

The Agent and Host Factors in Covid-19: Exploring Pathogenesis and Therapeutic Implications

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ABSTRACT

Infectivity and Pathogenesis

There is consensus that SARS-CoV-2 originated in horseshoe bats as it shares a high sequence similarity to bat CoVs, ~96% with the bat-CoV-RaTG13 and ~90% with bat-SL-CoVZC45 and bat-SL-CoVZXC21. But, the bat CoVs are not its direct ancestors, instead an intermediate host is involved, which subsequently transferred the virus to humans. The Malayan pangolin harbouring CoVs with a high similarity to SARS-CoV-2, is the most potential intermediate host. There is another consensus, that SARS-CoV-2 has higher infectivity and transmissibility as compared to SARS-CoV, related to a unique peptide (PRRA), four amino acid residue insertion on the S protein between its S1 and S2 subunits and involved in the proteolytic cleavage of the spike protein by cellular furin-like proteases widely expressed in lungs and respiratory tract and various other organs. This furin cleavage site is distinct from SARS-CoV and other CoVs including pangolin CoV which contain only a trypsin or TMPRSS2 cleavage site. This trait, the novel furin cleavage site on the S protein in SRS-CoV-2, has helped the its spill-over to humans and potentiated its transmissibility as well as pathogenicity.

The Perspective and Implications

The sequences of SARS-CoV-2 from various patients from different countries are almost identical, with greater than 99.9% sequence identity, suggesting that SARS-CoV-2 has originated from one source, from Wuhan and has spread relatively rapidly worldwide, within a short period. Through the novel furin cleavage site in its S protein, the SARS-CoV-2 utilizes and affects ACE in multiple ways. Apart from its entry through the ACE2, SARS-CoV-2 subsequently down-regulates ACE2 expression resulting in unopposed angiotensin II accumulation and local RAAS activation, which deranges homeostasis and exacerbates the inflammation and potentiates the tissue injury in lungs and other organs. In this perspective and in light of the studies and available data, it is advisable that an ACE inhibitor or ARB should be continued, rather than withdrawn in favour of another antihypertensive, in known or likely Covid-19 patients with stable condition.

Evolving Covid-19 Therapeutics

Presently there are no effective drugs for treatment of the Covid-19. The integrative network-based systems pharmacology methods have been used for rapid identification of repurposable drugs and drug combinations for the potential treatment. While new and repurposed drugs, including antiviral agents are being tested in various clinical trials, some of the promising drugs are simultaneously being recruited off-label for compassionate use or as experimental drugs to treat in desperate situations and otherwise dying patients. There has been a growing interest for the use of chloroquine and hydroxychloroquine as potential treatment in the interim till a specific treatment is available. There have been identified certain proteins in the host immune pathways which can be targeted for blocking viral replication by potential drugs or antibodies. Alternatively, the passive antibody transfer from pooled convalescent patient sera may seem another option. As obvious, not all clinical trials will be successful, but having so many efforts in the direction, some may succeed and provide plausible solutions.

Infectivity and Pathogenesis

The Agent Factors

Origin and Evolution of 2019-nCoV: The Coronaviruses (CoVs) belong to the subfamily Coronavirinae in the family of Coronaviridae of the order Nidovirales. The CoVs are enveloped RNA viruses, having the genome composed of a single-stranded positive-sense RNA (+ssRNA), ~30kb with 5'-cap structure and 3'-poly-A tail. The large CoV genome is related to the special features of the CoV RTC, which contains several RNA processing enzymes and encodes for several structural proteins (sps) and non-structural proteins (nsps) [1]. The sps having a critical role in viral RNA transcription and synthesis, are referred to as replicase-transcriptase proteins, whereas the nsps, also referred to as niche-specific proteins, though nonessential for virus replication but confer selective advantages for survival and tissue invasion [2]. The CoVs are further divided into four lineages, A to D. The lineage B includes the severe acute respiratory syndrome (SARS)-CoV and the novel SARS-CoV-2, whereas the lineage C includes Middle East respiratory syndrome (MERS)-related CoV. The six human CoVs

were identified till last year and included HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV and MERS-CoV. The SARS-CoV-2 or 2019-nCoV is the latest and most virulent member of the CoV family that can infect humans [3].

The SARS-CoV-2 shares a high sequence identity to SARS-CoV. The protein sequence analyses have documented that the amino acid similarity of the conserved nsps between SARS-CoV-2 and SARS-CoV is as high as 94.6%, suggesting that they are closely related. The homology between the SARS-CoV-2 genome and CoV bat viruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21 is approximately 90 percent, whereas it is approximately 96% with the bat SARS-like CoV (bat-CoV-RaTG13) genome [4]. There is consensus among the scientific community that the SARS-CoV-2 originated in horseshoe bats. But, the bat-SL-CoVZC45 and bat-SL-CoVZXC21 are not direct ancestors of 2019-nCoV. Instead the bat CoV may infected another animal, an intermediate host, which subsequently transmitted the virus to humans (Figure 1). The isolation of a CoV in the Malayan pangolins with high similarity to SARS-CoV-2 links these animals as being the potential intermediate host [5].

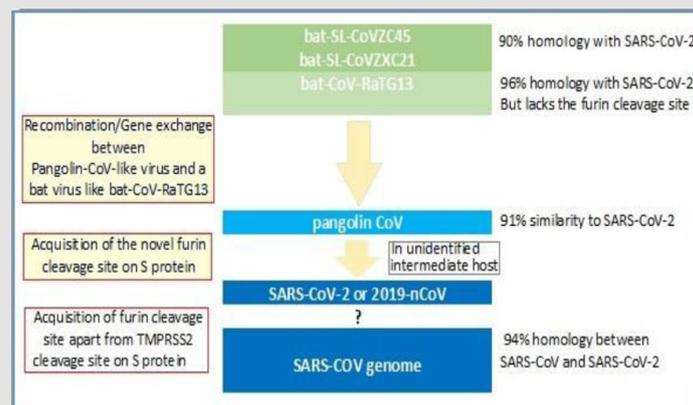


Figure 1: The Origin and Descent of SARS-CoV-2 or 2019-nCoV.

The genetic sequence of CoV, discovered in the lung samples of Malayan pangolins has a ~91% similarity to SARS-CoV-2. In addition, the pangolin CoV exhibits 100%, 98.2%, 96.7% and 90.4% amino acid identity with the SARS-CoV-2 E, M, N and S genes, respectively. The ancestor bat CoVs, have a 19 amino acids dissimilarity, whereas the pangolin CoV has only 5 amino acids dissimilarity for the S protein from SARS-CoV-2. In particular, the receptor-binding domain of the S protein of the Pangolin-CoV is virtually identical to that of SARS-CoV-2, with one amino acid difference. The comparison of available genomes appears to suggest that SARS-CoV-2 might have originated from the recombination of a Pangolin-CoV-like virus with a virus similar to bat-CoV-RaTG13, which has a striking 96% similarity to the SARS-CoV-2 virus but lacks the furin cleavage site. Further, the infected pangolins show clinical signs and histopathological features akin to SARS-CoV-2 in experimental models and harbour the antibodies which react with the S protein of SARS-CoV-2 [6]. The genetic sequence analysis of the bat CoVs,

pangolin CoV and SARS-CoV-2 appears to link the CoVs, suggesting that viruses from the bats and pangolin may have exchanged the genes at some point before spilling and infecting human-beings [7]. The pangolins are, thus, the most likely intermediate host, though other potential intermediate host(s), cannot be ruled out.

The pangolin CoV lacks a characteristic trait seen in SARS-CoV-2, that has helped it to leap to humans, the adaptation may have been acquired in another, yet unidentified, animal before the Wuhan epidemic spreading around the globe [8]. There has been identified a unique peptide (PRRA) insertion in the human SARS-CoV-2 virus in the S protein between its S1 and S2 subunits, which seems to be involved in the proteolytic cleavage of the spike protein by cellular furin-like proteases, which are widely expressed in a variety of organs including lungs and respiratory tracts, gastrointestinal tract, liver, pancreas and brain [9]. This furin cleavage site is distinct from SARS-CoV and other CoVs which only contain a trypsin or TMPRSS2 cleavage site. With the novel furin

cleavage site on the S protein, four amino acid residue insertion at the boundary between the S1 and S2 subunits, the SARS-CoV-2 appears to gain a potentially high infectivity and transmissibility as compared to SARS-CoV, due to the near-ubiquitous distribution of furin-like proteases in various tissues [10]. The sequences of SARS-CoV-2 from various patients from different countries are almost identical, with greater than 99.9% sequence identity. As a typical RNA virus, the average evolutionary rate for CoVs is approximately 10⁻⁴ nucleotide substitutions per site per year, with mutations arising during every replication cycle. This finding suggests that SARS-CoV-2 has originated from one source, from Wuhan and has spread relatively rapidly worldwide, within a short period [11].

The CoV Transcription and Expression: The CoVs have complex gene expression and replication cycle, encompassing ribosome frameshifting for genome translation, the synthesis of both genomic and sub-genomic components, and the assembly of progeny virions. Further, the CoV transcription involves the production of multiple sub-genomic mRNAs that contain sequences corresponding to both ends of the genome through a process of discontinuous transcription [12]. The expression of the CoV replicase-transcriptase (RT) protein genes is mediated by the translation of the genomic RNA. Whereas, the sub-genomic messenger RNAs (mRNAs) possess common 5'-leader and 3'-terminal sequences. The genome and sub-genomes of a typical CoV contain at least six open reading frames (ORFs). The first ORFs (ORF1a/b), comprising of about two-thirds of the whole genome length, produce two polypeptides, pp1a and pp1ab, which are processed by virally encoded protease into 16 nsps, 1 to 16. The other ORFs on the rest of one-third of the genome near the 3'-terminus encode 4 main sps - spike (S), membrane (M), envelope (E) and nucleocapsid (N) proteins. Besides the sps, accessory proteins, such as HE protein, 3a/b protein and 4a/b protein are also encoded. The sps and accessory proteins are translated from the sub-genomic RNAs (sgRNAs) [13].

The 2019-nCoV Structural Components: Spike (S) Protein: The glycosylated S protein is a fusion protein and mediates attachment of the virus to the host cell surface receptors and subsequently facilitate its entry through the cell membrane into the host cell. The S protein has two subunits: the globular S1 subunit is involved in receptor recognition and has a large receptor-binding domain (RBD) structure organized in four distinct domains, whereas the S2 subunit forms the stalk of the spike molecule and contains the key protein segments including the fusion peptide, two heptad-repeat regions (HR1 and HR2) that facilitate virus-cell fusion, and has been conserved across different genera of CoV species [14]. There are various receptors that bind to different CoVs, such as ACE2 for SARS-CoV29 and CD26 for MERS-CoV. There being a structural similarity between the RBDs of SARS-CoV and SARS-CoV-2, SRS-CoV-2 also uses ACE2 as its receptor, despite the presence of amino acid mutations in its S1 RBD [15].

The CoV S glycoprotein has N-terminal region (NTR, domain A) and the C-terminal region of S1 (CTR, domains B, C, and D) which bind to the host ACE2 receptors and function as RBDs [16]. The major difference in S1 subunit of SARS-CoV-2 as compared to SARS-CoV, is the 3 short insertions its N-terminal domain, which confer the sialic acid binding activity and 4 out of 5 key residues changes in the receptor-binding motif [17]. Other Major Structural Proteins: In addition, the CoV genome encodes other major sps, such as, nucleocapsid (N) protein, membrane (M) protein and the envelope (E) protein. The N protein is the most abundant protein in CoV and binds to the CoV RNA genome, leading to formation of the helical nucleocapsid. It is composed of two domains, an N-terminal domain (NTD) and a C-terminal domain (CTD). The genomic packaging signal binds specifically to the C-terminal RNA binding domain, whereas NTD binds to nsp3, a key component of the replicase complex and the M protein. The N protein is localised to the endoplasmic reticulum (ER)-Golgi region and involved in nascent virion assembly and budding. Its high hydrophilicity has been related to the host cellular response and potent immunity following the viral infection. The M protein, another major structural protein, helps in defining the shape of viral envelope. It is the central organiser of CoV assembly and interacts with all other major coronaviral sps. Interaction of S with M is necessary for retention of S in the ER-Golgi intermediate compartment (ERGIC)/Golgi complex and its incorporation into new virions. The binding of M to N stabilises the nucleocapsid (N protein-RNA complex) core.

The E protein is abundantly expressed inside the infected cell and localised at the ER, Golgi and ERGIC, and during the replication cycle a small portion of E protein is incorporated into the virion envelope along with a major contribution by M protein [18]. The E protein appears to be involved at multiple stages of the replication cycle, from assembly and induction of membrane curvature to budding and release, and inflammation, apoptosis and autophagy. The deletion of E from SARS-CoV attenuates the virus. The hemagglutinin-esterase (HE) protein is attached to the viral surface. It contains a lectin-binding domain that mediates binding to O-acetylated sialic acids. It possesses sialate-O-acetyl esterase receptor-destroying enzyme activity, though which viral attachment to non-permissive cells is prevented. The HE protein appears to facilitate virus entry into target cells after binding to the main entry receptor.

Receptor Tethering, Viral Invasion and Replication: The Viral Tethering and Cell Entry: Following receptor binding, the SRS-CoV-2 gains access to the host cell cytosol, accomplished by acid-dependent proteolytic cleavage of S protein by a furin like protease. The S protein cleavage occurs at two sites within the S2 portion of the protein, the first cleavage separates the RBD and fusion domains of the S protein and the second cleavage exposes the fusion peptide which inserts into the cell membrane, followed by joining of two heptad repeats, HR1 and HR2, in S2 forming an

antiparallel six-helix bundle. The formation of the bundle allows the blending of viral and cellular membranes, resulting in fusion and ultimately release of the viral genome into the cytoplasm. Replicase Protein Translation and Expression: The next step is the translation of the replicase gene from the viral genomic RNA. Viral RNA synthesis follows the translation and assembly of the viral replicase complexes and generation of genomic and sub-genomic RNAs. The replicase gene encodes two large ORFs, rep1a and rep1b, which

express co-terminal polyproteins (pps), pp1a and pp1ab. In order to express the pps, the virus utilizes an RNA pseudoknot that cause ribosomal frameshifting from the rep1a into the rep1b ORF. The pps are subsequently cleaved into the individual nsps by proteases encoded by the virus. Various nsps assemble into the replicase-transcriptase complex (RTC) for RNA replication and transcription of the sub-genomic RNAs. The sub-genomic RNAs serve as mRNAs to generate the structural and accessory proteins (Figure 2).

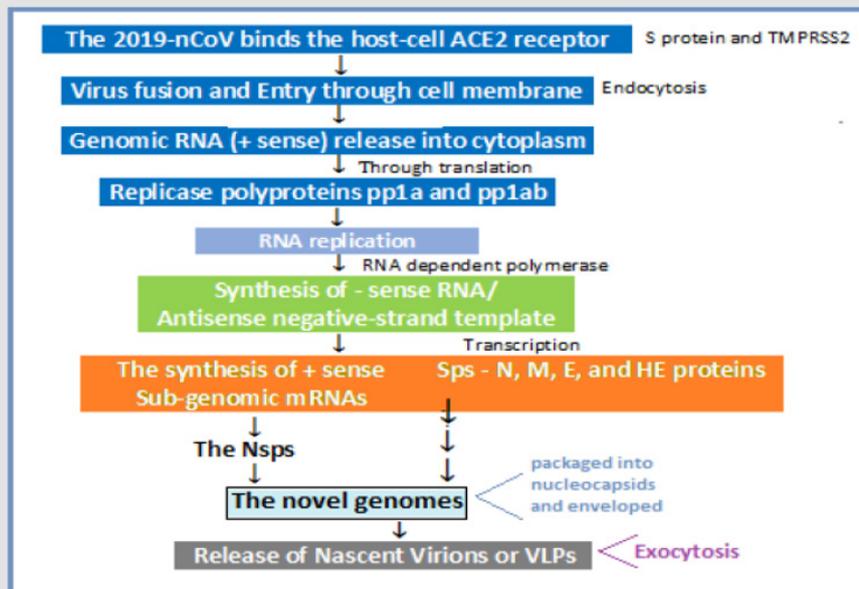


Figure 2: The Outline of the 2019-nCoV Replication Cycle.

Functions of CoV non-structural proteins: The nsps contain domains to carry out various genomic as well as supportive functions. The nsps promotes cellular mRNA degradation (nsp1) and blocks host cell innate immune response (nsp1 nsp3), cleave pps (nsp5), bind to prohibitin proteins (nsp2), interact with N protein (nsp3), act as processivity clamps for RNA polymerase (nsp7 and 8) and transmembrane scaffold proteins (nsp4 and 6). In addition, they promote cytokine expression (nsp3), stimulates viral 3'-5' exoribonuclease (nsp10 and 14) and N7 methyltransferase (nsp10 and 14), shield viral mRNA from recognition (nsp16) and bind viral RNA (nsp9). They also modulate the enzymes RNA-dependent RNA polymerase (nsp12), RNA helicase 5'triphosphatase (nsp13) and viral endoribonuclease (nsp15), and act as cofactor for nsp14 and 16 (nsp10).

Virion Assembly and Release: Following replication and sub-genomic RNA synthesis, the viral sps, S, N, M, E and HE are translated and inserted into the endoplasmic reticulum (ER). The sps move along the secretory pathway into the ERGIC. The viral genomes are encapsidated by the N protein bud into membranes of the ERGIC containing viral structural proteins, forming nascent virions or virus like particles (VLPs). The M protein then directs most protein-protein interactions required for assembly of virions and expressed along with E protein to produce virion envelope. The N protein

enhances VLPs formation and the S protein is incorporated into virions at this step. The M protein also binds to the nucleocapsid, promoting the completion of virion assembly. Following assembly, VLPs are transported to the cell surface in vesicles and released by exocytosis.

The Host Factors

Angiotensin-Converting Enzyme 2 (ACE2): The ACE2, also known as ACEH (ACE homolog), is an integral membrane protein and a zinc metalloprotease of the ACE family. The human ACE2 protein sequence consists of 805 amino acids, including a N-terminal signal peptide, a single catalytic domain, a C-terminal membrane anchor and a short cytoplasmic tail. ACE2 cleaves angiotensin I and II as a carboxypeptidase. ACE2 is located as an ectoenzyme on the surface of endothelial and other cells. There is an abundant presence of ACE2 in nasal mucosa and nasopharynx, oral mucosa and tongue, airways and lung alveolar epithelial cells. There is rich ACE2 surface expression on arterial and venous endothelial cells, and arterial smooth muscle cells. The abundant ACE2 expression has been linked with the pathogenesis involving vasculitis, deranged immune function, extensive pulmonary inflammation, and diffuse alveolar damage with hyaline membrane formation, and the severe clinical manifestations [19]. The oral cavity is a potentially high-risk route for SARS-CoV-2 infection and should be a part of future prevention

strategy in dental clinical practice as well as daily life [20]. Apart from this, there is a high ACE2 expression in oesophageal upper and stratified epithelial cells, absorptive enterocytes from ileum and colon, cholangiocytes, myocardial cells, renal proximal tubule cells and bladder urothelial cells, skin, lymph nodes and brain. The widespread ACE2 tissue distribution in various organs may explain the multi-organ dysfunction observed Covid-19 in patients.

The ACE2 is an important regulator of the renin-angiotensin system (RAS). Angiotensin I, having no direct biological activity, exists as a precursor to angiotensin II, and converted to the latter through removal of two C-terminal residues by the ACE2 primarily in the lung but also in kidneys, endothelial cells, and brain. Angiotensin II acts on venous and arterial smooth muscle to cause vasoconstriction and increases vasopressin production in the CNS. It also stimulates aldosterone secretion. It appears that apart from gaining its entry through the ACE2, the SARS-CoV-2 subsequently down-regulates the ACE2 expression leading to loss of its protective effects in various organs, which may have significant impact on the pathogenesis of the disease.

There is reduced ACE2 expression with aging in both genders. It is slightly reduced in young-adult and middle-aged groups but reduced significantly in the elderly age group. In animal studies, though there was no gender-related difference of ACE2 expression in adult mice, a higher ACE2 content was noticed in old female than male [21]. The epidemiological studies have documented that different sex and age groups have different susceptibility to SARS-CoV-2 infection, which may be linked to ACE2 expression. There is a skewed relationship with various host factors and the severity and mortality of the disease, with the male sex, elderly population and those with coexisting chronic diseases being the most affected. Further, as per the preliminary epidemiological data, there is an exponential increase in disease severity and mortality in those beyond the sixth decade of life with cardiovascular disease and diabetes [22]. The exaggerated proinflammatory profile is appears to be a salient feature in those suffering from hypertension, heart disease and diabetes. In addition, the elderly, especially those with hypertension and diabetes, have reduced ACE2 expression and upregulation of angiotensin II proinflammatory signaling. The SARS-CoV-2 binding to ACE2 may exaggerates this proinflammatory milieu, predisposing these population groups to greater disease severity and mortality.

S Protein-ACE2-Protease Host Cell Entry: The ACE2, expressed on the surface of various tissues including airway epithelial cells, binds with the S protein. The RBD on the spike, mediates the interaction with the host-cell receptor; ACE2 for both SARS-CoV and SARS-CoV-2. The spike RBD is capable of folding independent of the rest of the spike protein and contains the structural information for host receptor binding. The host protease processing during viral entry is a may be a significant barrier infection, but has been overcome by hCoV including SARS-

CoV-2 to the agent's advantage and after binding the receptor, a host protease cleaves the spike, releasing the spike fusion peptide and facilitates the virus entry [23]. The CoV entry into the host cell is a multi-step process. It involves several distinct domains in spike mediating the virus attachment to the cell surface, receptor engagement, protease processing and membrane fusion. After bonding with the host receptor, the host-cell protease cleaves spike and releases the fusion peptide, allowing for host-cell entry. Thus, armed with the SARS-CoV spike, the SARS-CoV-2 is capable of using human ACE2 efficiently, which may account for the human-to-human transmissibility of the virus [24].

The ACE2 is predominantly expressed in surfactant-secreting type II alveolar cells in lungs, bronchiolar epithelium, and endothelium and smooth muscle cells of pulmonary vessels. Further, it is more abundantly expressed in the apical than the basolateral areas of lungs. Furthermore, the ACE2 expression is proportionately correlated to the epithelial differentiation of the alveolar tissue. The undifferentiated cells poorly express ACE2, while well-differentiated cells richly express ACE2. The studies indicate that infection of human airway epithelia by SARS-CoVs correlates with the state of cell differentiation and ACE2 expression and localization. These findings have implications for understanding disease pathogenesis associated with the SARS-CoV infections [25].

The lungs appear to be the most vulnerable target organs for SARS-CoV and SARS-CoV-2 because of the vast surface area, making the lung highly susceptible to inhaled viruses. About 83 percent of ACE2-expressing cells are alveolar type II cells, which are exposed and can serve as a reservoir for the viral invasion. In addition, the ACE2-expressing alveolar type II cells contain high levels of viral process-related genes, including regulatory genes for viral processes, viral life cycle, viral assembly, and viral genome replication. Further, it has been claimed that ACE2 is not only the entry receptor of the virus but also protects from lung injury and high lethality associated with SARS-CoVs infection could be because of dysregulation of the pulmonary protective mechanisms [26].

Pathogenesis of CoV Infections: Coronaviruses cause a large variety of severe diseases in livestock and other animals such as pigs, cows, chickens, dogs and cats, which has led to significant research on these viruses. For instance, Transmissible Gastroenteritis Virus (TGEV) and Porcine Epidemic Diarrhoea Virus (PEDV) cause severe gastroenteritis in young piglets, leading to significant morbidity and mortality, and economic loss. The SARS-CoV, a 2b β -coronavirus, was identified as the causative agent of the Severe Acute Respiratory Syndrome (SARS) outbreak that occurred in 2002–2003 in the Guangdong Province of China, leading to approximately 8098 cases and 774 deaths, amounting to a mortality rate of 9%. The rate was much higher in elderly individuals, with mortality rates approaching 50% in individuals over 60 years of age [27]. It appears that the SARS-CoV originated in bats as a large number of Chinese horseshoe bats harbour sequences of SARS-related CoVs

and serological evidence for a prior infection with a related CoV. In fact, two novel bat SARS-related CoVs, bat-SL-CoVZC45 and bat-SL-CoVZXC21, were identified as having significant similarity to SARS-CoV. They were also found to use the same receptor, ACE2. Transmission of SARS-CoV was relatively inefficient, as it only spread through direct contact with infected individuals after the onset of illness, and the outbreak was controllable through strict quarantine.

Another novel human CoV, named Middle East Respiratory Syndrome-CoV (MERS-CoV), was found to be the causative agent in a series of highly pathogenic respiratory tract infections in Saudi Arabia and other countries in the Middle East, which emerged in the Middle East in 2012. It was thought that camel-to-human spill over contributed to the outbreak. The outbreak did not accelerate later in 2013, though sporadic cases continued throughout the rest of the year. From 2012 through May 31, 2019, MERS-CoV has infected 2,442 persons and killed 842 worldwide amounting to case fatality rate of ~29 percent [28]. The MERS-CoV utilizes Dipeptidyl peptidase 4 (DPP4) as its receptor in humans and certain species such as bats, camels, rabbits and horses to establish infection. The virus is unable to infect mouse cells due to difference in the structure of DPP4. Owing to the lack of effective therapeutics or vaccines, the best measures to control hCoVs, remain an efficient public health surveillance system coupled with rapid diagnostic testing, and isolation and quarantine. The past and likely future emergence of pathogenic zoonotic CoVs, the gross social and economic impact of hCoVs infections and the lack of effective antiviral strategies make it obvious that our preparedness to prevent and treat CoV infections is limited [29]. This highlights the importance of advancing our knowledge on the emergence, infectivity, replication and pathogenesis of the CoVs.

The Host Cell Immune Response: In general, the host cells respond to the virus infection by recruiting an innate antiviral response to limit the spread of the infection and resorting to induce an adaptive immune response to eventually clear the virus. In the case of CoVs and other +RNA viruses, the innate immune system is triggered by recognizing dsRNA and 5'-triphosphate-bearing RNA molecules arising as replication intermediates in the cytosol by the intracellular sensors of the Rig-I-like receptor (RLR) family, such as retinoic acid-inducible gene 1 (RIG-I)[30] and melanoma differentiation-associated protein 5 (MDA-5) which are expressed in various host cells [31]. The infections are recognized by the cytosolic sensors RIG-1, Mda5 and stimulator of interferon genes (STING) and result in the expression of IFN- β and an inflammasome response. The RLRs, RIG-1, Mda5 and LGP2 recognize the viral ligands. Mda5 is one of the pattern recognition receptors (PRRs) that recognize cytoplasmic viral ligands [32].

For recognition of CoV RNAs, MDA-5 seems the most important cytosolic sensor. The toll-like receptors (TLRs) which are expressed on the cell surface or reside in the endosomes of immune cells can

also recognize CoVs nucleic acids or proteins. Activation of one or more of these sensors generally leads to the activation of the transcription factors IFN-regulatory factor 3 and 7 (IRF3, IRF7) and NF- κ B, which stimulate the expression and secretion of Type-I IFN and pro-inflammatory cytokines. Following this, the JAK-STAT (Janus kinase/signal transducers and activators of transcription) signaling cascade is activated leading to expression of various antiviral interferon-stimulated genes (ISGs) resulting in an antiviral state of the infected cells.

The CoV infection and stages of replication are associated with the endoplasmic reticulum (ER) stress. The ER can sustain a high load of protein content without being overwhelmed. However, when the ER's capacity for folding and processing proteins is exceeded, unfolded or misfolded proteins rapidly accumulate in the lumen and the ER stress response is induced. The ER stress leads to activation of Unfolded Protein Response (UPR) pathways through the induction of protein kinase RNA-like endoplasmic reticulum kinase (PERK). The activated UPR leads to elevated level of phosphorylated Eukaryotic Initiation Factor 2 alpha (eIF2 α) resulting in the promotion of a pro-adaptive signaling pathway by the inhibition of global protein synthesis and selective translation of Activating Transcription Factor 4 (ATF4). In addition, during conditions of prolonged ER stress, several pro-apoptotic genes are induced, and there is failure of synthesis of anti-apoptotic Bcl-2 proteins. The ER stress-mediated leakage of calcium into the cytoplasm also leads to the activation of apoptosis effectors. These measures eventually trigger apoptosis, which is utilized by the host cells to inhibit viral replication. PKR (serine/threonine protein kinase) is a key player in the innate immune response to RNA virus infection by upregulating antiviral gene expression and enhancing synthesis of interferons (IFNs).

The CoVs Countermeasures: The viruses, on the other hand, have evolved strategies to suppress and overcome the immune responses, which influence the pathogenesis, course of the disease and persistence the virus in the host. The CoVs strategically counteract PKR-mediated signaling to prevent the translational shut-off due to eIF2 α phosphorylation. In case of the MERS-CoV, the ORF4a protein counteracts the PKR-induced formation of stress granules, probably by binding viral dsRNA to shield it from detection by PKR. The S proteins of both SARS-CoV and SARS-CoV-2 also interact with eIF3F, to modulate host translation, including the expression of the pro-inflammatory cytokines, and IL-6 and 8 at a later stage of infection. These interactions play an important regulatory role in CoV pathogenesis, and disease course and prognosis [33].

Apart from modulating eIF2 α phosphorylation, the hCoV manipulates the translation machinery through nsps. The nsp1 protein, an inhibitor at multiple steps of translation, inhibits 48S initiation complex formation and its conversion into the 80S initiation complex, apart from directly binding to the 40S ribosomal

subunit to stall translation. Further, the nsp1 and 40S complex induce cleavage of cellular mRNAs to suppress the translation. The viral nsps, especially nsp3, nsp4 and nsp6, appear to drive the formation of replication structures such as double-membrane vesicles (DMVs), tubules, zippered ER and convoluted membranes forming a reticulovesicular network in the cytosol of infected cells. In addition, various RNA-binding proteins in the infected host cells fail to interact and block the CoV 5' UTR, the 3' UTR and poly(A)-tail [34]. In addition, the CoV nsp15 inhibits retinoblastoma protein (pRb), a tumor suppressor protein, resulting in enhanced expression of genes normally repressed by pRb [35]. The overexpression of the SARS-CoV 3a protein leads to G1 arrest and inhibition of cell proliferation. Apart from this, SARS-CoV infection decreases p53 expression related to antiviral effect and able to enhance its replication in the cells lacking p53 depleted cells [36].

The Protein-Protein Interactions (PPIs): The PPIs can be host-host, virus-virus or virus-host PPIs. Through PIPs the viral proteins establish interactions with host proteins. The viral protein domains are basic units for viral-host protein interactions and the mutations at protein interfaces can reduce or increase the binding affinities. During the course of the viral invasion and infection, both the agent and cellular proteins are constantly competing for bonding. The protein-protein interactions (PPIs) in virus-host systems are mediated by domain-domain interactions (DDIs), involving molecular recognition via amino acid residues located at interfaces of interacting domains [37]. Apart from this, endogenous interfaces mediating viral-viral or host-host interactions, are constantly targeted and inhibited by exogenous interfaces mediating the interactions. In case of viral infection, the virus-host PIPs, the interacting proteins are constantly losing and regaining their binding sites in order to evade or optimize interspecific PPIs. The exogenous interfaces lend competition for such molecular reactions and interfere with host-host protein interactions leading to alterations in cellular metabolism [38].

The host factors evolve to retain or restore their recognition capabilities to bind and neutralize viral agent. Whereas, through virus-host interactions, the viral proteins tend to target more central and highly connected host proteins and maintain and adapt their capabilities via molecular evolutions and, including conservation, HGT, gene duplication and molecular mimicry [39]. Horizontal gene transfer (HGT) is a process of genome recombination by means of which the virus acquires genes from non-parental organisms, optimizes and integrates into its virus-host network. Through gene duplication, the virus acquires duplicated genes in the viral genomes to become more lethal. As the virus evolves at faster mutation rate, it can rapidly acquire new binding partners by mimicking and targeting interfaces of host proteins.

Immune Response/Inflammasome Activation: There is a complexity of the virus-host interactions that occur within the cell, tissues or the whole body. The SARS-CoV-infected peripheral blood mononuclear cells lead to the upregulation of expression

of various cytokines, including IL-8 and IL-17, and the activation of macrophages and the coagulation pathways. The protein kinases, the key regulators in signal transduction, control a wide variety of cellular processes. They are linked to cellular immune responses, like interleukin (IL) signaling, IL-6 and -8, and influence the CoV infection and CoV-induced inflammation. There occurs downregulation of a large number of mRNAs, including those encoding proteins involved in translation, leading to the host translational shut-off in CoV-infected cells due to a stress response and concomitant mRNA decay. In addition, the heterogeneous nuclear ribonucleoproteins (hnRNPs) influence the maturation of nascent nuclear RNAs into messenger RNAs (mRNAs) and stabilize their cellular transport and control their translation. In general, the difference in the immune and inflammatory responses determines the outcome of the infection and responsible for the differential activation of the Signal transducer and activator of transcription 3 (STAT3) pathway, involved in lung inflammation and cellular repair [40].

The CoV nucleocapsid (N) protein plays a multifunctional role in the virus life cycle, from regulation of replication and transcription to genome packaging and release of VLPs to modulation of host cell defence processes. In addition, various viral proteins interact with host ribosomal and nucleolar proteins, helicases and the hnRNPs [41]. The viruses encode proteins to modulate the cellular factors to modify the immune response pathways to avoid their detection and exploit the extensive network of the host cell's signalling pathways to promote their replication and propagation. The inflammasome activation by CoV E was first reported in porcine reproductive and respiratory syndrome virus (PRRSV), when it documented that PRRSV-encoded small envelope protein E, an ion channel-like protein, triggered the activation of inflammasomes. Blocking ion channel activity with amantadine significantly inhibited activation of the inflammasome [42]. Recently, the transport of Ca²⁺ by SARS-CoV E has been shown to trigger inflammasome activation [43]. Following which, there has been shown ER stress and apoptosis to occur, and the inflammatory lung damage in SARS-CoV-infected mice. Inhibiting the CoV E ion channel activity limited the CoV E viroporin induced CoV pathogenicity. Autophagy is a reparative cellular process that recycles excess or damaged cellular material inside the cell ensure its survival. The RNA viruses, including CoVs, appear to exploit autophagy for the purpose of viral replication and propagation.

Immunological Basis of Severe Covid-19: In the severely ill SARS-CoV-2 patients, the disease may progress rapidly from viral pneumonia to acute respiratory failure. The neutrophil-to-lymphocyte ratio (NLR) has been identified as an independent risk factor for severe illness in these patients. The patients with age \geq 50 and NLR \geq 3.13 denote a severe illness and should have rapid access to intensive care unit and respiratory support [44]. The pyroptosis, a form of inflammatory form of programmed cell death and appears to be the possible mechanism for the increased virulence of SARS-

CoV-2[45]. The SARS-CoV Viroporin 3a triggers the activation of the NLRP3 inflammasome and the secretion of IL-1 β by macrophages, suggesting SARS-CoV induced cell pyroptosis [46]. In addition, the patients infected with SARS-CoV-2 have increased IL-1 β in the serum, an indicator of the pyroptosis [47]. Further, the rising IL-1 β suggests activation of cell pyroptotic activity. The severe SARS-CoV-2 infection is likely to cause cell pyroptosis, especially in lymphocytes, through the activation of NLRP3 inflammasome.

About thirty percent of patients demonstrated neurological manifestations out of a sample of 214 newly diagnosed Covid-19 patients [48]. The central nervous system (CNS) involvement is indicated by headache, dizziness, disturbance of consciousness, acute cerebrovascular disease including epilepsy, and peripheral nervous system symptoms such as decreased taste, decreased smell, and appetite. The SARS-CoV-2 appears to invade the CNS through blood or retrograde neuronal circuits, leading to neurological damage and viral encephalitis.

Evolving COVID-19 Therapeutics

Outlining Solutions for Treatment

Presently there are no effective drugs for treatment of the Covid-19, which is now pandemic with millions of confirmed cases worldwide. While new and repurposed drugs are being tested in clinical trials, some of the promising drugs are simultaneously being recruited off-label for compassionate use or as experimental drugs to treat in desperate situations and otherwise dying patients. As obvious, not all clinical trials will be successful, but having so many efforts in progress, some may succeed and provide plausible solutions. Right now, though, there prevail confusion and despair. The future seems afflicted with dormant therapeutic options as well as faux *Espoir* or false hopes. See my review article – Preprint – “Exploring Pathophysiology of Covid-19 Infection: Faux *Espoir* and Dormant Therapeutic Options”. On www.researchgate.net DOI: 10.13140/RG.2.2.16995.09764. please visit- <https://www.researchgate.net/publication/340175439>. Exploring_Pathophysiology_of_COVID-19_Infection: Faux_Espoir_and_Dormant_Therapeutic_Options.

Understanding the PPIs offers an opportunity to target both viral-host and intraviral interactions to halt the viral replication and propagation. The virus binding and entry are the first steps of the replication cycle that can be targeted with inhibitors. There are inhibitors of endosomal acidification, such as ammonium chloride, chloroquine and hydroxychloroquine, which appear to block the entry of CoVs including SARS-CoV and SARS-CoV-2. In addition, peptides have been explored which can block recognition and fusion by interfering with the interaction between the HR1 and HR2 domains of the S protein. Apart from drugs acting as inhibitors directed at viral components, there are evolving therapeutic strategies involving host-directed approaches, based on the pathogenesis and virus–host interactions. The host-directed

approach can yield a broad-spectrum therapeutic strategy and may lower the chance of development of antiviral resistance. The interferon (IFN) has been shown to trigger the innate immune response in CoV-infected cells, leading to transcription of various interferon stimulated genes (ISGs) that may play a role in controlling and eradicating the infection [49].

Stalling ACE2-mediated COVID-19 Entry

Spike Protein-Based Vaccine: Development of a spike-1 subunit protein-based vaccine relies on the fact that ACE2 is the SARS-CoV-2 receptor [50]. The subunit vaccines for SARS-CoV may work by eliciting an immune response against the S-spike protein to prevent its docking with the host ACE2 receptor. In this context, the Novavax Inc, headquartered in Maryland, has developed immunogenic virus-like nanoparticles based on recombinant expression of the S-protein [51]. The Clover Biopharmaceuticals is developing a subunit vaccine consisted of a trimerized SARS-CoV-2 S-protein using their patented Trimer-Tag technology [52]. Another subunit vaccine being developed and tested is comprised of the receptor-binding domain (RBD) of the SARS-CoV S-protein [53]. Initial findings have documented that the SARS-CoV and SARS-CoV-2 RBDs exhibit more than 80% amino acid similarity and bind to the same ACE2 receptor offer an opportunity to develop either protein as a subunit vaccine.

Inhibition of TMPRSS2 Activity: The S protein of CoVs facilitates viral entry into target cells through attachment with ACE2 receptors. The priming of S protein by protease, namely, transmembrane protease serine 2 (TMPRSS2) is a prerequisite for S protein cleavage at the S1/S2, which frees S2 subunit essential for entry and translocation of the SARS-CoV-2 genome through interaction with the ACE2 receptor (Figure 3). The protease, TMPRSS2 is a potential target for therapeutic intervention and the serine protease inhibitor, camostat mesylate, has been shown to block TMPRSS2 activity and to prevent SARS-CoV-2 entry into the host cells [54]. In the animal study, Camostat protected 6 out of ten mice from lethal infection with SARS-CoV. The results, thus, indicate that camostat or similar serine protease inhibitors may be a potential treatment option for SARS-CoV and SARS-CoV-2 infections [55]. The drug, camostat mesylate is already approved in Japan for the treatment of a number of non-infectious conditions.

Convalescent SARS Sera: The convalescent SARS patients exhibit a neutralizing antibody response directed against the S protein, which can be detectable as long as 24 months or more following the infection [56]. It has been found that the antibodies found in the recovering SARS patients sera cross-neutralized SARS-2-S-driven entry as well, as apparent from being able to hamper the SARS-CoV-2 S protein-driven entry into Vero-E6 cells, *in vitro* [56]. The finding is important as it reveals an important commonality between SARS-CoV-2 and SARS-CoV infection and a potential therapeutic intervention.

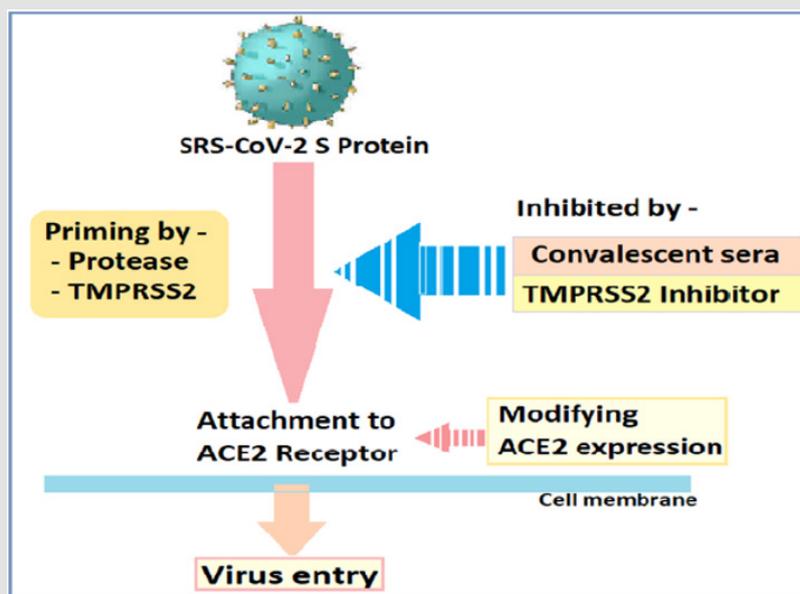


Figure 3: Therapeutic options to stall ACE2 mediated SRS-CoV-2 entry.

Modifying ACE2 Expression

The interaction between ACE2 and SARS-CoV is a therapeutic target. Theoretically, the anti-ACE-2 antibodies can block SARS-CoV-2 binding to the receptor, disrupt the interaction, prevent the virion entry and the alveolar cell damage. The treatment with anti-ACE-2 antibodies, thus, disrupts the interaction between virus and receptor. The same results can be achieved through enhancing the ACE2 expression by treatment with soluble ACE2.

It has been demonstrated in mice experiments that SARS-CoV downregulates ACE2 protein by binding its spike protein, contributing to severe lung injury [57]. It appears that, ACE2 competitively binds with SARS-CoV-2 not only to neutralize the virus but also rescue cellular ACE2 activity which negatively regulates the renin-angiotensin system (RAS) to protect the lung from injury [58]. Apart from this, angiotensin-(1-7) is generated by degradation of Ang II by ACE2 has vasoprotective functions. The enhanced ACE activity and decreased ACE2 availability may, thus, contribute to oxidative stress and subsequent lung injury. ACE2 deficiency may be critical in a variety of co-existing disease states, including hypertension, diabetes, renal impairment and cardiovascular disease, and in elderly with physiological reduced ACE2 availability.

Functionally, there are two forms of ACE2. The membrane-bound ACE2 contains a transmembrane domain and an extracellular domain, which is used by SARS-CoV-2 as the receptor. The other, soluble form of ACE2 lacks the membrane anchor and circulates in small amounts in the blood. In this context, treatment with a soluble form of ACE2 may slow the viral entry into host cells, as the soluble ACE2 acts as a competitive interceptor of SARS-CoV by preventing its binding to the membrane-bound ACE2, limits cell entry and

spread, and protects the lungs from injury. The recombinant human ACE2 (rhACE2; APN01, GSK2586881) has been found to be safe in healthy volunteers and in a small cohort of patients with ARDS [59].

The Stapled Peptides

Therapies using small-molecule drugs, allows the drugs to cross cell membranes but may lack selectivity. Further, the small-molecule drugs may not be able to disrupt PPIs efficiently. The larger molecules and protein-based therapies, including the engineered antibodies, on the other hand, are potent and more selective, but have restricted ability to reach the target tissues and cross the cell membranes. The stapled peptides are large enough to be specific and potent for viral infections to inhibit PPIs and can be modified to enhance their membrane permeability to reach the target cells [60]. The stapled peptides have the potential to act at multiple levels in virus replication cycle. Drug-development-wise, the development of stapled peptides can be expressed and has a short market time.

Blocking CD147 inhibits CoV Replication

The CD147 (basigin), transmembrane glycoprotein belonging to the immunoglobulin superfamily, is important factor in the host defence and regulates the expression of extracellular matrix metalloproteinases (EMMPs). It is expressed in various tissues and cell types, including leukocytes, platelets, epithelial cells and endothelial cells and fibroblasts. CD147 has been shown to play a prominent role in the induction of pro-inflammatory and pro-thrombotic events in various disease models [61]. The CD147-SP interaction enhances viral invasion for host cells and blocking CD147 on the host cells has shown an inhibitory effect on SARS-CoV-2 p [62]. It has been documented that Meplazumab, a humanized anti-CD147 antibody, can competitively inhibit the

binding of S protein and CD147, and prevent the viruses from invading host cells.

Developing/Repurposing Antiviral Drugs

The antiviral agents are being tested in clinical trials. Remdesivir, a broad-spectrum antiviral and nucleotide prodrug, inhibits replication of hCoVs in tissue cultures. The anti-retroviral and other investigational antiviral drugs are being tested for efficacy against COVID-19. Beta-D-N4-hydroxycytidine (NHC), plitidepsin and favipiravir are other anti-viral drugs in clinical trials. There have been efforts for using oligonucleotides against SRS-CoV-2 RNA genome or repurposing currently available various antiviral medications. The integrative network-based systems pharmacology methods have been used for rapid identification of repurposable drugs and drug combinations for the potential treatment of 2019-nCoV/SARS-CoV-2 [63].

In the cell culture infection models, a cyclophilin inhibitor, cyclosporin A (CsA), inhibited the replication of CoVs. CsA binds to cellular cyclophilins to inhibit calcineurin, a calcium-calmodulin-activated serine/threonine-specific phosphatase, and CoV replication [64]. The CK2 inhibitors Emodin and Rhein, anthraquinone compounds and chrysin, a flavonoid compound, target autophagy via different upstream pathways including the AKT/mTOR-axis and transcription of autophagy-related proteins. Whereas, emodin has been shown to block the S protein and ACE2 interaction in a dose-dependent manner. The translation inhibitors enable the ongoing translation of messenger ribonucleoproteins (mRNPs) that encode antiviral factors such as interferon-stimulated genes (ISGs) despite the arrest of bulk translation [65]. Whereas, the viral transcription inhibitors, like sirolimus, temsirolimus and everolimus (mTOR inhibitors) block the mTOR, a serine/threonine protein kinase that regulates protein synthesis, autophagy and transcription. The toll-like receptor 3 (TLR-3) agonist, rintatolimod is being tested as a potential treatment for Covid-19 in Japan.

Immuno-modulators and Other Drugs

The interleukin-6 inhibitors, such as tocilizumab (Genentech), may ameliorate severe damage to lung tissue caused by cytokine storm in patients with severe Covid-19. Tradipitant (Vanda Pharmaceuticals), a neurokinin-1 (NK-1) receptor antagonist, may have efficacy for inflammatory lung injury associated with severe SARS-CoV—19 infection. Corticosteroids are not generally recommended for treatment of Covid-19 or any viral pneumonia [66].

Conclusion: Perspectives And Implications

CQ and HCQ in Treating COVID-19

There has been a growing interest in the use of chloroquine (CQ) and hydroxychloroquine (HCQ) as potential treatments in the interim till a specific treatment is available, based on several in vitro and few in vivo studies reporting antiviral activity of CQ

and HCQ against SARS-CoV-2 [67]. It has been claimed that HCQ is more potent than CQ against SARS-CoV-2, *in vitro* [68]. There are some in vivo studies data at present, though limited [69]. A number of potential mechanisms of action of CQ/HCQ against SARS-CoV-2 have been postulated. CQ may reduce glycosylation of ACE2, thereby preventing SARS-CoV-2 from effectively binding to host cells. Furthermore, CQ might block the production of pro-inflammatory cytokines (such as IL-6), thereby blocking the pathway that may lead to acute respiratory distress syndrome (ARDS). CQ is also believed to raise the pH level of the endosome, which may interfere with virus entry and/or exit from host cells.

The prophylactic use of HCQ is not backed-up by research data and trials are underway relating to preexposure and post exposure prophylaxis [70]. Encouraging clinical results were reported from China in February 2020, revealing that the treatment of over 100 patients with chloroquine phosphate in China had resulted in significant improvements of pneumonia and lung imaging, with reductions in the duration of illness [71]. At present, considering all the data, there is insufficient evidence to endorse that CQ/HCQ are safe and effective treatments for Covid-19 [72].

In addition, HCQ has a potential QT-prolonging effect by blocking critical potassium channels in cardiac conduction system, with the possibility of arrhythmias and sudden cardiac death. The Covid-19 patients with a baseline QTc value greater than or equal to 500 milliseconds and those that experience an acute QTc reaction with a QTc greater than or equal to 60 milliseconds from baseline after starting treatment with one or more QTc-prolonging drugs are at greatest risk for drug-induced arrhythmias.

Interplay Between Covid-19 and the RAAS

The perspective that ACE inhibitors, like ramipril and ARBs, like losartan may increase ACE2 expression, have raised concerns about their safety in patients with Covid-19. The interaction between the SARS-CoVs and ACE2 has been proposed as a potential factor in their infectivity by some researchers, and there are concerns about the use of these drugs may alter the ACE2 expression and attended by its fallouts [73]. Currently, there are insufficient data available to translate it into clinical practice, though studies are underway. On the other hand, it is held that an abrupt withdrawal of these drugs in high-risk patients, having heart failure, coronary heart disease and hypertension may result in clinical instability and adverse outcomes. Further, despite substantial structural homology between ACE and ACE2, their enzyme active sites are distinct and the ACE inhibitors and ARBs in clinical use do not directly affect the ACE2 activity. Thus, it is advisable that an ACE inhibitor or ARB should be continued in known or likely Covid-19 patients with stable condition [74].

The SARS-CoV-2 utilizes and affects ACE in multiple ways. Apart from its entry through the ACE2, SARS-CoV-2 subsequently down-regulate ACE2 expression leading to loss of its protective effects

in various organs leading to uncontrolled angiotensin II activity, which may potentiate organ injury in Covid-19. The continued viral infection and replication contribute to reduced membrane ACE2 expression as the ACE2 sites are taken up and damaged by subsequent viral invasions. The reduced ACE2 activity in the lungs results in unopposed angiotensin II accumulation and local RAAS activation, which leads to inflammation and neutrophil infiltration. In the experimental mouse models, exposure to SARS-CoV-1 spike protein induced acute lung injury, which was prevented by RAAS blockade. Similarly, in the Covid-19 patients having elevated levels of plasma angiotensin II, which correlated with viral load and degree of lung injury and the restoration of ACE2 through the administration of recombinant ACE2 appeared to reverse the devastating lung-injury process [73]. SARS-CoV-2 binding to ACE2 creates an acutely exaggerated proinflammatory background and it is advised that clinicians continue treating patients with ACE inhibitors and ARB [75].

Convalescent Plasma and COVID-19 Antibodies

Considering the potential options to treat Covid-19, there have been identified certain proteins in the host immune pathways which can be targeted for blocking viral replication by potential drugs or antibodies [76]. These include antiviral pathways in the innate immune response, such as the stress granule protein, G3BP1, which is an antiviral protein that induces the innate immune antiviral response. The passive antibody transfer from pooled convalescent patient sera is another obvious therapeutic option in severe SRS-CoV-2 infection. The conceptual framework for using it as treatment for Covid-19 has been outlined in two recent review papers [77,78]. To date, two small case series have been published about its use and effectiveness by Chinese researchers [79,80].

Footnotes

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2. Disclosures – None.
3. The Figures 1-3 in this Review Article are subject to Copyright by Dr Vinod Nikhra.

Competing Interests

The author declare that they have no competing interests.

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