2), 873–882.

Siu, Y.L., *et al.*, 2008. The M, E, and N structural proteins of the severe acute respiratory syndrome coronavirus are required for efficient assembly, trafficking, and release of virus-like particles. *Journal of virology*, 82 (22), 11318–11330.

Stein, B.S., *et al.*, 1987. pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. *Cell*, 49 (5), 659–668.

Strauss, J. H., and Strauss, E. G., 2008. *Plus- strand RNA viruses*, Viruses and Human Diseases (Second edition). New York, USA.

Taguchi, F., 1995. The S2 subunit of the murine coronavirus spike protein is not involved in receptor binding. *Journal of virology*, 69 (11), 7260–7263.

Tooze, J., Tooze, S., and Warren, G., 1984. Replication of coronavirus MHV-A59 in sac-cells: determination of the first site of budding of progeny virions. *European journal of cell biology*, 33 (2), 281–293.

Turner, A.J., Hiscox, J.A., and Hooper, N.M., 2004. ACE2: from vasopeptidase to SARS virus receptor. *Trends in pharmacological sciences*, 25 (6), 291–294.

Van Marle, G., *et al.*, 1995. Regulation of coronavirus mRNA transcription. *Journal of virology*, 69 (12), 7851–7856.

Vennema, H., *et al.*, 1990. Biosynthesis and function of the coronavirus spike protein. In *Coronaviruses and their diseases* (pp. 9–19). Boston, MA: Springer.

Walls, A.C., *et al.*, 2016. Cryo-electron microscopy structure of a coronavirus spike glycoprotein trimer. *Nature*, 531 (7592), 114–117.

Wan, Y., et al., 2020. Receptor recognition by the Novel Coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS Coronavirus. *Journal of virology*, 94 (7). doi:10.1128/JVI.00127-20

Wang, C., *et al.*, 2020a. A novel coronavirus outbreak of global health concern. *The lancet*, 395 (10223), 470–473.

Wang, D., *et al.*, 2020b. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA*, 323 (11), 1061–1069.

Wang, C.J., Ng, C.Y., and Brook, R.H., 2020c. Response to COVID-19 in Taiwan. *JAMA*, 323 (14), 1341.

Wang, W., Tang, J., and Wei, F., 2020d. Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. *Journal of medical virology*, 92 (4), 441–447.

White, J., Matlin, K., and Helenius, A., 1981. Cell fusion by Semliki Forest, influenza, and vesicular stomatitis viruses. *The journal of cell biology*, 89 (3), 674–679.

Williams, G.D., Chang, R.Y., and Brian, D.A., 1999. A phylogenetically conserved hairpin-type 3′ untranslated region pseudoknot functions in coronavirus RNA replication. *Journal of virology*, 73 (10), 8349–8355.

Wu, Z., and McGoogan, J.M., 2020. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*, 323 (13), 1239.

Xu Z., Shi, and Wang, Y., 2020. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet respiratory medicine*. 8 (4), 420–422. doi:10.1016/S2213-2600(20)30076-X

Yang, X., *et al.*, 2020. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *The lancet. respiratory medicine*, 8 (5), 475–481.

Yoo, D., Parker, M.D., and Babiuk, L.A., 1991. The S2 subunit of the spike glycoprotein of bovine coronavirus mediates membrane fusion in insect cells. *Virology*, 180 (1), 395–399.

Zhang, B., *et al.*, 2020a. Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19. *Frontiers molecular biosciences,* 7, 157. doi:10.3389/fmolb.2020.00157

Zhang, H., *et al.*, 2020b. Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process. *Gut*, 69 (6), 1010–1018.

Zhang, J., *et al.*, 2020c. Therapeutic and triage strategies for 2019 novel coronavirus disease in fever clinics. *The lancet respiratory medicine*, 8 (3), e11.

Zhao, J., *et al.*, 2020. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clinical infectious diseases*. doi:10.1093/cid/ciaa344

Zhou, F., *et al.*, 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The lancet*, 395 (10229), 1054–1062.

Zou, L., *et al.*, 2020. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *The New England journal of medicine*, 382 (12), 1177–1179.