Basic Virology and Pathophysiology of COVID-19

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Abbreviations

ACE  Angiotensin-converting enzyme
CoV  Coronavirus(es)
COVID Coronavirus disease
E  Envelope protein
HCoV Human coronavirus
IFN Interferon
Ig Immunoglobulin
IL Interleukin
IRF Interferon response factor
JAK-STAT Janus kinase-signal transducer and activator of transcription
kDa Kilodaltons
M Membrane protein
Mpro Main protease
MCP Monocyte chemoattractant protein
MERS Middle East respiratory syndrome
MHC Major histocompatibility complex
N Nucleocapsid protein
ORF Open reading frame
PLpro Papain-like protease
PRR Pattern recognition receptors
RdRp RNA-dependent RNA polymerase
RNA Ribonucleic acid

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2.1 Introduction

A hitherto unknown virus emerged in Wuhan, China, during late December, 2019. Since then it has spread globally and has taken numerous lives. It is a highly transmissible virus which can afflict apparently healthy individuals even with momentary contact. The virus responsible for the current pandemic is the severe acute respiratory syndrome virus (SARS-CoV-2), and the disease it causes is known as COVID-19. The disease can have serious manifestations like respiratory distress, severe dry cough, high-grade fever, and in some cases even death [1]. Coronaviruses measure approximately 125 nm in diameter and contain single-stranded positive sense RNA. “Spikes” (club-like projections) on their surface give them the name Corona (crown) [2]. They are mainly zoonotic (only four coronaviruses are known in humans) and can be found in bats, birds, cats, dogs, mice, pigs, horses, and whales. Since the start of the twenty-first century, fatal pneumonia has been caused by three coronaviruses—severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2. All these are understood to have crossed over from animals to humans [3]. The following sections deal with the basic virology and pathophysiology of SARS-CoV-2 in detail.

2.2 Classification

The International Committee on Taxonomy of Viruses is responsible for giving out classification of all known viruses. Coronaviruses have been identified to be in the Coronaviridae family of the order Nidovirales. In order for a virus to be classified under Coronaviridae family, it should have the following characteristics [4]:

1. Enveloped virions having large (15–20 nm) surface projections.
2. A helical nucleocapsid, made up of genome and several copies of a single basic phosphoprotein species.
3. An envelope having varying number of viral membrane proteins, at least two of which are the same (conserved) family-wide and are essential for virion morphogenesis and/or infectivity.
4. A 200–250-aa triple spanning N\textsuperscript{exo} C\textsubscript{endo} integral membrane protein M.
5. A 1100–1600-aa class I fusion protein S which forms peplomers and is highly N-glycosylated.

6. A positive sense RNA, linear, unimolecular, infectious, 26–32 kb long, capped, polyadenylated, and structurally polycistronic genome.

7. Follows a 5′-UTR-replicase-S-M-N-UTR-3′ general genome organization with the genome acting as mRNA for replicase gene.

8. The replicase gene is made of overlapping open reading frames (ORFs) 1a and 1b which code for two huge polyproteins—pp1a and pp1ab. The synthesis of pp1ab should require a programmed 21 ribosomal frameshift, and both pp1a and pp1ab should be processed autoproteolytically.

9. Expression of the downstream ORF should be mediated by (−1) ribosomal frameshifting.

10. Virion assembly (morphogenesis) should take place through budding of preformed nucleocapsids of smooth intracellular membranes of endoplasmic reticulum/early Golgi compartments.

**Coronaviridae** has two subfamilies—**Coronavirinae** and **Torovirinae**. **Coronavirinae** has four genera: alpha (α), beta (β), gamma (γ), and delta (δ). α and β coronaviruses are known to infect only warm-blooded animals, while γ and δ coronaviruses mainly affect birds. Some γ and δ coronaviruses, however, are known to affect mammals [5]. The novel coronavirus (SARS-CoV-2) belongs to the subgenus **Sarbecovirus** of genus **Betacoronavirus** (Fig. 2.1).

### 2.3 Jump from Animals to Humans

Coronaviruses are mainly zoonotic and produce diseases in animals mostly. Coronaviruses were thought to be minor pathogens for humans that generally caused mild respiratory infections in otherwise healthy immunocompetent individuals. These infections usually passed off as common cold with the rare exceptions of severe illnesses in infants, young children, and the older population [6]. However, this understanding took a paradigm shift with the advent of the highly pathogenic severe acute respiratory syndrome (SARS) in 2002, which was caused by the severe acute respiratory syndrome coronavirus (SARS-CoV) [7]. Up until the novel coronavirus pandemic, only six known coronaviruses caused diseases in humans. These included HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, and Middle East respiratory syndrome coronavirus (MERS-CoV). These viruses along with their hosts and associated diseases are shown in Table 2.1. The novel coronavirus is the seventh coronavirus known to produce disease in humans.

Since coronaviruses are mainly localized to animals, the novel coronavirus is thought to have been transmitted from an animal host to humans. This “jump” is thought to have occurred via an intermediate host. Knowledge of this intermediate host is important in preventing further spread of the disease [8]. Based on codon usage, snakes were thought to be the possible source of the novel coronavirus [9]. However, it is now hypothesized that mammals or birds may be the source or
Fig. 2.1 Classification of coronaviruses. *Bat SL-CoV-WIV1* Bat SARS-like coronavirus WIV1, *BToV* bovine torovirus, *DKNV* Dak Nong virus, *EAV* equine arteritis virus, *FCoV* feline coronavirus, *HCoV-HKU1* human coronavirus HKU1, *HCoV-OC43* human coronavirus OC43, *HKU4* (human coronavirus), *HKU5* (human coronavirus), *IBV* infectious bronchitis virus, *MHV* mouse hepatitis virus, *PRRSV* porcine reproductive and respiratory syndrome virus, *PEDV* porcine epidemic diarrhea virus, *PToV* porcine torovirus, *TGEV* transmissible gastroenteritis coronavirus, *YHV* yellow head virus, *WBV* white bream virus. (Adapted from Cong Y, Verlhac P, Reggiori F. The interaction between nidovirales and autophagy components. Viruses. 2017;9 (7):182–196)
intermediate hosts. Genetic analysis of the genome of SARS-CoV-2 showed that it is related to the bat CoV RaTG13. It is, thus, a separate lineage than SARS and bat SARS-like CoVs. It is now suggested that the 2019 novel coronavirus is a new human-infecting coronavirus which most probably originated from bats and, thereafter, jumped to human via intermediate hosts [7]. Liu et al. [8] predicted the interaction between receptor binding domain of coronavirus spike protein and the host receptor after analyzing coronavirus genome sequences and spike protein residues. They suggest that bats are the natural host, while pangolins, snakes, and even turtles can serve as intermediate hosts for the novel coronavirus. A definite conclusion about the natural and intermediate hosts is, however, still missing.

### 2.4 Morphology of SARS-CoV-2

As mentioned above, a virus has to fulfill certain criteria to be called a coronavirus. Members of *Coronaviridae* family are relatively large, enveloped, single-stranded RNA viruses. In fact, they are the largest known RNA viruses with the virion sizes ranging from 118 to 136 nm in diameter and genomes ranging from 25 to 32 kilobase pair (kbp) in length. The virions are spherical in shape and are characterized by relatively large spikes that emerge from the virus envelope [10]. Park et al. [11] isolated and reported the morphology of the novel coronavirus in a 35-year-old female patient. For identification, monolayers of vero cells were inoculated with the oropharyngeal samples of the patient. Five days after this first inoculation, blind passage of the culture supernatant was done. Thereafter, vero cell monolayers showing cytopathic effects were fixed using 2% formaldehyde and 2.5% glutaraldehyde. These fixed cells were sectioned and observed under an electron microscope.
Observation revealed spherical particles with crown-like spikes. These spherical particles were 66–81 nm in diameter and were observed within the cytoplasmic vesicles and in the extracellular space next to the cell membrane.

Four main proteins, called the structural proteins, and other accessory proteins make up and define the visible structure of the novel coronavirus. The structural proteins starting from the inside out include nucleocapsid (N) protein, membrane (M) glycoprotein, envelope (E) glycoprotein, and the spike (S) glycoprotein [12].

The nucleocapsid also known as the N proteins functions primarily to bind to the CoV RNA genome, making up the nucleocapsid [13]. It is located in the endoplasmic reticulum-Golgi region which is bound to the nucleic acid of the virus. It is involved in functions of the viral genome, replication of the virus, and the host response to viral infections [12]. It is heavily phosphorylated and is thought to lead to structural modifications that enhance affinity for viral RNA. Transient expression of the nucleocapsid proteins has, however, been shown to increase the production of virus-like particles (VLPs) markedly in some coronaviruses, thus suggesting that this protein might be required for complete virion formation and not envelope formation [14].

The next is the membrane or M protein. It is the most well-structured and the most abundant of all structural proteins in SARS-CoV-2. It determines the shape of the viral envelope. It is considered to be the central organizer of coronavirus assembly. It can bind and interact with all other major structural proteins [14]. Homotypic interactions among M proteins are responsible for the formation of virion envelope, but the M protein is not sufficient for virion formation by itself [15]. Interactions between M and N proteins stabilize the nucleocapsid proteins (N) and help in completion of viral assembly [12]. The interaction of spike (S) and membrane (M) proteins is essential for retention of S in the endoplasmic reticulum-Golgi intermediate compartment and also for the incorporation of S into new virions. However, it is not required for the assembly process [14, 16]. The M and E proteins together make up the viral envelope, and their interaction leads to the production and release of VLPs [14].

The envelope (E) protein is the smallest among the major structural proteins. It is a short, integral membrane protein of 76–109 amino acid sequences and ranges from 8.4 to 12 kDa in weight [14, 17, 18]. E has a short, hydrophilic amino terminus containing 7–12 amino acids and a large hydrophobic transmembrane domain of 25 amino acids. It ends with a long, hydrophilic carboxyl terminus which makes up a majority of the protein [14, 19, 20]. The envelope protein is needed for production and maturation of the virus. It is copiously expressed inside an infected cell, but only a small portion of this is incorporated into the virion envelope [14]. It is mainly localized at sites of intracellular trafficking—the endoplasmic reticulum, Golgi apparatus, and the endoplasmic reticulum-Golgi complex. Here it partakes in the assembly and budding of the virus [21]. Its importance in virus production and maturation can be gauged from the fact that recombinant coronaviruses which lack the envelope protein display markedly reduced viral titers, affected viral maturation, or produce incompetent progeny [14, 22].
The outermost protein responsible for giving a characteristic appearance and name to the coronavirus family is the spike (S) protein. It is a transmembrane protein and has a molecular weight of around 150 kDa. It mediates the entry of the virus into the host cell and hence also receives particular attention from scientists. The S glycoprotein forms homotrimers that project up from the viral structure and promote binding to the host cells by attraction and attachment to the angiotensin-converting-enzyme 2 (ACE2) receptors [12]. This protein is made up of two functional subunits—S₁ and S₂. These can be recognized upon cleavage of the S protein by host cell furin-like protease [3, 16]. Subunit S₁ binds to the host cell receptor and thus determines the host range and the types of cells that a virus can affect. Subunit S₂ mediates fusion of virus with the host cell membrane. Both these subunits remain non-covalently bound in the prefusion state [3]. The distal S₁ subunit makes up the receptor-binding domains (RBDs) and stabilizes the S₂ subunit that has the machinery needed for fusion in the prefusion state [23]. The S homotrimers contain numerous N-linked glycans which are needed for achieving the correct structure and access to host proteases and neutralizing antibodies [3]. Compared to the S protein of SARS-CoV (and other beta coronaviruses), the spike protein of SARS-CoV-2 has 12 extra nucleotides at its cleavage site which is similar to a canonical furin-like cleavage site. The presence of this furin-like cleavage site might increase the efficiency of spread of SARS-CoV-2 as compared to other beta coronaviruses [24].

Some beta coronaviruses exhibit an additional structural protein called the hemagglutinin-esterase (HE) protein. This protein binds to sialic acid present on the virion surface, and this binding and the esterase activity together help the virus enter into the host cell. This entry is further facilitated by the spike protein. The HE protein also helps in mucosal spread of the virus [24]. A difference between SARS-CoV and SARS-CoV-2 is that HE proteins are present in SARS-CoV although they lack membrane fusion activity and are accessory to the spike protein. It is also debatable whether they help in virion attachment or not [25]. However, the genome of SARS-CoV-2 lacks the hemagglutinin esterase gene [26]. A graphical representation of the structural proteins and genomic material of the novel coronavirus is given in Fig. 2.2.

All these proteins along with other nonstructural proteins are coded for by the genome of the virus which is discussed in the next section.

### 2.5 Genome of the Novel Coronavirus

Coronaviruses possess a single-stranded positive sense RNA (+ssRNA). There is a 5′-cap and 3′-poly-A-tail. The novel coronavirus genome is about 30 kb long and has at least six open reading frames (ORFs). At the 5′ end, ORF1a and ORF1b, the first open reading frame genes, constitute around two thirds of the complete genome length and codes for pp1a and pp1b proteins, respectively. These together make up 16 nonstructural proteins (nsp1–nsp16) [27]. These nonstructural proteins are required for the maintenance, replication, and optimum function of the virion.
A summary of their purpose in the virion is presented in Table 2.2. The four structural proteins—N, M, S, and E. S protein mediates binding of the virus with the host cell surface receptor—ACE2. The M and E proteins are embedded in the host membrane-derived lipid bilayer which encapsulates the single-stranded helical (positive sense) viral RNA around the N protein.

![Structure of the novel coronavirus-SARS-CoV-2. SARS-CoV-2 has four structural proteins—N, M, S, and E. S protein mediates binding of the virus with the host cell surface receptor—ACE2. The M and E proteins are embedded in the host membrane-derived lipid bilayer which encapsulates the single-stranded helical (positive sense) viral RNA around the N protein.](image)

**Fig. 2.2** Structure of the novel coronavirus-SARS-CoV-2. SARS-CoV-2 has four structural proteins—N, M, S, and E. S protein mediates binding of the virus with the host cell surface receptor—ACE2. The M and E proteins are embedded in the host membrane-derived lipid bilayer which encapsulates the single-stranded helical (positive sense) viral RNA around the N protein.

The 30 bp long nucleotides of the virus can be broken down as follows [1]:

1. 8.903 (29.86%) Adenosines
2. 5.482 (18.39%) Cytosines
3. 5.852 (19.63%) Guanines
4. 9.54 (32.12%) Thymines.
Also, five mutations exist in this genome, namely [1]:

1. T8782C (ORF1, codons AGT to AGC-silent mutation).
2. T9561C (ORF1a, codons TTA to TCA-nonsilent mutation).
3. C15607T (ORF1b, codons CTA to TTA-silent mutation).
4. C28144T (ORF8b, codons TCA to TTA-nonsilent mutation).
5. T29095C (nucleocapsid, codons TTT to TTC-silent mutation).

It is also known that these mutations in the novel coronavirus exist at the nucleotide level in S gene, nsp1, nsp3, and nsp15, and not at the amino acid level [1].

Lu et al. [29] have described the genomic characteristics of the novel coronavirus in detail. Their analysis revealed that the novel coronavirus is most closely related to bat-SL-CoVZC45 and SARS-like betacoronavirus of bat origin, bat-SL-CoVZXC21. The sequence resemblances were more than 90% in five gene regions—E, M, 7, N, and 14. Out of these, the E gene displayed a sequence similarity of 98.7% (the highest). The lowest similarity, between the novel coronavirus

| Protein | Process mediated |
|---------|------------------|
| nsp1    | Inhibition of interferon signaling, degradation of cellular mRNA |
| nsp2    | Not known |
| nsp3    | Cleaving of papain-like protease domains, blocking host innate immune response, promoting cytokine expression, tether RNA genome to the replicase/transcriptase complex |
| nsp4    | Formation of double membrane vesicle (membrane remodeling), transmembrane scaffold |
| nsp5    | Inhibition of interferon signaling, splitting chymotrypsin like proteins and main protease |
| nsp6    | Formation of double membrane vesicle, restrict expansion of autophagosome |
| nsp7    | Acts as a co-factor of nsp8 and nsp12 |
| nsp8    | Acts as a co-factor of nsp7 and nsp12, primase, can function as a processivity clamp for RNA-dependent-RNA-polymerase (RdRp) |
| nsp9    | Dimerization and binding of single-stranded RNA |
| nsp10   | Acts as building protein for nsp14 and nsp16 |
| nsp11   | Not known |
| nsp12   | RNA-dependent-RNA-polymerase primer |
| nsp13   | RNA 5′ triphosphatase (cap synthesis), RNA helicase |
| nsp14   | N7-methyltransferase, exo N 3′-5′ exonuclease (provides proofreading function for coronavirus RdRp) |
| nsp15   | N endo U endonuclease (cleaves single- and double-stranded RNA downstream of uridylate residues, producing 2–3 cyclic phosphates), evasion of double-stranded DNA sensors |
| nsp16   | Methyltransferase, helps avoid MDA5 (melanoma-differentiation-associated protein 5) recognition, downregulates innate immunity |
and the other bat-SLCoVs, was seen in the S gene region being only around 75%. An interesting finding was the low genetic resemblance between SARS-CoV and SARS-CoV-2 (about 79%) and between SARS-CoV-2 and MERS-CoV (about 50%). Compared to previous coronaviruses—the SARS-CoV, MERS-CoV, and the bat SARS-like coronaviruses—SARS-CoV-2 codes for a longer spike protein. As mentioned earlier, the spike (S) protein facilitates binding to host receptor and fusion with cell membrane and is functionally composed of the S1 domain and S2 domain—the functions of them both have been discussed in the previous section. The S2 protein of the novel coronavirus exhibits a 93% similarity to bat-SL-CoVZC45 and bat-SL-CoVZXC21, while the S1 protein displays a similarity of only 68% with these viruses. It is seen that the receptor binding domain of the mentioned bat coronaviruses is located in the C-terminal domain of S1; however, despite similarities in the S1 and S2 domains, the receptor binding domain of SARS-CoV-2 falls within lineage B and is hence closer to that of SARS-CoV. Thus, in terms of the whole genome sequence, the novel coronavirus is closer to bat SARS-like coronaviruses than SARS-CoV. Based on the observations above, certain differences in the genome sequences of the novel coronavirus (SARS-CoV-2) and SARS-CoV are given in Table 2.3. However, it remains to be seen how these differences affect the pathogenesis and functionality of the novel coronavirus. A basic representation of the genomes of the novel coronavirus and that of SARS-CoV is given in Fig. 2.3.

Upon analysis and comparison of the genome of SARS-CoV-2, it is evident that it resembles the genome of bat coronaviruses. Hence, the host and intermediate hosts of this virus can also be identified. As seen earlier, bats are purported to be the natural host of this virus. However, there are several factors suggesting that the conjecture of a direct transmission from bats to humans is fraught with inadequacies as mentioned below:

1. An outbreak was reported in late December 2019. This is a period of winter when bats are hibernating.

| Table 2.3 | Differences in genome of SARS-CoV and SARS-CoV-2 |
| --- | --- |
| Trait | SARS-CoV | SARS-CoV-2 |
| Vulnerability to mutations in spike protein-cell receptor interface-associated amino acids | Low | High |
| Length of S protein | Shorter (1255 amino acids) | Longer (1273 amino acids) |
| Receptor-binding domain | Lineage B | Lineage B (similar) |
| Free-binding energy of S protein-human ACE2-binding complex | Low | 28 times more than that of SARS-CoV |
| Protein 8a | Present | Absent |
| Protein 8b | Shorter (84 amino acids) | Longer (121 amino acids) |
| Protein 3b | Longer (154 amino acids) | Shorter (22 amino acids) |
**Fig. 2.3** (a) Organization of the genome of SARS-CoV-2. The size of the genome can range from 26 to 32 kilobase pair (kbp) and contains 6–11 open reading frames (ORFs) which encode 9680 amino acids. The first ORF constitutes about 67% (nearly two-thirds) of the genome and codes for 16 nonstructural proteins (nsp), while accessory and structural proteins are coded by the remaining ORFs. The functions of nsps are given in Table 2.2. (b) Coding regions of SARS-CoV-2 and SARS-CoV. Only ORFs of more than 100 nucleotides have been shown.
2. No bats were sold or found in the wet seafood market which is supposed to be the starting point of the pandemic, although there were several nonaquatic animals being sold.

3. The overall sequence similarity between SARS-CoV-2 and other bat coronaviruses is less than 90%; hence, bat coronaviruses cannot be said to be the direct ancestors of the novel coronavirus.

4. Coronaviruses are mainly zoonotic. Even in previous SARS and MERS outbreaks, there were intermediate hosts (masked palm civet for SARS and dromedary camels for MERS), and bats were the natural hosts of the virus.

Hence, based on the above points, the hypothesis that bats are the natural hosts while some other animal acted as an intermediate host of the virus is further strengthened [29]. However, making conclusive remarks about definitive and intermediate hosts is not possible at the moment.

### 2.6 Life Cycle of SARS-CoV-2

Viruses are like parasites which need another living body to survive. Outside this body, they either lie dormant or cannot survive for long. Similarly, the novel coronavirus too needs the human body to survive. The virus can enter the body either through direct contact, indirect transmission through aerosols, or through droplets [30]. There are different steps involved in entry and propagation of the virus inside the human body which are given below:

1. **Attachment**: Angiotensin-converting enzyme 2 (ACE2) receptor is a zinc-binding carboxypeptidase receptor expressed on the cell surfaces of different organ systems, viz., lungs, heart, ileum, small intestine, kidney, bladder, and others [10, 31]. This receptor has been identified as the target receptor for SARS-CoV-2. Receptor recognition is the first step in viral infection. It is also a key determinant of host cell and tissue tropism. As discussed earlier, the spike (S) protein of SARS-CoV-2 is responsible for recognition and attachment to human ACE2 (hACE2) receptors. It contains a receptor binding domain (RBD) that specifically recognizes ACE2 as its receptor. The RBD contains a core and a receptor-binding motif (RBM) that mediates contact with the receptor. The surface of ACE2 contains two virus-binding hotspots that are essential for binding with the virus. While binding to the receptor, the S protein RBM forms a concave surface with a ridge on one side which binds to the exposed outer surface of the “claw-like” ACE2 receptor. As compared to SARS-CoV, the RBM of SARS-CoV-2 forms a larger binding interface and more contacts with its receptor. SARS-CoV-2 RBM contains a four-amino-acid residue motif: glycine-valine/glutamine-glutamate/threonine-glycine. Due to these bulky residues and flexible glycine, the binding loop takes a different conformation as compared to SARS-CoV. This structural difference leads to the formation of an additional main chain hydrogen bond between Asn487 and Ala475 in the RBM of the novel coronavirus. Due to
this the ridge takes an even more compact arrangement, and the loop containing Ala475 gets even closer to ACE2. As a result the ridge forms more contacts with the N-terminal helix of ACE2 [32]. Two virus-binding hotspots have been identified in ACE2-SARS-CoV-2 interface: hotspot Lys31 (also known as hotspot 31) and hotspot Lys353 (also known as hotspot 353). These hotspots are relatively weak in SARS-CoV; however, in SARS-CoV-2, they are stronger and well-stabilized due to different conformations. These hotspots are important for coronavirus binding [32].

2. Penetration: As mentioned earlier, the S protein is made of two subunits—$S_1$ and $S_2$—which are non-covalently bound. The presence of the four amino acid residue at the boundary between $S_1$ and $S_2$ subunits results in the introduction of a furin cleavage site. The cleavage of the subunits occurs by furin present in the Golgi compartment. One reason, why the novel coronavirus affects different organs and is highly transmissible, could be the presence of this polybasic cleavage site in the fusion glycoprotein (S) which is cleaved by furin—a protease which is found ubiquitously in the body [3]. Once attachment occurs, type II transmembrane serine protease (TMPRSS2), which is present on the host cell surface, clears the ACE2 and activates the receptor-attached S protein. S protein is also activated by furin via cleavage. Activation of the S protein causes conformational changes and allows the virus to enter host cell either by fusion of the envelope (E) protein with the host cell surface or by the endosomal pathway [12, 33]. If virions are taken up into endosomes, cathepsin L activates the spike protein. However, this cysteine protease can be blocked by lysosomotropic agents (like bafilomycin A1 or ammonium chloride inhibitors). Thus, it is not an efficient mode of viral replication. Alternatively, if the S protein is activated by TMPRSS2, the viral membrane fuses with the plasma membrane [34]. This fusion is less likely to activate host cell immunity and is thus a more efficient method of viral replication.

3. Biosynthesis: Once the virus enters a host cell, it releases its genetic material in the cytoplasm. The first synthetic event in the life cycle of coronavirus is the translation of viral genome by host cell ribosomes. As the released viral genome is positive stranded, 5′ capped, and 3′ polyadenylated, it can be directly translated to proteins by the host ribosomes. The virus has 14 open reading frames (ORFs) which code for a variety of proteins—structural as well as nonstructural. The gene segments which code the nonstructural proteins (nsp) are translated first into ORF1a and ORF1b. This translation produces two large overlapping polyproteins—pp1a and pp1ab through a ribosomal frame-shifting mechanism. These polyproteins are supplemented by protease enzymes—papain-like proteases (PLPro) and a serine-type Mpro [chymotrypsin-like protease (3CLpro)] protease which are coded in nsp3 and nsp5. Thereafter, cleavage occurs between pp1a and pp1ab into nsp1–nsp11 and nsp1–nsp16, respectively [12]. These nsps play important roles in various processes in the virus and host cells which are mentioned in Table 2.2. Several of these nsps form replicase-transcriptase complex (RTC) with RNA-dependent RNA polymerase (RdRp) in double membrane vesicles (DMVs). This complex leads to transcription of positive sense mRNAs, and this process is mediated by RdRp [12].
4. **Maturation**: During biosynthesis, the subgenomic proteins get translated to structural (and nonstructural and accessory) proteins—N, M, S, and E. These proteins are bound/formed on smooth-walled vesicles in the endoplasmic reticulum and then moved to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) [12]. Genomic RNA is bound by N protein. This associates with M protein and buds into endoplasmic reticulum/Golgi membranes. M packs into membranes and is thought to produce membrane curvatures which leads to budding. S and E are acquired during budding [10].

5. **Release of virions**: The synthesized virions are released from the host cell via exocytosis. The ion channel activity of E protein is that of a viroporin. It alters the cell secretory pathways to expedite the release of virions from the cell. It increases the pH of transport vesicles [10]. Once these vesicles fuse with the plasma membrane, virions are released to continue infecting other cells.

Figure 2.4 shows the different steps involved in the life cycle of the novel coronavirus.

### 2.7 Host Immune Response

(a) **Innate immunity**: Host immune response is triggered as the virus enters the host cell. The viral antigens are presented to the antigen-presenting cells (APCs) which include dendritic cells and macrophages. These constitute a central part of the body’s antiviral immunity. APCs possess pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I like receptors (RLRs), and other free molecules located in various places in host cells like the plasma membranes, endosomal membrane, lysosomes, endocytolysosomes, and cytosol. These recognize different molecules present in viruses such as the nucleic acids, carbohydrate moieties, glycoproteins, lipoproteins, and other molecules or intermediate products like dsRNA and bring about cascade signaling to produce immune system cell effectors. Each PRR is capable of inducing a different biological response to different proteins [35, 36].

TLR-4 recognizes the spike protein. Through mediation of MyD88, it triggers activation of NF-κB transcription factors and the pathogen-activated protein kinase pathway to produce pro-inflammatory proteins. Activation of endosomal receptors like the TLR-3 and TLR which can recognize the RNA or dsRNA genome of coronavirus leads to recruitment of TRIF adapter protein directly. TRIF in turn directs interferon response factor 3 (IRF 3) and nuclear factor NF-κB transcription factors to induce pro-inflammatory cytokines like interferon-α (IFN-α) and tumor necrosis factor-beta (TNF-β). Type I IFN complexes with its receptors and activates the JAK-STAT pathways. JAK1 and TYK2 kinases phosphorylate STAT1 and STAT2 followed by its complexing with IFN-9. Both these migrate to the nucleus to start transcription of IFN-stimulated genes and lead to suppression of viral replication and prevent the severity of the disease [12]. This process can cause exhaustion, weakness, and
Binding and viral entry via membrane fusion or endocytosis

1. TMPRSS2-ACE2 receptors
2. SARS-CoV-2

Endocytosis

Membrane fusion and virus release

Release of viral RNA

Host ribosomes on +ve viral RNA

Translation

pp1a and pp1ab

Proteolysis

S
E
M
6
7a and 7b
8a and 8b
3a and 3b

mRNAs

N and 9b

Translation

Transcription

N

Replicase

−ve Viral RNA

+5' 3'

+5'

RNA replication and packaging (S,M,E
proteins combine with N)

Golgi body assembly and budding

Endoplasmic reticulum

SM E

ERE

Golgi body

Assembly and budding

RNA replication and packaging (S,M,E proteins combine with N)

Mature virion (inside Golgi vesicle)

Virus released out of the cell

Exocytosis

Mature virion (inside Golgi vesicle)

NUCLEUS

ERGIC

−3'

SM E

Endoplasmic reticulum

Fig. 2.4 SARS-CoV-2 life cycle inside a host cell
cough in patients. An important observation in case of SARS-CoV-2 infection is that immune response by type 1 IFN is suppressed [37]. It is seen that SARS-CoV-2 infection produces an aberrant immune response wherein the release of pro-inflammatory cytokines like IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, and TGFβ, and chemokines CCL2, CCL3, CCL5, CXCL8, CXCL9, and CXCL10 in excessive quantities from immune effector cells leads to hyperinflammation leading to acute respiratory distress syndrome (ARDS) [12].

(b) **Cell-mediated immunity:** The APCs present the CoV antigens to CD4+ T-helper (Th) cells via major histocompatibility complex (MHC) class 1. This causes release of IL-12—a stimulatory molecule which stimulates Th1 cell activation. In addition to the stimulation of Th1, other processes like the release of IL-12 and IFN-α, increase in MHC class I expression, and activation of natural killer (NK) cells are also required for thwarting viral replication and eradication of virus-infected cells. Antigen presentation also causes production of pro-inflammatory cytokines via the NF-κB signaling pathway. These cytokines recruit neutrophils and monocytes to the infection site and activate other pro-inflammatory cytokines and chemokines including IL-1, IL-6, IL-21, TNF-β, and MCP-1 [12]. Activation of Th1 cells stimulates CD8+ T cells which target and kill cells infected with the coronavirus. Simultaneously, CD4 T cells stimulate humoral immune response by activating T-dependent B cells [12, 38].

It is seen that in COVID-infected patients, the number and function of CD8+ T cells is greater than CD4+ T cells. Also, virus-specific T cells from severely infected patients tend to possess a central memory phenotype with a significantly higher polyfunctional CD4+ and CD8+ T cells. Strong T cell responses have been shown to have higher neutralizing antibody, while more serum Th2 cytokine secretion (e.g., IL-4, IL-5, IL-10—which increase production of antibodies) have been observed in deceased patients [37].

(c) **Humoral immunity:** The production of neutralizing antibodies plays a protective role in limiting infection. It also prevents re-infections in the future. The antibodies produced against SARS-CoV-2 infection are IgM and IgG which display a unique presence pattern. Usually IgM produced against SARS-CoV-2 lasts for only 12 weeks, but IgG will last longer [12]. SARS-CoV-2 infection induces production of IgG against the N protein, and this can be detected as early as day 4 after the onset of disease with most patients seroconverting by day 14 [37]. In addition to antibody formation, exposure to the novel coronavirus also leads to formation of CD4 T cells and CD8 memory cells as seen above, and these can last for up to 4 years [12].

2.8 **Immune Evasion by Coronaviruses**

Over the course of evolution, viruses have developed mechanisms to protect them from immune system cells. This ability enables them to survive and infect host cells efficiently. Such an avoidance strategy can be applied to different processes
both before and after entering a host cell. As seen earlier, host immune cells recognize a virus by various PRRs. The virus can avoid recognition by forming double membrane vesicles which lack these PRRs. This way they can replicate inside such vesicles without their dsRNA being detected [39]. Additionally, the virus has proteins that block IFN and thus avoid the immune system onslaught. nsp1 can suppress IFN 1 by inactivating the host translation mechanism, degrading host RNA and inhibition of STAT1 phosphorylation. This mechanism can cause IFN 1 failure and can lead to dissemination of viruses at an early stage and hence an increased severity of the disease [12, 40]. nsp14 and nsp16 can help the virus mimic the host capping mechanism. nsp14 initiates cap formation (5' end similar to host cell RNA), and nsp16 later modifies it to viral RNA. This way the virus seems similar to host cell RNA and thus can escape being recognized by immune cells [12, 41, 42].

nsp3 is another nonstructural protein which encodes two functional proteins, macro-domains and PLpro (which cleaves nsps). This nsp also helps the virus evade host cell immune response. Besides nonstructural proteins, the virus may also use accessory proteins to escape immune response. As an example, a protein coded for by ORF3b can antagonize the IFN signaling pathway and thus lead to inhibition of effector cell activation cascade responsible for eradication and inhibition of viral replication. Similarly, proteins coded in ORF6 can inactivate JAK-STAT signaling pathway by complexing with karyopherin-α2 and tethering karyopherin-β1 on internal membranes thus blocking nuclear translocation of transcription factor STAT-1 [12].

The novel coronavirus is a new virus, and limited literature is available on its different attributes. Hence, conclusive remarks about its life cycle or immune responses cannot be made at the present moment. Much understanding of how it behaves inside the human body comes from research on SARS-CoV which has been studied in detail in the past years. The immune evasion methods described above are also based on SARS-CoV research. As such, readers are encouraged to update themselves about immune mechanisms and responses of SARS-CoV-2 as and when additional information is added in the literature about it.

2.9 Pathogenesis

Knowledge about the life cycle and immune response to the novel coronavirus can help us better understand the progression of the disease that it causes. Various hypotheses have been proposed to explain the course of COVID-19 in the human body. From a cellular biology perspective, COVID-19 can be divided into three stages [43]:

1. First stage: asymptomatic period (the first 1–2 days of infection).

   The virus which has gained entry inside the body most likely binds to epithelial cells in the nasal cavity and begins multiplying. Through research conducted on SARS-CoV, it is surmised that ciliated cells are the first cells to be infected in
conducting airways. However, this understanding remains to be validated as conducting airways show low levels of ACE2 expression. At this stage, the virus progresses locally, and there is a limited innate immune response. The virus can be detected via nasal swabs during this period.

The clinical significance of this period is that in spite of having low viral load, the infected individuals can transmit the virus to others.

2. Second stage: upper airway and conducting airway period (the next few days).

The virus multiplies and migrates downward along the conducting airways. A stronger immune response is elicited at this stage. For testing purposes, both nasal swabs and sputum samples should theoretically yield the virus. The disease COVID-19 manifests itself clinically at this stage. The host immune responses given in the previous section are activated and amplified during this stage. Thus biochemical markers (cytokines, interferons, antibodies, etc.) can be tested for during this period. A majority of the infected patients (about 80%) experience the disease till this stage only, and they can be monitored at home via symptomatic therapy.

3. Third stage: hypoxia, pulmonary infiltrates, and progression to respiratory distress.

Around 20% infected patients progress to this stage of COVID-19. In this stage, the virus reaches the alveoli (gas exchange units) and infects type II pneumocytes. The virus proliferates in these cells releasing large number of viral particles which can affect other organs. The infected cells, however, undergo apoptosis and die. This way, large areas of the lungs may lose their type II cells, and thus epithelial regeneration is triggered. COVID-19 causes diffuse alveolar damage with fibrin-rich hyaline membranes and a few multinucleated giant cells. An aberrant wound healing leads to even more scarring and fibrosis.

**Cytokine storm in COVID-19:** The viral infection, if not controlled at stage 2 or 3, can lead to the death of the infected individual. A major cause of death from COVID-19 is the acute respiratory distress syndrome (ARDS). A severely lethal, uncontrolled systemic inflammatory response due to the release of large amounts of pro-inflammatory cytokines and chemokines (refer to Innate immunity under host immune response section) is called a “cytokine storm.” This cytokine storm triggers a violent attack to the body by its own immune system which can lead to ARDS, multi-organ failure, and eventually death [44].

Some other investigation parameters which have been reported from COVID-19 patients are given in Table 2.4.

The clinical presentation of COVID-19 varies with age—most severely affecting the elderly [43] while the pediatric age group remains relatively unaffected [31]. It is interesting because infants and young children are a high-risk group for other respiratory infections like the respiratory syncytial and influenza virus infection. The reason for this finding remains unknown; however, some possible explanations have been proposed [30, 31]:
Immature and quantitatively less ACE2 receptors in children.

Growing immune system in children reacts differently to SARS-CoV-2 as compared to adults.

Presence of other viruses in airways and lungs of children which can limit growth of SARS-CoV-2.

The target receptor for the novel coronavirus (ACE2) is found abundantly in the lungs and small intestine and is highly expressed in endothelial cells and smooth muscle cells of virtually all organs. Thus, SARS-CoV-2 is a threat not only to the respiratory system but also to the gastrointestinal, central nervous, and circulatory systems [45]. These systems along with their specific pathophysiology and symptoms are discussed below:

1. **Respiratory system**: The mechanism of infection of type II pneumocytes in lungs has been discussed previously. Three classical symptoms of COVID-19 related to the respiratory system are fever, cough, and shortness of breath. Other symptoms may include sore throat, nasal congestions, and dyspnea.

   Radiographically, bilateral lung involvement in the form of sub-pleural and peripheral areas of ground glass opacity and consolidation is seen [46].

   Histopathological examination reveals pulmonary edema, cellular fibromyxoid exudates with diffuse alveolar damage, pneumocyte desquamation, and formation of hyaline membrane. Lungs also exhibit interstitial mononuclear patchy inflammatory infiltrates dominated by lymphocytes. Intra-alveolar spaces show multinucleate syncytial cells with atypical enlarged pneumocytes showing virus-induced cytopathic effect [33].

2. **Gastrointestinal system**: The clinical features representing involvement of the digestive system are not very specific. Diarrhea, vomiting, and abdominal pain have been reported by affected patients [47]. Though these symptoms are not

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**Table 2.4** Blood investigation parameters from COVID patients [37]

| Parameter                                                                 | Percentage of patients |
|--------------------------------------------------------------------------|------------------------|
| Lymphopenia                                                              | 89.2                   |
| Neutrophilia                                                             | 74.3                   |
| Thrombocytopenia                                                         | 24.3                   |
| High (>5) neutrophil to lymphocyte ratio                                 | 94.5                   |
| High (>500) systemic immune inflammation index                          | 89.2                   |
| Increased C-reactive protein level                                       | 100                    |
| Increased lactate dehydrogenase                                          | 93.2                   |
| Increased d-dimer                                                        | 97.1                   |
| High level (>10 pg/ml) of IL-6                                           | 100                    |
| High levels of pro-inflammatory cytokines (IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, TNF-α) during cytokine storm | 100                    |
very severe and are also not reported by all patients, they do point to the fact that
the gastrointestinal system might get affected and also contribute in transmission
of the virus. The intestinal epithelium comes in direct contact with exogenous
pathogens, and, hence, it possibly gets affected first after consumption of a
SARS-CoV-2-infected animal. The nucleic acids of SARS-CoV-2 have also been
detected in stool samples of the affected patients strengthening the assumption
that gastrointestinal system is a potential route of transmission [45].

Other than this, patients can also suffer from liver injury with raised enzymes
found in blood tests. It is assumed that liver injury can occur in three ways—
direct viral infection of hepatocytes, immune-related injury, or due to drug hepa-
totoxicity. The possible mechanism of liver involvement could be the binding of
the virus to ACE2 receptors on cholangiocytes. Histopathology of liver samples
from deceased COVID-19 patient has revealed microvesicular steatosis and mild
lobular activity. However, no viral inclusions were seen [47].

3. Urogenital system: COVID-19 patients exhibit increased serum creatinine, urea
nitrogen, and urine protein indicating renal damage. Different studies have
shown that 3–10% of COVID patients have renal insufficiency and 7% have
acute kidney injury. Viral nucleic acids have also been isolated from urine sam-
ples of these patients. The CT scan of kidneys in COVID patients suggests
inflammation and edema in parenchymal region [45]. Apart from renal tubular
and mesenchymal cells, ACE2 is expressed in testicular and vas deferens cells as
well. It is thought that SARS-CoV-2 binds to these receptors and leads to dys-
function of kidneys and testis [48]. It is, therefore, important for clinicians to
assess the risk of testicular lesions in young patients to lessen the impact of
COVID-related reproductive injury.

4. Central nervous system (CNS): It is known that viruses can travel along infected
nerve endings. As the nasal mucosa is usually the first affected part of the body,
it is possible that the olfactory tract can act as a channel for viral transmission to
brain. The potential invasion of the brainstem by the novel coronavirus can lead
to acute respiratory failure. Headache, epilepsy, and confusion have been
reported by some COVID patients all of which point to intracranial infection.
Pulmonary injury can lead to hypoxia in the CNS leading to interstitial edema,
obstruction of cerebral blood flow, congestion, and even coma. CNS can also get
damaged from the cytokines released by glial cells as a result of SARS-CoV-2
infection [45].

5. Cardiovascular system: COVID patients have been known to present with circu-
latory system symptoms like palpitations, chest tightness, and shortness of
breath. Elevated creatinine kinase, creatinine kinase-MB, and hs-cTnl have been
also reported in COVID patients. It is presently believed that SARS-CoV-2
causes myocardial injury through three possible mechanisms:
(a) The virus infects the heart and causes direct myocardial injury.
(b) The virus binds to ACE2 receptors in the cardiovascular system and causes
myocardial injury via signaling pathways.
(c) The cytokine storm that occurs in COVID patients causes myocardial injury.
Additionally, hypoxemia and respiratory dysfunction caused by the virus can also damage myocardial cells [45]. Biopsies performed on COVID patients have revealed infiltration of myocardium by interstitial mononuclear inflammatory cells [46].

6. **Oral cavity:** ACE2 receptors are ubiquitously present in the respiratory tract as well as salivary gland duct epithelium in the mouth [30]. As such, the oral cavity and its functions can also be affected in COVID-19. Symptoms like irregular ulcers on dorsal surface of the tongue [49], hyposmia/anosmia and dysgeusia [50], glossodynia, unilateral ulcers (resembling recurrent herpetic stomatitis) on keratinized as well as non-keratinized mucosa, pain in the palate, and sore throat [51] have been reported. Some authors have also speculated that these might be the first manifestations of COVID in the human body [50]. However, this claim remains to be validated.

7. **Ocular manifestations:** It has been previously suggested that respiratory disease could be transmitted through the nasolacrimal system. It is known that the ocular mucosal system clears and drains fluid from the eye to the inferior meatus of the nose. Hence, it is possible that if a respiratory droplet is deposited in the eye, the infected ocular fluid can enter the respiratory system. Moreover, ACE2 receptors have been demonstrated on corneal and conjunctival cells. Conjunctival hyperemia, chemosis, epiphora, unilateral or bilateral conjunctivitis, foreign body sensation, tearing without blurred vision, inferior palpebral conjunctival follicles, and tender palpable preauricular lymph nodes have all been reported as the ocular manifestations of COVID-19. These findings are consistent with acute viral conjunctivitis. However, they have not been reported in all patients, and patients who presented with these complaints have not consistently tested positive for COVID. This paradox has been attributed to low viral load in ocular secretions and improper collection techniques [52, 53].

8. **Dermatological manifestations:** The commonly reported skin anomalies include chilblain-like lesions, maculopapular lesions, vesicular lesions, urticarial lesions, livedoid/necrotic lesions, pain, and burning. Skin lesions have been reported especially on acral sites like the digits of feet. These lesions begin as erythematous violaceous patches which turn to purpuric lesions, blisters, and necrotic lesions and finally return to normal [54]. Improvement in skin lesions has also been reported to be concomitant with improvement in laboratory markers (bilirubin transaminases and coagulation parameters) [55]. Once again the exact mechanisms of dermatological manifestations are not fully understood; however, a few plausible explanations have been suggested [56]:

   (a) Viral particles in cutaneous blood vessels lead to a lymphocytic vasculitis induced by immune complexes which activate cytokines. Immune reaction to COVID infection activates Langerhans cells leading to vasodilation and spongiosis.

   (b) Accumulation of microthrombosis from other organs can reduce blood flow to cutaneous microvascular system.
Accumulation of deoxygenated blood in veins due to hypoxia and low-grade generalized intravascular coagulation can also cause such lesions.

(d) Pauci-inflammatory thrombogenic vasculopathy with deposition of C5b-9 and C4d can also cause such manifestations.

None of the above given theories can explain the cutaneous manifestations on its own, and such features are most likely a result of these mechanisms acting together. Cutaneous manifestations could also be late manifestations of the inflammatory phase of a primary respiratory infection [55].

Thus, it can be appreciated that once the novel coronavirus reaches the bloodstream, it can affect any organ in the body; however, its target remains the same—the ACE2 receptor. This is of concern because patients might present with symptoms other than the recognized classical features of COVID, and misclassification or incorrect diagnosis of patients can negatively impact community transmission control efforts. Therefore, a sound knowledge and understanding of the pathophysiological mechanisms and manifestations of SARS-CoV-2 can help in its early diagnosis and management.

2.10 Conclusion

The novel coronavirus has ravaged the world since it first came to light in December 2019. It is a beta-coronavirus approximately 66–81 nm in diameter. Having originated in bats, it has jumped from its natural host into humans via an intermediate host which is still not known. Four main structural proteins—N, M, E, and S—provide the necessary framework for establishing its characteristic morphology and also define its pathologic ability. These as well as other nonstructural and accessory proteins are coded for by the genome of the virus which displays significant differences from its predecessor—the SARS-CoV. The virus can enter the body by direct contact, aerosols, and droplet transmission and attaches to its target receptor ACE2 via the spike protein. This spike protein exhibits considerable differences from other coronaviruses which might explain its increased transmissibility. Post attachment, it multiplies and also escapes detection by host immune cells via specific strategies. SARS-CoV-2 can affect pulmonary as well as extrapulmonary sites due to the ubiquitous distribution of ACE2 receptors. An understanding of its structure, immune response, and pathophysiology can help in better management of affected patients and possibly arrest the spread of this pandemic.

References

1. Dagur HS, Dhakar SS. Genome Organization of Covid-19 and emerging severe acute respiratory syndrome Covid-19 outbreak: a pandemic. EJMO. 2020;4(2):107–15.
2. Malik YA. Properties of coronavirus and SARS-CoV-2. Malays J Pathol. 2020;42(1):3–11.
3. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020;180(2):281–92.
4. International Committee on Taxonomy of Viruses. ICTV 9th Report 2011. Available from: https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/222/coronaviridae. Accessed 31 May 2020.

5. Jaiswal NK, Saxena SK. Classical coronaviruses. In: Saxena SK, editor. Coronavirus disease 2019 (COVID-19). 1st ed. Singapore: Springer; 2020. p. 141–50.

6. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol. 2017;39(5):529–39.

7. Salata C, Calistri A, Parolin C, Palu G. Coronaviruses: a paradigm of new emerging zoonotic diseases. Pathog Dis. 2019;77(9):1–5.

8. Liu Z, Xiao X, Wei X, Li J, Yang J, Tan H, et al. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. J Med Virol. 2020;92(6):595–601.

9. Ji W, Wang W, Zhao X, Zai J, Li X. Homologous recombination within the spike glycoprotein of the newly identified coronavirus may boost cross-species transmission from snake to human. J Med Virol. 2020. (Accepted manuscript).https://doi.org/10.1002/jmv.25682.

10. Payne S. Chapter 17- Family Coronaviridae. In: Viruses. Academic Press. 2017. pp. 149–158.

11. Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, et al. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol. 2011;174(1):11–22.

12. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Coronaviruses. 2015;1282:1–23.

13. Li Y, Surya W, Claudine S, Torres J. Structure of a conserved Golgi complex targeting signal in coronavirus envelope proteins. J Biol Chem. 2014;289(18):12535–49.

14. Liao Y, Yuan Q, Torres J, Tam J, Liu D. Biochemical and functional characterization of the membrane association and membrane permeabilizing activity of the severe acute respiratory syndrome coronavirus envelope protein. Virology. 2006;349(2):264–75.

15. Nieto-Torres JL, DeDiego ML, Álvarez E, Jiménez-Guardeño JM, Regla-Nava JA, Llorente M, et al. Subcellular location and topology of severe acute respiratory syndrome coronavirus envelope protein. Virology. 2011;415(2):69–82.

16. Kuo L, Hurst KR, Masters PS. Exceptional flexibility in the sequence requirements for coronavirus small envelope protein function. J Virol. 2007;81(5):2249–62.

17. Li Y, Surya W, Claudine S, Torres J. Structure of a conserved Golgi complex targeting signal in coronavirus envelope proteins. J Biol Chem. 2014;289(18):12535–49.
26. Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microb Infect. 2020;9(1):221–36.
27. Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication, and pathogenesis. J Med Virol. 2020;92(4):418–23.
28. Mirza MU, Froeyen M. Structural elucidation of SARS-CoV-2 vital proteins: computational methods reveal potential drug candidates against main protease, Nsp12 polymerase and Nsp13 helicase. J Pharm Anal. 2020 (Accepted manuscript). https://doi.org/10.1016/j.jpha.2020.04.008.
29. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395(10224):565–74.
30. Singh V, Lehl GK, Talwar M, Luthra A. The novel coronavirus and challenges for general and paediatric dentists. Occup Med (Oxford, England). 2020 (Ahead of print). https://doi.org/10.1093/occmed/kqaa055.
31. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: a review. Clin Immunol. 2020;215(108427):1–7.
32. Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, et al. Structural basis of receptor recognition by SARS-CoV-2. Nature. 2020;581(7807):221–4.
33. Kumar S, Nyodu R, Maurya VK, Saxena SK. Morphology, genome organization, replication, and pathogenesis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In: Saxena SK, editor. Coronavirus disease 2019 (COVID-19). 1st ed. Singapore: Springer; 2020. p. 23–31.
34. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, 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.
35. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. J Med Virol. 2020;92(4):424–32.
36. Yi Y, Lagniton PN, Ye S, Li E, Xu R. COVID-19: what has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci. 2020;16(10):1753–66.
37. Rokni M, Ghasemi V, Tavakoli Z. Immune responses and pathogenesis of SARS-CoV-2 during an outbreak in Iran: comparison with SARS and MERS. Rev Med Virol. 2020;30(3):e2107. https://doi.org/10.1002/rmv.2107.
38. Rabi FA, Al Zoubi MS, Kasasbeh GA, Salameh DM, Al-Nasser AD. SARS-CoV-2 and coronavirus disease 2019: what we know so far. Pathogens. 2020;9(3):231–44.
39. Snijder EJ, Meer Y, Dobbe J, Onderwater JM, Meulen J, Koerten H, et al. Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. J Virol. 2006;80(12):5927–40.
40. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol. 2020;38(1):1–9.
41. Chen Y, Cai H, Xiang N, Tien P, Ahola T, Guo D. Functional screen reveals SARS coronavirus nonstructural protein nsp14 as a novel cap N7 methyltransferase. Proc Natl Acad Sci. 2009;106(9):3484–9.
42. Daffis S, Szretter KJ, Schriewer J, Li J, Youn S, Errett J, et al. 2′-O methylation of the viral mRNA cap evades host restriction by IFIT family members. Nature. 2010;468(7322):452–6.
43. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J. 2020;55:2000607. https://doi.org/10.1183/13993003.00607-2020.
44. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal. 2020;10(2):102–8.
45. Zhang Y, Geng X, Tan Y, Li Q, Xu C, Xu J, et al. New understanding of the damage of SARS-CoV-2 infection outside the respiratory system. Biomed Pharmacother. 2020;127:110195.
29

46. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. JAMA Cardiol. 2020:E1–E10 (Ahead of print). https://doi.org/10.1001/jamacardio.2020.1286.

47. Wong SH, Lui RN, Sung JJ. Covid-19 and the digestive system. J Gastroenterol Hepatol. 2020;35(5):744–8.

48. Fan C, Li K, Ding Y, Lu WL, Wang J. ACE2 expression in kidney and testis may cause kidney and testis damage after 2019-nCoV infection. BMJ. 2020 (Pre-print). https://doi.org/10.1101/2020.02.12.20022418.

49. Chaux-Bodard AG, Deneuve S, Desoutter A. Oral manifestation of Covid-19 as an inaugural symptom? J Oral Med Oral Sur. 2020;26(2):18.

50. Vinayachandran D, Balasubramanian S. Is gustatory impairment the first report of an oral manifestation in COVID-19? Oral Dis. 2020;00:1–2.

51. Presas M, Sánchez A, López AF, Jané E, Pérez ML. Oral vesiculobullous lesions associated with SARS-CoV-2 infection. Oral Dis. 2020: 1–3 (Ahead of print). https://doi.org/10.1111/odi13382.

52. Aiello F, Afflitto GG, Mancino R, Li JP, Cesareo M, Giannini C, et al. Coronavirus disease 2019 (SARS-CoV-2) and colonization of ocular tissues and secretions: a systematic review. Eye. 2020:1–6 (Ahead of print). https://doi.org/10.1038/s41433-020-0926-9.

53. Dockery DM, Rowe SG, Murphy MA, Krzystolik MG. The ocular manifestations and transmission of COVID-19: recommendations for prevention. J Emerg Med. 2020 (Article in press). https://doi.org/10.1016/j.jemermed.2020.04.060.

54. Guarneri C, Rullo EV, Pavone P, Berretta M, Ceccarelli M, Natale A et al. Silent COVID-19: what your skin can reveal. Lancet Infect Dis. 2020 (Ahead of print). https://doi.org/10.1016/S1473-3099(20)30402-3.

55. Morey-Olivé M, Espiau M, Mercadal-Hally M, Lera-Carballo E, Garcia-Patos V. Cutaneous manifestations in the current pandemic of coronavirus infection disease (COVID 2019). Anales De Pediatría. 2020 (Ahead of print). https://doi.org/10.1016/j.anpede.2020.04.002.

56. Sachdeva M, Gianotti R, Shah M, Lucia B, Tosi D, Veraldi S, et al. Cutaneous manifestations of COVID-19: report of three cases and a review of literature. J Dermatol Sci. 2020;98(2):75–81.