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SARS-CoV-2 Molecular Peculiarities are Armaments Employed by the Virus but could be Potential Targets for Drugs

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Abstract

Background: SARS-CoV-2 (nCoV) is the causative virus of COVID-19 disease. It belongs to the Coronaviridae family which includes other members causing other forms of severe acute respiratory diseases. Although nCoV shares great genetic similarity with its relatives, it possesses specific structural components that render it peculiar in replication, pathogenicity, and response to drugs.

Objectives: To elaborate the molecular structures of nCoV and correlate them with the COVID-19, reveal the mechanism of disease pathogenicity towards rationalizing the use of the old drugs, and establishment of the base of development of novel drugs.

Methods: Different search engines such as Google, PubMed-PMC-NCBI, Elsevier Coronavirus Research Hub, Wikipedia, Microsoft Academic Search, RefSeek-Academic Search Engine, were examined using the main search stream as the nCoV, its structural domains, molecular bases of pathogenicity, and authors' names as s keyword in the search.

Results: Detailed molecular studies of different structural and nonstructural components of nCoV had been extensively elaborated to an extent made it possible to pinpoint the drug binding sites, revealed the mechanism by which nCoV induces pathological changes.

Conclusion: Our knowledge of the molecular structure of nCoV is of great importance in the determination of mutation sites, gain an insight into the pathology of the disease, and in providing data that could be the basis for systematic drug repurposing approaches. It also constitutes the cornerstone for the development of novel drugs and establishes a solid COVID-19 treatment strategy in the future.

Keywords: SARS-CoV-2; COVID-19; Angiotensin-converting enzyme 2; Spike protein; RNA-dependent RNA polymerase; Cytokine storm; Pneumonia; Coagulopathy; Antiviral therapy

Abbreviations

ACE2: Angiotensin-Converting Enzyme 2; B^0AT1: amino acid transporter (SLC6A19); 3CLpro: 3C-like protease; CD4+ and CD8+: helper and cytotoxic T-lymphocyte; CLD: Collectrin-Like Domain; COVID-19: Coronavirus Disease; CT: C-terminal; CTD: C-terminal dimerization domain; HE: Hemagglutinin Esterase; HR: Heptad Repeat; IL: Interleukin; E: Envelope protein; ER: Endoplasmic Reticulum; ERGIC: ER-Golgi Intermediate Compartment; M: membrane/matrix protein; Mpro: Main protease; N: Nucleocapsid protein; N7-MTase: guanine-N7-methyltransferase; NLR: Nucleotide-binding domain leucine-rich repeat; NLRP3: pyrin domain-containing receptor 3 inflammasome; nsp: niche-specific proteins, nonstructural protein; NT: N-terminal; NTD: N-terminal RNA-binding domain; O-MTase: O-methyltransferase; ORF: Open-Reading Frame; PAI-1: Plasminogen Activator Inhibitor-1; PLpro: Papain-Like cysteine protease; RAS: Renin-Angiotensin system; RBD: Receptor-Binding Domain; RdRp; RNA-dependent RNA polymerase (replicase); RNP: viral nucleocapsid+ protein ribonucleoprotein; (+) and (-) ssRNA: positive and negative-single-strand (sense) RNA; gRNA and sgRNA: genomic and subgenomic RNA; RTC: Replication-Transcription Complex; SARS: Severe Acute Respiratory Syndrome; S: Spike glycoprotein; SARS-CoV-2: new coronavirus (nCoV); TMPRSS2: Transmembrane Protease, Serine 2; TPC2: two-pore channel subtype 2; VLP: Virus-Like Particles

Introduction

The COVID-19 disease is caused by Severe Acute Respiratory Syndrome (SARS) coronavirus

(CoV), SARS-CoV-2 (referred to as nCoV hereafter). The infection source of COVID-19 maybe bats, pangolins, murine, etc. It is a major health concern implicated in an alarming pandemic global outbreak. The COVID-19 patients have symptoms of varying degrees, ranging from fever or a mild cough to pneumonia and extensive multiple organ (brain, cardiovascular, renal) dysfunctions. The number of confirmed global COVID-19 cases exceeded 16.5 million with more than 655.112 deaths, 3.96% of total cases [1].

It has been reported that the nCoV is different from MERS-CoVs in terms of the whole genome sequence. Moreover, nCoV is structurally and functionally different from its other SARS-CoVs relatives. While there are no amino acid substitutions in nonstructural proteins, envelope, or accessory proteins, 380 amino acid substitutions had been detected between the amino acid sequences of nCoV and the corresponding SARS and SARS-like viruses, which may shed light into how differs structurally and functionally from SARS-CoVs [2].
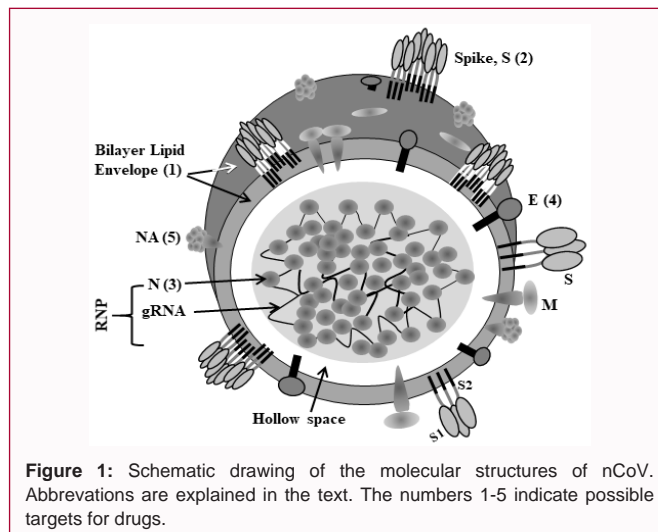
Various animal species had been incriminated as a source of human COVID-19 sources. The virus potentially recognizes the Angiotensin (Ang)-Converting Enzyme 2 (ACE2) receptor from a diversity of animal species, except mice and rats, because mouse or rat ACE2 contains a histidine at the position 353, which does not fit into the virus-receptor interaction. The nCoV Receptor-Binding Domain (RBD) likely recognizes ACE2 from pigs, ferrets, cats, orangutans, monkeys, and humans with similar efficiencies, because these ACE2 molecules are identical or similar in the critical virus-binding residues, implicating these animal species as possible intermediate hosts for nCoV infections. The situation involving bat ACE2 is complex because of the diversity of bat species. It is likely that nCoV's RBD recognizes bat ACE2 from *Rhinolophus sinicus* bats as its receptor, but the same is not valid for other species [3].

Although the structure of the virus is a complex one, the molecular components are extensively elaborated. Our knowledge of the structure of nCoV is indispensable for the development of effective antiviral drugs and vaccines. Indeed, the knowledge of the molecular structures of the virus, and the seriousness of the disease prompted greater investment for drug development that may be useful not only for the present pandemic but for the future possible outbreaks too.

This review aims to reveal the molecular structures of the virus that are used by the virus to invade human cells, replicate, multiply, harm the cell, and spread out. A plethora of researches and publications relevant to epidemiology and molecular biology of COVID-19 and nCoV can be found in the literature. However, the mechanistic correlation of these structures with virus infectivity awaits more elucidation. Accordingly, we tried in this review to highlight the molecular structures of nCoV that are responsible for the virus's pathological effect and could be potential targets for anti-coronavirus drugs.

Methods

Based on publications (especially recently published literature, official documents, and selected up-to-date preprints) we reviewed the virology, pathology, and treatment of COVID-19 infection using two main search streams: 1. the virus nCoV structure, virulence, and susceptibility to antivirals; 2. the disease-COVID-19, clinical manifestations, pathology, and treatment. The authors' names were referred to as an additional keyword in the search. The search was conducted using several search engines including Google, PubMed-PMC-NCBI, medRxiv, Elsevier Coronavirus Research Hub,

Figure 1: Schematic drawing of the molecular structures of nCoV. Abbreviations are explained in the text. The numbers 1-5 indicate possible targets for drugs.

Wikipedia, Microsoft, and RefSeek-Academic Search Engines.

Structural characteristics of nCoV

The nCoV is lipid-enveloped, spherical, or oval particles measuring 80-120 nm (average 100 nm) in diameter. The viral envelopes are acquired from membranous structures of various components of the host cell including the plasma membrane, internal cell membranes such as the Endoplasmic Reticulum (ER), and the Golgi complex. For most viruses, the envelope lipids are expected to contain the components of these membranes such as phospholipids, sphingolipids, and some cholesterol. However, the lipid composition is not the same across sub-cellular membranes. The mammalian plasma membranes having higher cholesterol and sphingolipid content, whereas ER-Golgi Intermediate Compartment (ERGIC) contains more phosphatidylcholine, but less cholesterol and sphingolipids than the plasma membrane. Since coronaviruses are known to bud from ERGIC before being transported by exocytosis, it is expected that nCoV envelop may contain more phosphatidylcholine [4].

nCoV is a positive (+)-single-strand (sense) RNA (+ssRNA) virus, belongs to Lineage b, Genus β that is a member of Coronavirinae subfamily of the family Coronaviridae which in turn comprises the order Nidovirales [5]. The genomic RNA (gRNA) and non-genomic structural component of nCoV had been determined. The structural peculiarities are shown in Figure 1 and detailed in the following sections.

Structural proteins and glycoproteins

The viral genome encodes more than 20 proteins. Of these four major structural proteins play important role in nCoV infectivity: the spike (S) glycoprotein, nucleocapsid (N) protein, membrane (M) protein, and the envelope (E) protein. All of these proteins are required to produce a structurally complete integrated viral particle. Individually, each protein primarily plays a role in the structure of the virus particle, but they are also involved in other aspects of the replication cycle.

Spike glycoprotein unit (S)

It is class I fusion protein, densely glycosylated, and integrated over the surface of the virus. S is a globular club-like, pear-shaped, or petal-shaped transmembrane structure protrudes some 17-20 nm from the viral surface. The individual S domains assemble into groups of three on the outer membrane, giving the nCoV its characteristic

"corona-like" appearance. Structurally, each S is composed of two non-covalently bound subunits, S1 and S2, each assembled into trimmers (Masters). S mediates attachment and fusion of the virus with the host cell membranes, and ensures viral entry into the host cell [6].

The S1-subunit contains the RBD. It binds to the extracellular Peptidase Domain (PD) of ACE2 by its polar residues. This binding initiates fusion of the nCoV with cell membrane by induction of conformational changes in the S2 subunit. S1 should be first activated in order to modify S2 and induce fusion. Moreover, to engage a host-cell receptor, the RBD of S1 undergoes hinge-like conformational movements that transiently hide (down receptor-inaccessible state) or expose (up conformation-(receptor-accessible state) the determinants of receptor binding [7].

On the other hand, the S2-subunit contains amino acid sequences necessary for membrane fusion and endocytosis. A critical proteolytic cleavage event within the S2 subunit, acting in concert with the S1-S2 cleavage site to mediate membrane fusion and virus infectivity had been proposed as a novel priming mechanism for a viral fusion protein. The S2 is composed of two Heptad Repeats (HR) HR1 and HR2 (heptad repeat is a structural motif that consists of a repeating pattern of seven amino acids containing hydrophobic, charged and polar residues). HR1 forms a homotrimeric assembly forms a six-helix bundle with HR2, exposes the site for HR2 binding on the cell membrane, and helps bring the viral and cellular membranes into close proximity, allowing the virus to bind with the cell membrane and thence enter into the cell. On the other hand, HR2 is located close to the membrane anchor. The conformation changes induced after binding of S1 to ACE2 bring fusion peptide, located in the N-terminal (NT) of HR1, and the transmembrane anchor into close proximity [8]. During viral infection, the trimeric S unit is cleaved into S1 and S2 subunits, and S1 subunits are released in the transition to the post-fusion conformation [9,10]. When S1 binds to the host receptor ACE2, another cleavage site on S2 is exposed and is cleaved by host proteases, such as Transmembrane Protease, Serine 2 (TMPRSS2), a process that is critical for viral infection [9,11]. The S glycoproteins, especially S1, maybe a potential target for specific drugs.

Envelope protein (E)

The E is the smallest integral membrane protein of the major structural proteins and is found in relatively low numbers in the virus particle. The nCoV possesses a unique and characteristics E. This E contains at least one α-helical Transmembrane Domain (TMD). In particular, arginine, positively charged amino acid, replaces Glutamate or Glycine, negatively charged and neutral respectively [12]. E proteins present at low concentration in the nCoV virus but found abundantly in internal membranes of infected cells, from ER to Golgi where it participates in the virus assembly and budding. The majority of the protein is localized at the site of intracellular trafficking, compared to the ER, Golgi, and ERGIC, where it participates in nCoV assembly and budding [13]. The E protein is thought to have several functions that contribute to virus growth and its ability to cause disease. The E protein-PDZ-Binding Motif (PBM), a domain involved in protein-protein interactions, is a major determinant of viral virulence. The PBM of E protein was found to be directly involved in SARS-CoV pathogenesis by binding the host's syntenin protein resulting in overexpression of inflammatory cytokines contributing to the so-called "cytokine storm" [14]. Furthermore, the E-formed channel can be embedded into the ERGIC-Golgi membranes and facilitate

Ca^{2+} transport resulting in the activation of the nucleotide-binding domain Leucine-rich Repeat (NLR) and pyrin domain-containing receptor 3 (NLRP3) inflammasome followed by an overproduction of interleukin (IL)-1 β [13].

The E is involved in important stages of the virus's morphogenesis and life cycle, such as assembly, budding, envelope formation, pathogenesis [15], membrane permeabilizing activity [16,17], and interactions with other viral and host cell proteins [15]. Absence, or inactivation, of E protein results in attenuated viruses, due to alterations in either virion morphology or tropism [16]. During the replication cycle, E protein is abundantly expressed inside the infected cell, but only a small portion is incorporated into the virus envelope [18]. Coronaviruses are distinct from other enveloped viruses in that they bud in the ERGIC, from where they acquire their membrane envelope. Once in the lumen of the ERGIC, infectious viruses make their way through the host secretory pathway to, ultimately, be released from the infected cell [19]. Accordingly, the E protein is localized mainly to the ER and Golgi complex where it participates in the assembly, budding, and intracellular trafficking of infectious viruses [20]. Additionally, the E-protein participates in the formation of viroporin-viral proteins with ion channel activity that plays an important role in several processes including virus replication and alters the properties of host cell membranes. Coronaviruses have three types of ion channels: pentameric assemblies composed of single TMD (E and 8a protein), and 3a with three TMD. The E protein has short hydrophilic amino terminus followed by a large hydrophobic TMD. The hydrophobic region can generate oligomerization and form an ion-conductive pore in membranes. The E viroporin is cation-selective, demonstrating a preference for the monovalent cations Na^+ and K^+ [21]. It has been suggested that the viral viroporins and PBMs are suitable targets for antiviral therapy and for the mutation in attenuated SARS-CoV vaccines. Mutation of the E-protein could disrupt the ion-conductivity and the normal viral assembly [22], hence control the E protein dynamics is a promising target for preventing pathogenesis associated with the COVID-19. In this respect, blockade of E–protein ion channels by hexamethylene amiloride [23], belachinal, macaflavanone E, and vibsanol B [17], or deletion of E-protein by inhibitor [24] all had been shown to block the E-protein ion channel conductance in the cell membrane and inhibit replication of coronavirus.

Nucleocapsid (N) protein

The N protein consists of three distinct but highly conserved parts: NT RNA-binding domain (NTD), C-terminal dimerization domain (CTD), and an intrinsically disordered central Ser/Arg (SR)-rich linker. The NTD is responsible for RNA binding, CTD for oligomerization, and (SR)-rich linker for primary phosphorylation [25].

Unlike the other major structural proteins, N is a multifunctional protein that interacts with other viral membrane proteins during the assembly of the virus particles. N is the only protein that functions primarily to bind to the nCoV RNA genome, making up the capsid around the enclosed RNA. Multiple copies of the N link together to form a spiral that tightly wraps and coils the RNA. This allows the long RNA molecule to fit into the small virus particle and forms a protein coat around the RNA that protects it from damage. It also assists in RNA folding and release. Moreover, N protein participates in RNA package, budding and release. N protein has a role in determining the ratio of the genome- to sub-genome-length minus strands and,

in the absence of N, this ratio is perturbed, with the underproduction of genome-length templates. N protein does not function as a replication-transcription switch. It may act as an RNA chaperone, where chaperone activity results in the transient dissociation of RNA structures, important for the initiation of minus-strand synthesis. The coronaviruses possess helical nucleocapsids. This is not common among plus-stranded RNA viruses but is typical of minus-stranded RNA viruses. It is possible that the role of the coronavirus N protein is to associate with the gRNA to produce a template that is "configured" to balance the ratio of Replication-Transcription Complexes (RTCs) engaged in either transcription or replication [26].

N protein has an important function in the early stages of infection when the RNA molecule is first released into the host cell, acting to reduce the cell's natural defenses against the virus. Moreover, N induces the intrinsic apoptotic pathway, resulting in cleavage of N by cysteine-aspartic proteases, caspase-6 and/or caspase-3. Of note, caspase activation is highly cell type-specific in nCoV [27]. Three lines of evidence support implication of N in virus RNA synthesis: first: N protein binds to the leader RNA sequences present at the 5' end of gRNA and subgenomic RNA (sgRNA) [28]; second: in addition to a cytoplasmic distribution in the host cell, at least a fraction of the N protein colocalizes with the RTCs early in infection [29]; third: there is clearly a requirement for sustained translation of the N protein in trans or in cis form for optimal replication of nCoV RNA [30]. Although N is largely involved in processes relating to the viral genome, it is also involved in other aspects of the nCoV replication cycle and the host cellular response to viral infection [31]. Interestingly, localization of N to the ER-Golgi region has proposed a function for it in assembly and budding [32]. Moreover, N protein is required for synthesis and release of Virus-Like Particles (VLPs) and incorporation of S into C-like protease, suggesting that requirements for nCoV differ from those of other coronaviruses, which require M and E but not N protein [33].

Membrane protein (M, Matrix protein)

The M protein is the most abundant membrane-embedded structural protein and it is believed to be the central organizer for the coronavirus assembly. It defines the shape and integrity of the viral envelope, but alone, it is not sufficient for virus formation [34]. It interacts with all other major coronavirus structural proteins. Interaction of M with S is necessary for retention of S in the ERGIC, and assembly of new viruses [35]. On the other hand, binding of M to N stabilizes the nucleocapsid (N protein-RNA complex), as well as the internal core of the virus, and ultimately promotes the completion of viral assembly [36]. Together, M and E make up the viral envelope and their interaction is sufficient for the production and release of VLPs [37].

Nonstructural components

The coronavirus gRNA encodes both structural proteins of the virus and nonstructural proteins important for viral RNA synthesis. These proteins are known as replicase-transcriptase proteins. Moreover, there are nonstructural proteins that are nonessential for virus replication but confer a selective activity in the viral pathogenicity. These are referred to as niche-specific proteins, nonstructural protein (nsp). In CoVs, nsps are recruited to RTCs that synthesize both gRNA and sgRNA. The nsps display a variety of activities such as proteinase, polymerase, endonuclease, and methyltransferase [26]. At least one nsp (nsp 12), and one structural protein (N) are involved in viral RNA synthesis. The other vital components are proteases. Proteases

are very important for virus entry to cells and replication. They represent potential targets for antiviral drugs. The proteases are 2 types: host/human- and virus-originated proteases. The proteases are important in polyproteins processing, replication, and maturation of the structural proteins.

The nCoV uses two host proteolytic enzymes to prim S glycoprotein. S can be cleaved by both the furin (at the of S1 and S2 and within S2) and the TMPRSS2 (at proteolytic cleavage site within S2). Furin belongs to the proprotein convertases, responsible for the maturation of a huge number of inactive protein precursors. It cleaves SARS-CoV-2 spike, inactive protein precursors, into their biologically active products, thus facilitating viral entrance into host cells. TMPRSS2, a human cell surface serine protease together with furin enhances S1 binding to ACE2, and S2 fusion with the cell membrane, resulting in membrane fusion and endocytosis of nCoV. ACE2 and TMPRSS2 are essential in airway cells for nCoV infection [38]. On the other hand, while other coronaviruses utilize three proteases for proteolytic processing, SARS-CoV is known to encode only two proteases, which include a papain-like cysteine protease (PLpro) [39] and a chymotrypsin-like cysteine protease known as 3C-like protease (3CLpro, main protease, M pro) [40]. These proteases are vital to virus replication; they cleave the two translated polyproteins (PP1A and PP1AB) into individual functional components [41]. The 3CLpro enzyme, is indispensable to the viral replication and infection process, thereby making it an ideal target for antiviral therapy.

Additional-accessory structures

An additional, accessory structural protein may be present not in nCoV but in other related lineages. Hemagglutinin Esterase (HE) is one of these enzymes. It is a homodimeric N-glycosylated class I membrane glycoprotein [24]. It occurs only in a subset of closely related coronaviruses; these viruses, designated group 2 coronaviruses, including human coronavirus OC43 (HCoV-OC43). Conceivably, HE synthesis might exert a negative effect on viral replication in cultured cells by drawing on the cell's economy or even by directly interfering with the production of other viral proteins, in particular the S protein, e.g., by competing for folding enzymes, chaperones, and other resources in the ER. Still, this does not seem to be the case. The HE is not required for replication, but it aids in the release of virus particles from an infected cell, may be involved in virus entry, and appears to be important for infection of the natural host-cell [42].

The other accessory protein that had been detected in other coronavirus strains but not in nCoV is M2. The M2 protein of influenza A and B viruses forms tetrameric proton channels that are important for the viral life cycle. After the virus enters the infected cell by endocytosis, the M2proton channel opens in response to the low pH of the endosome, allowing proton flux into the virus, which triggers the dissociation of the viral RNA from the membrane proteins, and fusion of the viral and endosomal membranes. These events release the viral RNA to the cytoplasm for replication by the host cell. In a later stage of virus replication, it is the M2 protein that maintains the high pH of the trans-Golgi network and prevents premature conformational changes [43].

Many RNA viruses, including the coronaviruses, have evolved mechanisms for generating cap structures at the 5' ends of viral gRNA and sgRNA, through consecutive methylations by virally encoded guanine-N7-methyltransferase (N7-MTase), at the N7 position of the capped guanine, and by 2'-O-methyltransferase (2'-O-MTase) at

the ribose *2'*-O-position of the first nucleotide. This mechanism plays a critical role in pre-mRNA splicing, mRNA export, RNA stability (via the blocking of degradation by *5'-3'* exoribonuclease), translation initiation (by promoting host eukaryotic translation initiation factor 4E binding), and escaping the host's innate immune system.

nCoV structure as drug target

The repurposed-old antiviral drugs display their effects on various structural and nonstructural targets in nCoV (Figure 1):

Lipid bilayer: The ability of the virus to infect host cells could be modified by disrupting the virus membrane which eventually impacts on transmembrane protein structures. The lipid envelope is highly sensitive to agents that disrupt lipid bio-membranes. It has been reported that ethanol (60-70%) causes interdigitation of phospholipids, whereas chlorhexidine (0.12%), fatty acids, hydrogen peroxide (0.5%) and povidone-iodine (0.23-1%) disrupt envelope [4].

S glycoprotein: Furin inhibitors, and piperazine carboxamide interfere with ACE2 recognition by S. Moreover, Ritonavir>Remdesivir>Lopinavir has high docking affinity for human furin protease [44]. CP-1 and a pan-coronavirus fusion inhibitor lipopeptide (EK1C4) target S2 and interfere with the conformational changes leading to blockade of the virus-cell fusion process [45]. Phthalocyanine and hypericin (derived from Hypericum perforatum-St. John's Wort) bind to a pocket in S glycoprotein and prevent fusion [46].

Viral nucleocapsid protein ribonucleoprotein (RNP): RNP is formed by the interaction of N protein with RNA in a process known as Protein-Protein Interaction (PPI), in which N-NTD is associated with the *3'*-end of the viral RNA genome. It serves multiple critical functions during the viral life cycle including expression of gRNA packing, viral transcription, and assembly in the infectious cell. The NTD of SARS-CoV-2 contains a potential hydrophobic pocket that could be a unique excellent drug-targeting candidate [24]. 5-benzyloxygramine stabilizes the N-NTD dimers through simultaneous hydrophobic interactions with both partners (N protein and RNA), resulting in abnormal N protein oligomerization that disrupts PPI [47]. Moreover, cepharanthine inhibits N, S, NFκB transcription factor, and reduces the cell membrane fluidity. Ergot alkaloids dock to SARS-CoV-2 N protein [48].

E protein: Nocardamine is a macrocyclic siderophore (binding Fe^{3+} ions with extremely high affinity). Nocardamine- and its noncyclic relative deferoxamine-complex fit to and bind with high affinity at E channel mouth, and effectively block it. Moreover, E is also blocked by other compounds such as hexamethylene amiloride, and glycyrrhetic acid, glycyrrhizic acid hydrolysis product aglycone [23].

Neuraminidase (NA): Oseltamivir, Peramivir and Zanamivir are available neuraminidase inhibitors used in the treatment of influenza.

Virus invasion of human cells

The main infection route is known to-day is either enteral or nasal. In the case of the enteral route the virus attaches to the pharyngeal mucosa, then travels en route to either down the gastrointestinal system (diarrhea) or to the lungs (pneumonia) and from there to the general circulation. Although the respiratory symptoms prevail, the gastrointestinal system is also a target of nCoV. The involvement of the digestive system in COVID-19 may be underestimated due to the vast majority of patients initially develop respiratory symptoms.

Vomiting and diarrhea could be seen in some patients (5-6%). Two factors could determine the nCoV infection via the gastrointestinal system: gastric pH and disease. It has been reported that SARS-CoV is completely inactivated by a highly acidic (pH 1-3) gastric environment. Accordingly, in order for the SARS-CoV-2 virus to invade epithelial cells without being inactivated in the stomach, the gastric pH must be neutral. Patients who tested positive on respiratory specimens but tested negative on the stool are generally younger than older patients who are virus-positive in stool samples, suggesting that aging is involved in the ease of virus enteral invasion. This is expected as the pH at gastric mucosa surface increases in the elderly (pH 5-7) and those with atrophic gastritis due to Helicobacter pylori (pH=3). In these cases, nCoV is not inactivated but instead enters the epithelial cells of the stomach, and further invades the epithelial cells of the small and large intestines [49]. The virus could find its way to the brain either from the nasal olfactory area (close to the brain) or from the circulation. Wherever the nCoV meets ACE2 it hijacks it and initiates an eight-phase cycle intracellularly: 1. Attachment to cell membrane; 2. ACE2 and S priming fusion, endocytosis 3. Uncoating and release; 4. RdRp polymerase-translation and replication; 5. ER, ERGIC assembly, packing, and storage; 6. Nucleocapsid formation-RNA folding; 7. Vesicular storage, budding, and release (Figure 2).

1) Attachment to cell membrane: although different components of the viral envelope glycoproteins are essential for membrane fusion to occur, attachment of S to ACE2 initiates the invasion process.

2) ACE2 and S priming, fusion, endocytosis: in order for fusion to take place, ACE2 and the S protein needs to be cleaved and activated by proteases found in the cell membrane (furin, TMPRSS2). This step is critical, as it allows for the fusion sequences to be exposed. Following binding of RBD in S1 subunit to the ACE2 receptor on the target cell, HR1 and HR2 domains in S2 subunit of S protein interact with each other to form a six-helix bundle (6-HB) fusion core, bringing viral and cellular membranes into close proximity for fusion and infection [50].

Host proteases (trypsin and furin) participate in virus-plasma membrane fusion. They cleave coronavirus S protein in two sites located at the boundary between the S1 and S2 subunits (S1/S2 site). This stage is followed by cleavage of the S2 domain (S2' site), fusion and endocytosis. The fusion processes are enhanced at low pH in severe infections. Moreover, Phosphatidylinositol 3-phosphate 5-kinase, two-pore channel subtype 2 (TPC2), and cathepsin L are important for entry into cells [51].

3). Uncoating and release: once entered into the cytoplasm, it is most likely that COVID-19 employs a unique three-step method for membrane fusion to enter early endosome involving receptor binding and induced conformational changes, intracellular proteases and further activation of membrane fusion mechanism [52]. Then, early endosome transfers to the late endosome and finally to the lysosome. At this stage, the envelope, structural proteins, and the viral nucleocapsid had been digested and removed. Finally, the viral genetic material, ssRNA, is fully released into the cytoplasm [53]. On the other hand, nCoV may also find its way to the double-membrane vesicles, which are substrates for autophagy and conversion to the autophagosome. The latter combines with lysosomes to form autosomes where the viral components are also processed and the gRNAs are released.

4). RdRp polymerase-translation and replication: being a (+) ssRNA is an important property of nCoV, because this enables it to
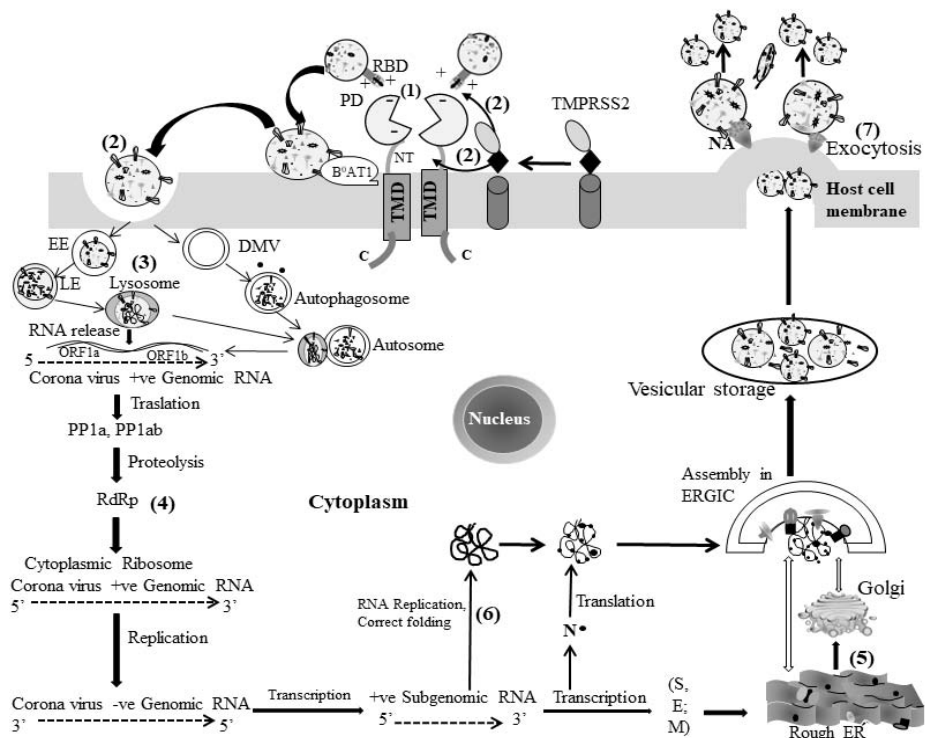
Figure 2: Schematic illustration of nCoV entry to and release cycle from human cells with the possible sites of antivirals. The numbers and abbreviations are explained in the text.

harbor RdRps sequences (replicase) within it. These sequences are typically capped and polyadenylated (polyA) and used directly to synthesize proteins in the cytoplasm but not the nucleus, and without the help of a complementary RNA intermediary. RdRps catalyze RNA-dependent formation of phosphodiester bonds between ribonucleotides. The nCoV RdRp (also named nsp12) is a key component of the replication/transcription machinery. Interestingly, nCoV shares high homology with SARS-CoV, suggesting that its function and mechanism of action might be well conserved once released from the gRNA to cytoplasm by proteolysis. RdRp firstly converts (+) ssRNA to a (-) ssRNA) complementary to the viral (+) ssRNA. It is noteworthy to mention that (-) ssRNA cannot be translated into protein directly. Instead, it must first be transcribed into a (+) ssRNA. Moreover, the expression of the coronavirus replicase-transcriptase protein genes is mediated by the translation of the gRNA. RdRps are considered among primary targets for antiviral drugs including Favipiravir and Remdesivir.

The nCoV genome possesses 14 Open-Reading Frames (ORFs) encoding 27 proteins. The replicase-transcriptase proteins are encoded in ORF1a and ORF1b and are synthesized initially as two large polyproteins, PP1a and PP1ab. The synthesis of pp1ab involves programmed ribosomal frame shifting during translation of ORF1a [54]. During or after synthesis, these polyproteins are cleaved by virus-encoded proteinases with PLpro and 3CLpro [55]. The replicase-transcriptase proteins, together with other viral and cellular proteins, assemble into membrane-bound RTC copying, gRNA, or sgRNA. RTCs accumulate at peri-nuclear regions and are associated with double-membrane vesicles [56]. Moreover, autophagy is also implicated in the replication of the virus, a process partly related to the formation of double-membrane vesicles inside the host cell.

5). ER, ERGIC assembly, packing, and storage: the (-) ssRNA either replicates to (+) ssRNA (which if correctly folded will be introduced to ER-Golgi compartment), or to discontinuous transcription yielding the structural proteins (M, S, and E) in ER, then transfer to ERGIC.

6). Nucleocapsid formation-RNA folding: nucleocapsids are formed from the encapsidation of correctly replicated genomes by N protein in the cytoplasm, and as a result, they coalesce within the ERGIC membrane in order for self-assembly into a new virus.

7). Vesicular storage, budding, and release: finally, the newly formed mature viruses transfer to smooth-walled vesicles that transport to the cell membrane, and leave the cell *via* exocytosis (Figure 2). In the meantime, the stress of viral production on the ER eventually leads to cell death-apoptosis [57].

Angiotensin-converting enzyme 2 (ACE2)

The ACE2-MasR forms the "good" arm of the renin-angiotensin system (RAS; RAAS in case aldosterone is added) against the "bad" arm formed by Ang II-AT1R [58]. ACE2 cleaves Ang II directly to the vasodilator peptide Ang-(1-7), and indirectly to Ang I through the production of Ang-(1-9) which is then processed by ACE/Neprilysin to Ang-(1-7), thus acting as a pivotal element in balancing the vasoconstrictor effects of these peptides and preventing hypertension. However, ACE2 is now well known to play an important role in nCoV infection, because it serves as the entry point into cells for the virus. ACE exists in two isoforms: somatic ACE, and testis-specific ACE, tACE [59]. The architecture of the ACE2 structure is very similar to that of tACE. ACE2 is zinc metallopeptidase, homodimeric type I integral-membrane protein. Each of its protomer is composed of a single TMD a short cytoplasmic CT tail, and NT ectodomain-extracellular with one Zn^{2+} containing claw-like negatively charged active site of the PD. Moreover, there is a deep pouch containing the

catalytic site on the ectodomain (in ACE there are two active sites). Typically the virus receptors contain ridges that bind to structures containing loops, cavities, and ridges in the proteins mediating entry [60].

ACE2 functions as the chaperone for the membrane trafficking of the amino acid transporter B^0AT1, which mediates uptake of neutral amino acids into cells in a sodium-dependent manner. ACE2 and B^0AT1 interact with each other and the two assemble as a dimer of heterodimers, with the Collectrin-Like Domain (CLD) of ACE2 mediating homo-dimerization. The expression of B^0AT1depends on the presence of ACE2. However, the expression and distribution of ACE2 are broader than B^0AT1. In addition to kidneys and intestine, where B^0AT1 is mainly expressed, ACE2 is more widely expressed. On the alignment of B^0AT1 complex with nCoV's S1 RBD, a ternary complex is formed enabling simultaneous binding of two S proteins to ACE2 homodimer [61].

ACE2 is more than a door-way for nCoV to enter cells. ACE2 has been identified in various tissues. High ACE2 gene expression was initially reported in the heart, kidney, and testis [62]. Later studies showed ACE2 expression in a wide variety of tissues, including the brain [63] where ACE2 is expressed predominantly in glial cells [64], although nCoV has been detected almost exclusively in neurons in the brains of infected patients, suggesting the distribution of ACE2 to the CNS [65]. Moreover, ACE2 is also extensively expressed in most of the cardiovascular-relevant tissues [66]. It is also widely expressed especially in type II alveolar epithelial cells, bronchiolar epithelial cells, endothelial cells, and arterial smooth muscle cells of the lung [67].

In addition to its importance in regulating and protecting the cardiovascular system, ACE2 also plays a protective role in acute lung injury. Down-regulation of ACE2 was found in the lungs after acute lung injury, including SARS-CoV infection [68]. One of the main targets employed by nCoV to gain entry into host human cells is the ACE2 receptors [69]. The S protein of nCoV binds ACE2 with higher affinity than does SARS-CoV [61]. The negatively charged ridges surrounding the channel within ACE2 may provide a possible binding site for the positively charged RBD of the S-glycoprotein [59]. In addition to cleavage of S, TMPRSS2 also cleaves ACE2 arginine and lysine residues in specific clusters present in C terminals close to the TM domain of ACE2. Cleavage of the ACE2 by TMPRSS2 enhances viral uptake, whereas cleavage of the spike S glycoprotein activates the latter for membrane fusion [70].

The expression of ACE2 is subject to variation, which may be one of the factors involved in the difference in the severity of COVID-19 among patients. Moreover, ACE2 polymorphism could be one of the causes of individual variation with regard to infection rate and severity. Also, it is still debated whether smoking predisposes to COVID-19 or helps prevent the disease. It is well known that smoking is associated with increased ACE2 in the lungs [71]. This is expected to be in favor of the virus entry to cell initially, but as the disease progresses nCoV depletes ACE2. Accordingly, increased ACE2 could avert the nCoV-induced depletion of ACE2, or it may substitute for the nCoV-shedded ACE2. However, smoking (especially chronic) is well known to inflict extensive pulmonary and cardiovascular damages (lung edema, inflammation, DNA damage, activation of proteases, increased secretions) that all increase severity of COVID-19 [72]. While ACE level is not changed by age or sex, it has been reported that men are more prone to being affected by COVID-19, maybe because of a high smoking rate.

Pathogenicity

The route of transmission and pathogenesis of COVID-19 is still debated. Although the respiratory route is salient, mounting evidence supports the involvement of the gastrointestinal system as a route of infection. The gastrointestinal tract (intestine) tropism of SARS-CoV was verified by the viral detection in biopsy specimens and stool even in discharged patients, which may partially provide explanations for the gastrointestinal symptoms such as nausea, vomiting and diarrhea, and potential recurrence, and transmission of nCoV from persistently shedding human [73]. Whereas up to 60% of patients suffering from SARS had a liver impairment, mild to moderate liver injury, including elevated aminotransferases, hypoproteinemia, and prothrombin time prolongation, has been reported in the existing clinical investigations of COVID-19. However, the exact mechanism of COVID-19-induced gastrointestinal symptoms largely remains elusive. Previously, the prevalent persuasion was that nCoV-2 could not infect the liver due to its lack of ACE2. However, recent studies had revealed a significant enrichment of ACE2 expression in cholangiocytes (59.7% of cells) instead of hepatocytes (2.6% of cells), suggesting that COVID-19 might lead to direct damage to the intrahepatic bile ducts [74].

Mitochondrial dysfunction is another important pathogenic change that is involved both in COVID-19 pathogenicity and effect of some drugs. The nCoV sequesters the protons, generated from the electron transfer chain, and uses them for release and replication. Sequestration of the protons leads to disruption of mitochondrial oxidative phosphorylation and impairment of the electrochemical gradient required for ATP synthesis. The dcrease of ATP production that is caused by disrupted oxidative phosphorylation compensates by upregulation of glycolysis with subsequent increased production of lactic acid and acidosis that also results in virus's favor. On the other hand, drugs like chloroquine also sequester mitochondrial protons. This mechanism is implicated in chloroquine-toxicity, but it is expected to be involved in chloroquine's antiviral activity because the virus will be deprived of protons [75]. The mitochondrial effect may explain the resistance of the youngs to COVID-19 or their asymptomatic experience of the disease, possibly due to their better adaptation to ATP requirement and their capability to synthesize more mitochondria [76]. It may also provide an answer to the better clinical effects obtained with early chloroquine treatment [77].

When it comes to the respiratory route, three stages could be delineated according to the clinical picture of the disease and laboratory findings.

Stage 1: Asymptomatic incubation period; 1-2 days after inhalation of the virus. The virus likely binds to epithelial cells in the nasal cavity and starts replicating. There is local propagation of the virus but with a limited innate immune response. The extent of SARS-CoV-2 binding to the ACE2 receptors is limited to the nasal, oral and pharyngeal epithelial cells. The virus is likely to cause mild symptoms that may be confused with a common cold or flu. At this stage, the virus can be detected by nasal and pharyngeal swabs. Although the viral burden may be low, these individuals are infectious. The patients may experience smell or taste impairments but often recover without any interventions.

Stage 2: Non-severe symptomatic period; a few days after the first stage 80% of the infected patients exhibit mild clinical symptoms that are mostly restricted to the upper and conducting airways. The

virus propagates and migrates down the respiratory tract along the conducting airways, and a more robust innate immune response is triggered in this stage and leads to primarily respiratory symptoms such as persistent cough, shortness of breath, and low blood oxygen level. A moderate pulmonary involvement with or without hypoxia is probable. Nasal swabs or sputum yields the virus. Moreover, CXCL10 is a chemokine secreted by several cell lines including alveolar type II cells in response to both SARS-CoV and influenza. CXCL10 plays a crucial role in the development of COVID-19-related symptoms. The level of CXCL10 (or some other innate response cytokine) may be predictive of the subsequent clinical course [78]. About 20% of the infected patients will progress to stage 3.

Stage 3: Severe respiratory symptomatic stage; characterized by pulmonary infiltrates, lung injuries, and severe pulmonary symptoms such as dyspnea. The virus now reaches the gas exchange units of the lung and infects alveolar type II cells because SARS-CoV preferentially infects type II cells, where ACE2 receptors are abundant, compared to type I cells [79]. The virus replicates intracellularly, the infected cells undergo apoptosis and die, and the released viral particles infect type II cells in adjacent units. These cells in the end will die and secondary pathway for epithelial regeneration will be triggered. The pathological result is diffuse alveolar damage with fibrin rich hyaline membranes and a few multinucleated giant cells. One of the cardinal diagnostic pulmonary changes observed in CT images is accumulation of fluid and the hazy lung opacity-characteristic white patches "ground glass powder", composed of the exudates in the alveoli. Hyaline membranes formation is another pulmonary pathological finding observed in COVID-19 [80].

Inflammation and immune response

The core inflammatory reaction that takes place in COVID-19, cytokine storm, is different from that caused by prostaglandins. Accordingly, in order for the selected treatment to be effective, it should target the cytokines. S-glycoprotein induces pyroptosis where cells release interleukins and mitochondrial cytc. IL-6 is one of the main cytokines involved in COVID-19 pathologies. It is produced by various cell types, including T- and B-cells, lymphocytes, monocytes, and fibroblasts. IL-6 is a proinflammatory cytokine that is involved in diverse physiological processes such as T-cell activation, induction of immunoglobulin secretion, initiation of hepatic acute-phase protein synthesis, and stimulation of hematopoietic precursor cell proliferation and differentiation. Moreover, the caspases have identified roles not only in apoptosis but also in pyroptosis. However, in the case of COVID-19, they are not essential in apoptosis [81]. Of the caspases, caspase 1 is required for pyroptosis [82]. It has been reported that nCoV activates caspase 1 *via* NLRP3 inflammasome [83]. Active caspase-1 cleaves and activates the inflammatory cytokines, such as pro-IL-1β and pro-IL-18, and the pore-forming protein gasdermin D that causes inflammatory cell death [84]. Glyburide and parthenolide inhibit NLRP3 [85]. The molecular effects of nCoV are the conduit for the laboratory findings of inflammation in COVID-19 patients such as elevated C-reactive protein, IL-6, D-dimer, serum ferritin, and lactate dehydrogenase during the first week after illness onset.

Pulmonary effects

The respiratory system is the main route implicated in COVID-19 human-to-human transmission, possibly due to its high ACE2 expression. The transmission is believed to occur through respiratory droplets (particles>5-10 μm in diameter) from coughing and sneezing. Aerosol transmission is also possible in case of exposure to elevated aerosol concentrations in closed spaces. The nCoV mainly settles into type II alveolar cells [79]. Following the nasal entrance of nCoV, a two-phased immune/inflammation response takes place [86]. The first phase is the immune-based protective phase. An innate immune response is required to eliminate the virus and to preclude disease progression to the severe stages. Therefore, strategies to boost immune responses (antisera or pegylated IFNα) at this stage are certainly important. The second phase is inflammation-phase. The severe pulmonary inflammatory reactions are the main cause of life-threatening respiratory disorders. However, the protective immune response is impaired, nCoV propagates, induces massive destruction of pulmonary tissues, and induces extensive cytokines release largely by pro-inflammatory macrophages and granulocytes. The severe inflammatory reaction and fever are attributed to cytokine storm that is caused by IL-6, but not by T cells because lymphocytopenia is evident in this stage. Moreover, nCoV inhibits type 1 interferons, which together with cytokine storm may orchestrate infiltration of mononuclear cells (monocyte, macrophages) and neutrophils in lung parenchyma [87].

Cardiovascular and hematological system

Several studies suggest an association between pre-existing cardiovascular diseases and severe COVID-19. Pre-existing cardiovascular diseases may also increase the adverse effects of drugs. Moreover, nCoV can result in heart dysfunction due to the intensively expressed ACE2 in the myocardial cells that act as receptors for this virus. Hemoglobin and neutrophil count decrease in many COVID-19 patients and the index values of serum ferritin, erythrocyte sedimentation rate, C-reactive protein, albumin, and lactate dehydrogenase of many patients increase significantly. Each hemoglobin molecule contains four hem moieties, each surrounded by one of the four globins (2α and 2β) forming a tetrahedral structure. Each hem is composed of one iron bound to four porphyrins. It has been reported that some structural and nonstructural components of nCoV could bind porphyrin. The ORF8, possibly coordinately with other components, could bind the porphyrin and detach it from the iron [88] leading to the release of iron. This will lead to nonfunctional hemoglobin because the oxygen and carbon dioxide carrying capacity of hemoglobin will be reduced, and eventually lead to alveolar cells degeneration and formation of the ground-glass-like images in the lung. Moreover, excessive iron will accumulate in the tissues. Consequently, reactive oxidant species will increase and lead to tissue (pulmonary, cardiac, kidney, intestine) damage, and fibrin and thrombus accumulation in lung tissue. Moreover, hyperferritinemia linked to fatality takes place. Accordingly, iron chelators (e.g. desferrioxamine) may be useful and could be added to treatment protocols.

The other important pathological change observed in various stages of COVID-19 patients is coagulopathy that could take place as hypercoagulation or extra- and intra-pulmonary bleeding. It depends on the interaction of hyperinflammatory mediators and the immune system and the degree of the cell and organ damage. The pathways that lead to coagulopathy include: 1. hyperinflammation-induced endothelitis and endothelial damage. These changes result in deterioration of endothelial function, down-regulation of ACE2, an increase of AngII, vascular permeability, excessive thrombin generation [89], and elevation of tissue factor and plasminogen activator inhibitor-1, PAI-1 [90]; 2. hypoxemia: triggers expression of hypoxia-inducible factors which may promote further inflammation, increase blood viscosity, induce platelets and coagulation factors

activation, increase tissue factor expression, increase PAI-1, and inhibit the endogenous anticoagulant proteins. On the other hand, necrotizing hemorrhage in the brain [91] and colon [92] had been reported in some COVID-19 patients. Moreover, hemorrhage and infarction secondary to microhemorrhage induced by inflammation- or infection-linked vascular damage are all detected in some COVID-19 patients' lungs.

COVID-19 also involves macrophage activation syndrome that triggers immuno-thrombosis characterized by diffuse pulmonary intravascular coagulopathy, increased D-dimer and cardiac enzyme levels and hyperferritinemia [93]. Hyaline membrane formation is also expected to be involved in the coagulopathy and alveolar degeneration [80]. The initial coagulopathy of COVID-19 presents with a prominent elevation of D-dimer and fibrin/fibrinogen degradation products, while abnormalities in prothrombin time, partial thromboplastin time, and platelet counts are relatively uncommon. The increased D-dimer could indicate plasmin hyperactivity. This is expected to result from decreased pH that is produced by excessive hypoxia, and correlate with a parallel rise in markers of inflammation (e.g. C-reactive protein). Unlike the pattern seen in classic disseminated intravascular coagulation from bacterial sepsis or trauma, prothrombin time in COVID-19 patients is not elongated, and D-dimer value is lesser, but platelet count is higher than non-COVID-19 patients [94].

Lymphopenia and thrombocytopenia are the other two prominent hematological changes observed in COVID-19 patients. The nCoV can induce lymphopenia and thrombocytopenia, directly or indirectly, *via* apoptosis and inhibition of the hematopoietic stem or progenitor cells by induction of antibodies, immune complexes, and CD13 or CD66a cells. Moreover, COVID-19 is associated with CD4+ and CD8+ T-cell lymphopenia, which may result from a combination of virus-induced direct cytopathic effects, as well as enhanced T-cell apoptosis due to a dysregulated cytokine milieu [95]. While depletion of CD8+T cells at the time of infection has no effect on viral replication or clearance, depletion of CD4+T cells results in an enhanced immune-mediated interstitial pneumonitis and delayed clearance of nCoV from the lungs. These effects are associated with reduced neutralizing antibody, cytokine production, and reduced pulmonary recruitment of lymphocytes [96]. On the other hand, the pulmonary damage due to nCoV-ventilation disturbance will decrease pulmonary healthy capillary bed, which will lead to decreased platelet production. The lung damage could also induce platelet aggregation and pulmonary thrombosis which increases platelet consumption. These effects collectively decrease the circulating platelets and eventually cause thrombocytopenia.

Hypokalemia

Hypokalemia prevails in COVID-19 patients. The degree of hypokalemia is correlated with several clinical features reflecting the severity of COVID-19 [97]. It is well known that sufficient and appropriate levels of plasma K+ have a protective role in preventing myocardial failure through weakening cellular hyperpolarity and depolarization. Therefore, frequent plasma/serum K+ blood test is useful for determination of K+ level and maintain it between 4.0-5.5 mmol/L in plasma, or 4.5 and 5.5 mmol/L in serum, serum generally has more K+ than plasma. The levels below these values indicate hypokalemia (3-3.5 mM) or severe hypokalemia (<3 mM) [98]. A low prevalence of optimal concentration of plasma K+ implies a massive risk for the patients' heart to when it occurs in COVID-19 patients. Moreover, higher prevalence (28%) of pH values over 7.45 had been reported in the patients with severe hypokalemia because severe hypokalemia led to alkalosis due to H+-K+ exchange between intracellular and extracellular fluid [99].

The prevalence of hypokalemia among COVID-19 patients and its association with the severity of the disease, necessitates elucidation of the mechanisms of hypokalemia, in order to understand its cause and correct it. In the current situation, two probable causes of hypokalemia are proposed: increased gastrointestinal and/or urinary loss. Gastrointestinal loss of K+ might not contribute much to hypokalemia in COVID-19 because only a small proportion (31%) of patients with hypokalemia show diarrhea, and no significant difference had been detected for K+ level in these patients. Therefore, hypokalemia might principally result from increased urine loss, because urine K+ excretion extensively increases in the hypokalemic patients. Additionally, increased urine K+ excretion as the primary cause of hypokalemia is consistent with the pathogenesis of nCoV. This virus, by degrading ACE2, drives RAAS towards enhanced ACE-Ang II-AT1R. The final effect of this disturbance of RAS is the increased distal delivery of sodium and water to collecting tubule of the kidney and enhanced potassium secretion in exchange for Na+ absorption. This effect is similar to the effect of aldosterone that stimulates water and sodium reabsorption and potassium excretion and thus increases body water and blood pressure. It is noteworthy to mention that continual K+ supplement has very little effect on the return of normokalemia when the urine loss of K+ persists in severe COVID-19 patients. However, the patients who are inclined to recovery responded well to K+ supplement treatment and steadily return to normokalemia. This phenomenon indicates the end of K+ loss in urine due to disordered RAAS balance, indicating retrieval of the ACE2 function. It could be suggested that return of normokalemia might be a reliable biomarker for monitoring ACE2 function [97].

Brain infection

The nCoV is neuroinvasive, detected in CSF, exhibits neurotropic properties, with the potential to induce CNS damage and neurological diseases. Based on an epidemiological survey on COVID-19, the median time from the first symptom to dyspnea is 5.0 days, to hospital admission is 7.0 days, and to the intensive care is 8.0 days [100]. This latency period may be enough for the virus to get access to the brain via nasal mucosa and circulation. The juxtaposition of the nasal cavity with the brain's olfactory nerves and olfactory bulb makes it a very important channel for nCoV to reach the brain from the nose. This renders the nasal cavity a pathway for brain infection besides the circulation. In case nCoV contrive a way to the brain through the nose, its entrance could be through the olfactory tract in the early stages of infection, then reach the entire brain (including the brainstem where centers regulating important cardiovascular and respiratory functions are located) and cerebrospinal fluid through the olfactory nerve and olfactory bulb within 7 days [101]. Brain infection exhibits a three-stage pathogeny:

Stage 1: limited to the nasal and gustatory epithelial cells, with possible smell or taste impairment.

Stage 2: robust immune response leading to cerebral blood vessels inflammation, the formation of blood clots in cerebral arteries and veins leading to stroke, fatigue, sensory loss, hemiplegia or tetraplegia, or ataxia in some patients.

Stage 3: During the cytokine storm, the integrity of the blood-

brain barrier is disrupted, leading to the entry of cytokines, blood components, and viral particles into the brain. This stage can be characterized by seizures, confusion, coma, loss of consciousness, or death.

The coronavirus, like other neurotropic viruses, causes three main diseases in the nervous system [102]. These include: 1. viral encephalitis: acute onset involving neuronal damage; 2. acute toxic encephalitis-refers to a type of reversible brain dysfunction syndrome caused by factors such as systemic toxemia, metabolic disorders, and hypoxia during the process of acute infection. The basic pathological changes in this disease include cerebral edema, with no evidence of inflammation; 3. acute viral cerebrovascular diseases expected to arise from the cytokine storm and hyperfibrinolysis. The diseases may be caused by several mechanisms: a) direct injury caused by inflammatory and immunologic responses induced by interleukins that disrupt the blood-brain barrier and induce neuronal damage and demyelination; b) ACE2 downregulation/or loss; c) hypoxic injury (anaerobium and metabolic acidosis). Subsequent to pulmonary infection and dysfunction, COVID-19 patients often suffer from severe hypoxia [103]. This will lead to brain mitochondrial disorder, acidosis, cerebral vasodilatation and disruption of circulation, neuronal and interstitial edema, intracranial hypertension, and injury to the nervous system, and various neurological signs ranging from light (headache, nausea, and vomiting, etc.) to more severe neurologic manifestations (epileptic seizures), acute cerebrovascular diseases and impaired consciousness [104].

Conclusion

The COVID-19 outbreak continues, the number of cases and deaths are gradually increasing all over the world, of course by changing the spreading center. Our knowledge of the molecular structure of nCoV is of great value for determining the sites which could be targets for selective and effective novel antiviral drugs. Several drugs that had been used to treat other coronaviruses and diseases are being used in different parts of the world with different success degrees and efficacy. Moreover, our knowledge of details of nCoV structure is of great importance in determining the sites where the virus mutates, and those which could be useful for development of effective and specific vaccines.

References

1. WHO Coronavirus Disease (COVID-19) Dashboard. 2020.

2. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe. 2020; 27: 325-328.

3. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. J Virol. 2020; 4: e00127-e00220.

4. O'Donnell VB, Thomas D, Stanton R, Maillard JY, Murphy RC, Jones SA, et al. Potential role of oral rinses targeting the viral lipid envelope in SARS-CoV-2 infection. Function. 2020;1: zqaa002.

5. Pillaiyar T, Manickam M, Namasivayam V, Hayashi Y, Jung SH. An overview of severe acute respiratory syndrome–coronavirus (SARS-COV) 3CL protease inhibitors: peptidomimetics and small molecule chemotherapy. J Med Chem. 2016; 59: 6595-6628.

6. Kirchdoerfer RN, Cottrell CA, Wang N, Pallesen J, Yassine HM, Turner HL, et al. Pre-fusion structure of a human coronavirus spike protein. Nature. 2016; 531: 118-121.

7. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020; 367: 1260-1263.

8. Bosch BJ, van der Zee R, de Haan CAM, Rottier PJM. The coronavirus spike protein is a class i virus fusion protein: structural and functional characterization of the fusion core complex. J Virol. 2003; 77: 8801-8811.

9. Belouzard S, Chu VC, Whittaker GR. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. PNAS. 2009; 106: 5871-5876.

10. Simmons G, Reeves JD, Rennekamp AJ, Amberg SM, Piefer AJ, Bates P, et al. Characterization of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry. PNAS. 2004; 101: 4240-4245.

11. Millet JK, Whittaker GR. Host cell proteases: critical determinants of coronavirus tropism and pathogenesis. Virus Res. 2015; 202: 120-134.

12. Bianchi M, Benvenuto D, Giovanetti M, Angeletti S, Ciccozzi M, Pascarella S. Sars-CoV-2 envelope and membrane proteins: structural differences linked to virus characteristics?. Bio Med Res Int. 2020.

13. Nieto-Torres JL, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA, Castaño-Rodriguez C, et al. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. Virology. 2015; 485: 330-339.

14. Jimenez-Guardeño JM, Nieto-Torres JL, DeDiego ML, Regla-Nava JA, Fernandez-Delgado R, Castaño-Rodriguez C, et al. The PDZ-binding motif of severe acute respiratory syndrome coronavirus envelope protein is a determinant of viral pathogenesis. PLOS Pathog. 2014; 10: e1004320.

15. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J. 2009; 16: 69.

16. Pervushin K, Tan E, Parthasarathy K, Lin X, Jiang FL, Yu D, et al. Structure and inhibition of the SARS coronavirus envelope protein ion channel. PLoS pathog. 2009; 5: e1000511.

17. Gupta MK, Vemula S, Donde R, Gouda G, Behera L, Vadde R. In-silico approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel. J Biomol Struct Dyn. 2020.

18. Venkatagopalan P, Daskalova SM, Lopez LA, Dolezal KA, Hogue BG. Coronavirus envelope (E) protein remains at the site of assembly. Virology. 2015; 478: 75-85.

19. Westerbeck JW, Machamer CE. A coronavirus E protein is present in two distinct pools with different effects on assembly and the secretory pathway. J Virol. 2015; 89: 9313-9323.

20. 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: 69-82.

21. Wilson L, Mckinlay C, Gage P, Ewart G. SARS coronavirus E protein forms cation-selective ion channels. Virology. 2004; 330: 322-331.

22. Verdiá-Báguena C, Nieto-Torres JL, Alcaraz A, DeDiego ML, Torres J, Aguilella M, et al. Coronavirus E protein forms ion channels with functionally and structurally-involved membrane lipids. Virology. 2012; 432: 485-494.

23. Chernyshev A. Pharmaceutical Targeting the Envelope Protein of SARS-CoV-2: the screening for inhibitors in approved drugs. ChemRxiv. 2020.

24. Boopathi S, Poma AB, Kolandaivel P. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. J Biomol Struct Dyn. 2020.

25. Kang S, Yang M, Hong Z, Zhang L, Huang Z, Chen X, et al. Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. Acta Pharm Sin B. 2020; 10: 122-1238.

26. Sawicki SG, Sawicki DL, Siddell SG. A contemporary view of coronavirus transcription. J Virol. 2007; 81: 20-29.

27. Diemer C, Schneider M, Seebach J, Quaas J, Frösner G, Schätzl HM, et al. Cell type-specific cleavage of nucleocapsid protein by effector caspases during SARS coronavirus infection. J Mol Biol. 2008; 376: 23-34.

28. Nelson GW, Stohlman SA, Tahara SM. High affinity interaction between nucleocapsid protein and leader/intergenic sequence of mouse hepatitis virus RNA. J Gen Virol. 2000; 81:181-188.

29. Bost AG, Carnahan RH, Lu XT, Denison MR. Four proteins processed from the replicase gene polyprotein of mouse hepatitis virus colocalize in the cell periphery and adjacent to sites of virion assembly. J Virol. 2000; 74: 3379-3387.

30. Almazán F, Galán C, Enjuanes L. The nucleoprotein is required for efficient coronavirus genome replication. J Virol. 2014; 78: 12683-12688.

31. McBride R, Van Zyl M, Fielding BC. The coronavirus nucleocapsid is a multifunctional protein. Viruses. 2014; 6: 2991-3018.

32. Klumperman J, Locker JK, Meijer A, Horzinek MC, Geuze HJ, Rottier PJ. Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding. J Virol. 1994; 68: 6523-6534.

33. Siu YL, Teoh KT, Lo J, Chan CM, Kien F, et al. 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. J Virol. 2008; 82: 11318-11330.

34. 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; 11-22.

35. Opstelten DJ, Raamsman MJ, Wolfs K, Horzinek MC, Rottier PJ. Envelope glycoprotein interactions in coronavirus assembly. J cell Biol. 1995; 131: 339-349.

36. Narayanan K, Maeda A, Maeda J, Makino S. Characterization of the coronavirus M protein and nucleocapsid interaction in infected cells. J Virol. 2000; 74: 8127-8134.

37. Baudoux P, Carrat C, Besnardeau L, Charley B, Laude H. Coronavirus pseudoparticles formed with recombinant M and E proteins induce alpha interferon synthesis by leukocytes. J Virol. 1998; 72: 8636-8643.

38. 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: 271-280.

39. Ratia K, Saikatendu KS, Santarsiero BD, Barretto N, Baker SC, Stevens RC, et al. Severe acute respiratory syndrome coronavirus papain-like protease: structure of a viral deubiquitinating enzyme. PNAS. 2006; 103: 5717-5722.

40. Chen S, Chen L, Tan J, Chen J, Du L, Sun T, et al. Severe acute respiratory syndrome coronavirus 3C-like proteinase N terminus is indispensable for proteolytic activity but not for enzyme dimerization. Biochemical and thermodynamic investigation in conjunction with molecular dynamics simulations. J Biol Chem. 2005; 280: 164-173.

41. Chen C, Qi F, Shi K, Li Y, Li J, Chen Y, et al. Thalidomide combined with low-dose glucocorticoid in the treatment of COVID-19 pneumonia. Preprints. 2020.

42. Lissenberg A, Vrolijk MM, Van Vliet ALW, Langereis MA, de Groot-Mijnes JDF, Rottier PJM, et al. Luxury at a cost? Recombinant mouse hepatitis viruses expressing the accessory hemagglutinin esterase protein display reduced fitness in vitro. J Virol. 2005; 79: 15054-15063.

43. Cady SD, Luo W, Hu F, Hong M. Structure and function of the influenza A M2 proton channel. Biochemistry. 2009; 48: 7356-7364.

44. Cubuk H, Ozbil M. Comparison of Clinically Approved Molecules on SARS-CoV-2 Drug Target Proteins: A Molecular Docking Study. ChemRxiv. 2020.

45. Gil C, Ginex T, Maestro I, Nozal V, Barrado-Gil L, Cuesta-Geijo MÁ, et al. COVID-19: Drug Targets and Potential Treatments. J Med Chem. 2020.

46. Romeo A, Iacovelli F, Falconi M. Targeting the SARS-CoV-2 spike glycoprotein prefusion conformation: virtual screening and molecular dynamics simulations applied to the identification of potential fusion inhibitors. Virus Res. 2020; 286: 198068.

47. Lin SM, Lin SC, Hsu JN, Chang CK, Chien CM, Wang YS, et al. Structure-based stabilization of non-native protein-protein interactions of coronavirus nucleocapsid proteins in antiviral drug design. J Med Chem. 2020; 63: 3131-3141.

48. Kadioglu O, Saeed M, Greten HJ, Efferth T. Identification of novel compounds against three targets of SARS CoV-2 coronavirus by combined virtual screening and supervised machine learning. Bull World Health Org. 2020.

49. Uno Y. Why does SARS-CoV-2 invade the gastrointestinal epithelium?. Gastroenterology. 2020.

50. Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, et al. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res. 2020; 30: 343-355.

51. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020; 11: 1-12.

52. Simmons G, Reeves JD, Rennekamp AJ, Amberg SM, Piefer AJ, Bates P, et al. Characterization of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry. PNAS. 2004; 101: 4240-4245.

53. Yamauchi Y, Greber UF. Principles of Virus Uncoating: Cues and the Snooker Ball. Traffic. 2016; 17: 569-592.

54. Namy O, Moran SJ, Stuart DI, Gilbert RJ, Brierley IA. A mechanical explanation of RNA pseudoknot function in programmed ribosomal frameshifting. Nature. 2006; 441: 244-247.

55. Ziebuhr J, Snijder EJ, Gorbalenya AE. Virus-encoded proteinases and proteolytic processing in the Nidovirales. J Gen Virol. 2000; 81: 853-879.

56. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, Onderwater JJ, van der Meulen J, Koerten HK, et al. Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. J Virol. 2006; 80: 5927-5940.

57. Masters PS. The molecular biology of coronaviruses. Adv Virus Res. 2006; 66: 193-292.

58. Hatip-Al-Khatib I, Bölükbaş Hatip F, Matsunaga Y. The protective tributary angiotensin members of renin-angiotensin system display beneficial effects in the central nervous system disorders. Am J Pharmacol. 2018.

59. Prabakaran P, Xiao X, Dimitrov DS. A model of the ACE2 structure and function as a SARS-CoV receptor. Biochem Biophys Res Commun. 2003; 314: 235-241.

60. Dimitrov DS. Virus entry: molecular mechanisms and biomedical applications. Nat Rev Microbiol. 2004; 2: 109-122.

61. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2. Science. 2020; 367: 1444-1448.

62. Kleta R, Romeo E, Ristic Z, Ohura T, Stuart C, Arcos-Burgos M, et al. Mutations in SLC6A19, encoding B 0 AT1, cause Hartnup disorder. Nature Genet. 2004; 36: 999-1002.

63. Xia H, Lazartigues E. Angiotensin converting enzyme 2 in the brain:properties and future directions. J Neurochem. 2008; 107: 1482-1494.

64. Gallagher PE, Chappell MC, Ferrario CM, Tallant EA. Distinct roles for ANG II and ANG-(1-7) in the regulation of angiotensin-converting enzyme 2 in rat astrocytes. Am J Cell Physiol. 2006; 290: C420-C426.

65. Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol. 2004; 203: 622-630.

66. Igase M, Strawn WB, Gallagher PE, Geary RL, Ferrario CM. Angiotensin II AT1 receptors regulate ACE2 and angiotensin-(1–7) expression in the aorta of spontaneously hypertensive rats. Am J Physiol Heart Cell Physiol. 2005; 289: H1013-H1019.

67. Hamming I, Timens W, Bulthuis MC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004; 203: 631-637.

68. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nat Med. 2005; 11: 875-879.

69. Xiao X, Chakraborti S, Dimitrov AS, Gramatikoff K, Dimitrov DS. The SARS-CoV S glycoprotein: expression and functional characterization. Biochem Biophys Res Commun. 2003; 312: 1159-1164.

70. Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pöhlmann S. TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. J Virol. 2014; 88: 1293-1307.

71. Cai G. Tobacco-Use Disparity in Gene Expression of ACE2, the Receptor of 2019-nCoV. Medrxiv. 2020.

72. Olds JL, Kabbani N. Is nicotine exposure linked to cardiopulmonary vulnerability to COVID-19 in the general population? FEBS J. 2020.

73. Leung WK, To KF, Chan PK, Chan HLY, Wu AKL, Lee N, et al. Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. Gastroenterology. 2003; 125: 1011-1017.

74. Chai XQ, Hu LF, Zhang Y, Han W, Lu Z, Keet A, et al. Specific ACE2 expression in cholangiocytes may cause liver damage after COVID-19 infection. bioRxiv. 2020.

75. Sheaf RJ. A New Model of SARS-CoV-2 Infection Based on (Hydroxy) Chloroquine Activity. bioRxiv. 2020.

76. Sun N, Youle RJ, Finkel T. The mitochondrial basis of aging. Mol Cell. 2016; 61: 654-666.

77. Moore N. Chloroquine for COVID-19 infection. Drug Saf. 2020; 43: 1-2.

78. Tang NLS, Chan PKS, Wong CK, To KF, Wu AKL, Sung YM, et al. Early enhanced expression of interferon-inducible protein-10 (CXCL-10) and other chemokines predicts adverse outcome in severe acute respiratory syndrome. Clin Chem. 2005; 51: 2333-2340.

79. Mossel EC, Wang J, Jeffers S, Edeen KE, Wang S, Cosgrove GP, et al. SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells. Virology. 2008; 372: 127-35.

80. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Resp Med. 2020; 8: 420-422.

81. Favreau DJ, Meessen-Pinard M, Desforges M, Talbot PJ. Human coronavirus-induced neuronal programmed cell death is cyclophilin d dependent and potentially caspase dispensable. J Virol. 2012; 86: 81-93.

82. Ball DP, Taabazuing CY, Griswold AR, Orth EL, Rao SD, Kotliar IB, et al. Caspase-1 interdomain linker cleavage is required for pyroptosis. Life Sci Alliance. 2020; 3: e202000664.

83. Shah A. Novel coronavirus-induced NLRP3 inflammasome activation: a potential drug target in the treatment of COVID-19. Fron Immunol. 2020; 11: 1021.

84. Shi J, Gao W, Shao F. Pyroptosis: Gasdermin Mediated Programmed Necrotic Cell Death. Trend Biochem Sci. 2017; 42: 245-254.

85. Zahid A, Li B, Kombe AJK, Jin T, Tao J. Pharmacological Inhibitors of the NLRP3 Inflammasome. Front Immunol. 2019; 10: 2538.

86. Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, et al. COVID-19 infection: the perspectives on immune responses. Cell Death Differ. 2020; 27: 1451-1454.

87. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, et al. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microbe. 2016; 19: 181-193.

88. Wenzhong L, Hualan L. COVID-19: attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism. ChemRxiv. 2020.

89. Frantzeskaki F, Armaganidis A, Orfanos SE. Immunothrombosis in acute respiratory distress syndrome: cross talks between inflammation and coagulation. Respiration. 2017; 93: 212-225.

90. Ozolina A, Sarkele M, Sabelnikovs O, Skesters A, Jaunalksne I, Serova J, et al. Activation of coagulation and fibrinolysis in acute respiratory distress syndrome: a prospective pilot study. Front Med. 2016; 3: 64.

91. Poyiadji N, Shahin G, Noujaim D, Stone M, Patel S, Griffith B. COVID-19-associated acute hemorrhagic necrotizing encephalopathy: Imaging features. Radiology. 2020; 296: E119-E120.

92. Carvalho A, Alqusairi R, Adams A, Paul M, Kothari N, Peters S, et al. SARS-CoV-2 gastrointestinal infection causing hemorrhagic colitis: implications for detection and transmission of COVID-19 disease. Am J Gastroenterol. 2020.

93. McGonagle D, O'Donnell JS, Sharif K, Emery P, Bridgewood C. Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. Lancet Rheumatol. 2020; 2: E37-E45.

94. Yin S, Huang M, Li D, Tang N. Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. J Thromb Thrombolys. 2020.

95. Wang X, Xu W, Hu G, Xia S, Sun Z, Liu Z, et al. SARSCoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion. Cell Mol Immunol. 2020.

96. Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, et al. Cellular Immune Responses to Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Infection in Senescent BALB/c Mice: CD4+ T Cells Are Important in Control of SARS-CoV Infection. J Virol. 2010; 84: 1289-1301.

97. Chen D, Li X, Song Q, Hu C, Su F, Dai J, et al. Hypokalemia and Clinical Implications in Patients with Coronavirus Disease 2019 (COVID-19). medRxiv. 2019.

98. Macdonald JE, Struthers AD. What is the optimal serum potassium level in cardiovascular patients? J Am Coll Cardiol. 2004; 43: 155-161.

99. Unwin RJ, Luft FC, Shirley DG. Pathophysiology and management of hypokalemia: a clinical perspective. Nat Rev Nephrol. 2011; 7: 75-84.

100. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA. 2020; 323: 1061-1069.

101. Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, et al. Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun. 2020; 87: 18-22.

102. Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, et al. Nervous system involvement after infection with COVID-19 and other coronaviruses.

Brain Behav Immun. 2020; 87: 18-22.

103. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. Mil Med Res. 2020; 7: 1-10.

104. Mao L, Wang M, Chen S, He Q, Chang J, Hong C, et al. Neurological manifestations of hospitalized patients with COVID-19 in Wuhan, China: a retrospective case series study. MedRxiv. 2020; 77: 683-690.