Molecular Insights on the Pathophysiology and Treatment of COVID-19

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Abstract

The SARS-CoV-2 infection has been considered a global pandemic due to its widespread transmission and high rate of fatality. As of April 11, 2020, globally, there are 1.76 million confirmed cases of COVID-19, of which 108,281 people succumbed to the disease. In the absence of therapeutic intervention and a possible vaccine candidate, the spread of the disease and associated fatalities are on the rise. The epidemiological data indicate age and country-specific bias in the spread and severity of COVID-19. In this review, we discussed the recent update on the pathogenesis of SARS-CoV-2 among men and women, including children. Further, we also discuss the role of the cellular receptors and co-receptors used by the virus to enter host cells in the virus pathogenesis on differential infection among men and women. Further, highlighted the co-morbidity of COVID-19 with cardio-metabolic disease, and the potential treatments to control SARS-CoV-2 infection. Finally, we summarize the prospective treatment options that have been evaluated or are in the pipeline at different stages of clinical trials to fight against COVID-19.

Introduction to SARS-COV-2

SARS-CoV-2, which causes COVID-19 illness, represents the seventh member of the coronavirus family that infects humans and has been classified under the orthocoronavirinae subfamily. The SARS-CoV-2 forms a clade within the subgenus sarbecovirus (Zhu et al., 2020). Based on the genetic sequence identity and the phylogenetic reports, SARS-CoV-2 is sufficiently different from SARS-CoV, and it can thus be considered as a new betacoronavirus that infects humans. The SARS-CoV-2 most likely developed from the bat origin coronaviruses. Another piece of evidence that supports the SARS-CoV-2 is of bat origin is the existence of a high degree of homology of the angiotensin-converting enzyme 2 (ACE2) receptor from a diversity of animal species, thus implicating these animal species as possible intermediate hosts or animal models for COVID-19 infections (Wan, Shang, Graham, Baric & Li, 2020). Moreover, these viruses have a single, intact open reading frame on gene 8, which is a further indicator of bat-origin CoVs (Ren et al., 2020). However, the amino acid sequence of the tentative receptor-binding domain resembles that of SARS-CoV, indicating that these viruses might use the same receptor (Ren et al., 2020). The susceptibility to the infection with SARS-COV-2 and disease severity varied amongst individuals factoring age, sex, and health conditions. The SARS-COV-2 infection also varies based on symptomatic and asymptomatic infections, where the symptomatic include mild and severe infections. Thus, it is the rationale that the treatment/disease management of COVID-19 illness should be applied, taking in mind the mentioned groups of people and infection categories. Here, we discuss the epidemiology of the COVID-19 illness based on patient groups and the severity of the infection. Also, we discuss the role of the cellular receptors and co-receptors in virus infectivity, the co-morbidity with cardio-metabolic syndromes, the overlap between cardio-metabolic treatments and COVID-19 infection, and the potential pre and post exposure therapeutics, including those in clinical trials to treat the COVID-19 illness.

COVID-19 infection in children

Infections with coronavirus SARS-CoV-2 that appeared in December 2019 (COVID-19) in China have become a global public health threat worldwide (Rothan & Byrareddy, 2020). COVID-19 targets the lower respiratory airway causing severe acute syndrome resulting in increasing death cases among people. The epidemiological reports shown that the death cases of COVID-19 infection are higher in healthy or older adults compared to children. A study showed that among 44,672 COVID-19 confirmed cases, 549 (1.2%) were between 10–19 years, and 416 (0.9%) were between 0–10 years (Novel Coronavirus Pneumonia Emergency Response Epidemiology, 2020). About 4% of children were asymptomatic, 51% had a mild illness, and 39% had a moderate illness. Another study showed that the percentage of severe COVID-19 infection among children was 6% compared to 18.5% of adults(Dong et al., 2020). Generally, infected children show milder to asymptomatic COVID-19 infection. Adults with severe COVID-19 suffer from deadly pneumonia and insufficient supply of oxygen throughout the body, probably due to disruption of hemoglobin by SARS-CoV-2 as reported by several frontline clinicians from New York City; on the other hand, clinical reports showed the children are insusceptible to pneumonia caused by COVID-19 infection (2020; Huang et al., 2020; Li et al., 2020; Lippi & Mattiuzzi, 2020; Wang et al., 2020a). With the help of in-silico molecular modeling and docking analysis, it is predicted that Orf1ab, ORF10, and ORF3a proteins of SARS-CoV-2 binds with the heme of the beta chain of hemoglobin resulting in dissociation of iron from the porphyrin ring such studies still need further in vitro and in vivo investigations. The considerable variation in COVID-19 infection between children and adults raises a question that could help to understand the mechanism of COVID- 19 pathogenesis.

COVID-19 infection in women

Interestingly, the reports showed that the rate of COVID-19 infection is higher in men than women, and the severity of the illness is much higher in men. The percent of disease fatality in men is 2.8%, while in women, it is 1.7% (Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19) [Pdf] - World Health Organization; The Epidemiological Characteristics of an Outbreak of 2019 Novel Coronavirus Diseases (COVID-19) - China CCDC). An early study from China showed that of the 99 patients with COVID-19 pneumonia where the average age of the patients was 55·5 years, the average age for men was 67 years and for women was 32 years (Chen et al., 2020). Another report showed that of the total 55,924 COVID-19 confirmed cases reported in February 2020, the men comprised 51.1%, women comprised 21.6%, and the median age was 51 years (Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19) [Pdf] - World Health Organization).

Viral cellular receptor and the severity of COVID-19 illness

The binding of the viral spike protein to the specific cellular receptor on the membrane is the first step of viral infection, followed by fusion with the cell membrane. The lung epithelial cells represent the primary target of coronaviruses. The binding of the virus spikes initiates SARS-CoV entry into target cells via receptor-binding domain to the cellular receptor, which has been identified as ACE2 (Jaimes, Millet, Stout, Andre & Whittaker, 2020; Wan, Shang, Graham, Baric & Li, 2020). Importantly, the sequence of the receptor-binding domain in spikes of SARS-CoV-2 is similar to that of SARS-CoV. The sequence data shows similarities between SARS-CoV-2 and SARS-CoV and the recent reports strongly suggest that the entry of SARS-CoV-2 to the host cells is via ACE2 receptor (Hoffmann et al., 2020; Wan, Shang, Graham, Baric & Li, 2020). The ACE2 gene is mapped on the human X chromosome (Xp22) (Egan, 2013; Tipnis, Hooper, Hyde, Karran, Christie & Turner, 2000). The hormone 17 β -estradiol increased ACE2 protein, and gene expression in ovariectomized female rats with the renal wrap model of hypertension. It also prevented glomerular and tubular injury in this experimental hypertensive model (Ji, Menini, Zheng, Pesce, Wu & Sandberg, 2008). Previous studies on SARS-CoV reported that the binding of viral Spike (S) protein to ACE2 down-regulates the expression of ACE2, resulting in a diminished protective role of ACE2 and, subsequently, acute respiratory failure (Kuba et al., 2005). Furthermore, ACE2 expression is dramatically reduced with aging in both genders, (Xie, Chen, Wang, Zhang & Liu, 2006). Taken together, the malfunction or the down-regulation of the ACE2 receptor on the cell membrane during SARS-CoV-2 represents one of the leading causes of developing severe COVID-19 illness.

Additionally, ACE2 receptor is co-expressed with TMPRSS2, a cellular trans-membrane protease that cleaves the S protein of SARS-CoV and SARS-CoV-2 into two fragments S1, which is essential for virus attachment, and S2, for virus fusion into the target cells (Bertram et al., 2013; Glowacka et al., 2011; Hoffmann et al., 2020; Zmora, Moldenhauer, Hofmann-Winkler & Pohlmann, 2015). TMPRSS2 protein is expressed in many tissues, including the lungs. In lung tissues, TMPRSS2 protein is expressed primarily in the epithelial cells (Jacquinet, Rao, Rao & Hoidal, 2000; Lin et al., 1999). The expression levels of TMPRSS2 protein are regulated by levels of androgen and androgen receptors (Chen et al., 2010; Lin et al., 1999; Tomlins et al., 2008; Yu et al., 2010), suggesting sex-related expression levels of TMPRSS2 protein. Both women and children have a lower level of androgen and androgen receptors than men, and therefore, TMPRSS2 may play a critical role in the severity of COVID-19 pathogenesis in men. This pattern is followed when we looked at percentage of age and gender distribution of people died out of total deaths in United States due to COVID-19 (**Fig. 1**) (CDC, 2020). Where we saw a positive correlation in increase in % of people died out of total death due to COVID-19 with increase in age group distribution. In USA, the percentage of death of male is also higher compared to female COVID-19 patients. Thus, it could be possible that the expression levels of ACE2 and TMPRSS2 have an impact on virus infectivity and pathogenesis among different groups of individuals, considering the variation in the expression levels in older men compared to the women and children.

The possible role of viral secondary receptors and cell-to-cell virus transmission could have an impact on the severity and mortality of COVID-19 infection. Wang et al. reported that SARS-CoV-2 is able to infect T-lymphocyte cell lines that express a deficient level of ACE2 surface receptor, but failed to reproduce progeny viruses, which indicates some other cellular receptors may play a role in the cellular entry of the virus (Wang et al., 2020c). Currently, a clinical trial (NCT04275245) is going on to test the efficacy of Meplazumab against SARS-CoV-2.

Co-morbidity of COVID-19 with cardio-metabolic syndromes

The severity and mortality of COVID-19 infection is positively correlated with the co-morbidity of lung disease, diabetes, cardiovascular diseases, and cerebrovascular diseases. A study reported that 173 patients from 1,099 confirmed cases of COVID-19, had co-morbidities of hypertension (23·7%), diabetes mellitus (16·2%), heart diseases (5·8%), cerebrovascular disease (2·3%), and all of the 173 patients showed severe COVID-19 illness(Guan et al., 2020). Another report showed that of a group of 52 COVID-19 infected patients, 32 patients died with co-morbidities of diabetes (22%) and cerebrovascular diseases (22%)(Yang et al., 2020). Another study reported that of 140 severe cases of COVID-19, about 30% of the patients experienced chronic hypertension, and 12% had diabetes(Zhang et al., 2020a).

SARS-CoV-2 infection and ACE2 expression in cardio-metabolic patients

ACE cleaves angiotensin (Ang) I to form Ang II within the renin-angiotensin system (RAS). Ang II has been recognized as the main active peptide that is cleaved by ACE2, a homolog of ACE, to form Ang-(1-7). ACE2 has high catalytic efficiency, suggesting an essential role in preventing Ang II accumulation, while enhancing Ang-(1-7) formation (Kalea & Batlle, 2010; Wysocki et al., 2010). ACE2 alterations have been described in experimental models of hypertension and diabetic kidney disease, and ACE2 levels were found to be decreased in the setting of hypertension (Mizuiri et al., 2008; Soler, Wysocki, Ye, Lloveras, Kanwar & Batlle, 2007; Wysocki et al., 2006; Ye, Wysocki, William, Soler, Cokic & Batlle, 2006). ACE2 expression is dramatically reduced with aging in both genders, young-adult vs. old (Xie, Chen, Wang, Zhang & Liu, 2006). Thus, ACE2 overexpression improves pancreatic islet-cell function, cardiovascular health, blood pressure, and renal protective arm of the RAS (Ferrario, 2006). ACE2 has a therapeutic effect for diabetes, cardiovascular conditions, kidney disease, and several other conditions in which the overactivity of Ang II is undesirable. Previous studies on SARS-CoV reported that the binding of viral S protein to ACE2 down-regulates the expression of ACE2, resulting in a diminished protective role of ACE2 and, subsequently, acute respiratory failure(Kuba et al., 2005). Downregulation or malfunction of ACE2 leads to accumulation of Ang II, resulting in a significant reduction in insulin secretion from the pancreas as well as glomerular filtration rate in the kidney (Batlle, Jose Soler & Ye, 2010).

Aging decreased expression of the ACE2 which also leads to accumulation of Ang II levels, in turn affecting other body organs like the heart, pancreas, and kidney. These factors suggest that treatment with ACE2 activating compounds could help to enhance hypertension and diabetic kidney disease during infection. However, the impact of ACE2 activators/inhibitors on the COVID-19 illness require urgent investigation. The current therapies should be continued at this point of COVID-19 illness as the withdrawing of the ARB therapies may not be wiser since there is no clinical evidence for the interaction of the ARB therapies and COVID-19 illness.

Based on current reports and pieces of evidence, we hypothesize that for the severe COVID-19 illness, drugs that activate the ACE2 function could help alleviate the reduced expression of ACE2 due to virus infection. For example, both Xanthenone and resorcinolnaphthalein were found to increase ACE2 activity in a dose-dependent manner. Xanthenone showed a remarkable antihypertensive effect, as it acutely decreased blood pressure by a massive 71 mmHg, however, its long-term antihypertensive effect was only moderate. Xanthenone was also found to improve cardiac function and reverse myocardial, perivascular, and renal fibrosis (Hernandez Prada et al., 2008). Additionally, a chemical compound, diminazene aceturate (DIZE), has been promoted as a potential ACE2 activator to treat ischemia-induced cardiac pathophysiology, pulmonary hypertension, and ischemic stroke (Qi et al., 2013). Wysocki et al. tested whether a soluble human recombinant ACE2 (rACE2) may be used to decrease ANG II and increase ANG (1–7) levels in plasma and tissues, and whether rACE2 may be used to prevent ANG II-induced hypertension in mice. Interestingly, this study found that rACE2 infusion induced a dose-dependent increase in serum ACE2 activity, but had no effect on kidney or cardiac ACE2 activity (Wysocki et al., 2010).

Role of Bacillus Calmette-Guerin (BCG) vaccination in protection against COVID-19

Bacillus Calmette-Guerin (BCG) vaccine contains a live attenuated strain of Mycobacterium Bovis, which is globally used to prevent Tuberculosis (TB) (Luca & Mihaescu, 2013). In a recent epidemiological study, Miller et al. described that the severity of the COVID-19 pandemic is more devastating in countries that do not have a universal BCG vaccination policy (USA, Italy) compared to countries that implement BCG vaccination at birth as a part of their routine vaccine policy for new borns (China, India, Portugal). Several pieces of evidence suggest that BCG vaccination provides protection against various DNA and RNA viruses that causes lower respiratory tract infections including Influenza virus (Moorlag, Arts, van Crevel & Netea, 2019). It is probable that BCG vaccine mediated 'the trained immunity' plays a vital role in providing partial non-specific protection against SARS-CoV-2. The trained immunity involving innate immune memory confers protection against secondary infections independent of the adaptive immune response (Covian et al., 2019). It was reported that innate immune cells like macrophages and natural killer cells (NK cells) involved in the development of 'the trained immunity' are functionally reprogrammed through epigenetic changes mediated by NOD2 that involves histone methylation (H3K4me3) to develop non-specific protection against viral infections after BCG vaccination (Kleinnijenhuis et al., 2012). To understand BCG vaccine-mediated protection against SARS-CoV-2 infection and or reduction in the severity of COVID-19, two open-label clinical trial were initiated in Australia and Netherlands. These trials are recruiting frontline health care providers who are providing medical support to patients suffering from COVID-19 (NCT04327206 and NCT0432844). The final outcome of these trials will provide more insight into the molecular mechanism of BCG vaccine-mediated protection against COVID-19.

Prospective on the treatment/management of COVID-19 illness

Treatment of COVID19 illness should be customized based on the stage of infection and the health conditions. The early stage of virus infection involves viral replication resulting in tissue damage and immune system activation. At this stage, the body relies on the immune system to combat virus replication. Thus, antiviral treatment would be a promising avenue in addition to non-immunosuppressive pain and fever management drugs, such as acetaminophen.

For mild infection, the treatment should include antiviral drugs and moderate anti-inflammatory drugs. Auranofin is a useful anti-inflammatory drug that reduces cytokine production and stimulates cell-mediated

immunity (Walz, DiMartino, Griswold, Intoccia & Flanagan, 1983). It is a gold salt used in treating inflammatory arthritis and has antiviral activity (Roder & Thomson, 2015). Targeting specific, prominent cytokines in COVID-19 disease represents another method of controlling the acute inflammation. Several cytokine inhibitors could be useful in treating acute inflammation during severe COVID-19 infection. The cytokine inhibitors include: 1) Tumour necrosis factor (TNF)-α inhibitors (monoclonal antibodies infliximab, adalimumab, the TNF-α-receptor fusion protein etanercept). 2) the interleukin (IL)-1 inhibitor (anakinra) (Doan & Massarotti, 2005). 3) IL1β inhibitors (rilonacept and monoclonal antibodies canakinumab and gevokizumab) (Peiro, Lorenzo, Carraro & Sanchez-Ferrer, 2017). 4) IL6 inhibitor (tocilizumab), a humanized monoclonal antibody specific for the IL-6 receptor (IL-6R) (Hennigan & Kavanaugh, 2008). Since children experience such mild COVID-19 symptoms, the antiviral and pain management drugs can be used even at the mild infection.

The severe or late stage of COVID-19 illness involves a devastating inflammatory lung disorder due to cytokine storm that is associated with multiple organ dysfunction leading to high mortality. Therefore, targeting prominent cytokines like TNF-α, IL-6 could be useful. The malfunction of ACE2 due to SARS-COV-2 infection leads to accumulation of ANG II in the blood, especially in older men and patients with metabolic syndromes. Increasing ACE and decreased ACE2 activity in human lung epithelial cells contribute to lung injury (Wosten-van Asperen et al., 2011; Zhang et al., 2015). A very recent study described the transcriptional signature of the SARS-COV-2 infection showing that infection increased ACE expression and decreased ACE2 (Daniel Blanco-Melo & https://doi.org/10.1101/2020.03.24.004655). Thus, treatment with ACE2-activating compounds could be helpful to attenuate lung injury, hypertension, and diabetic kidney disease during the infection (some of these activators are mentioned above). A recombinant human ACE2 (GSK2586881) was used in phase IIa of the clinical trial to treat acute respiratory disorder syndrome. The use of twice-daily doses of GSK2586881 infusion resulted in a rapid decrease in plasma Ang II levels and an increase in Ang 1-7 and Ang 1-5 levels, as well as a trend towards a decrease in plasma IL-6 concentrations (Khan et al., 2017). Such treatment could be beneficial to COVID-19 patients at severe stage of disease. IL-6 concentrations have especially been noticed to be elevated at this stage of infection.

CytoDyn's, the manufacturer of leronlimab (A humanized IgG4 mAb that antagonist CCR5) claims that their clinical trial data from cancer patients indicate this mAb can block Treg and macrophages that translate into an immunomodulatory response. They also claim that in a pilot study at a New York City hospital, some of the severally ill COVID-19 patients responded positively after given leronlimab. The molecule reduces cytokine storm by lowering IL-6 and TNF-a. It also imparts immune restoration in the CD8+ T lymphocytes (2020, April 9.; 2020.). The manufacturer is working with US-FDA to initiate a clinical trial of eronlimab to treat COVID-19 patients. In **Table 1** , we included all ongoing trials related to therapeutic intervention against COVID-19 that have completed recruiting, are currently recruiting, or have yet to start recruiting patients. Hydroxychloroquine/ Chloroquine, in combination with Azithromycin is included in most of the trials. While Azithromycin is an antibacterial drug having anti-inflammatory actions, Hydroxychloroquine enters the lysosomes of malaria parasites, inhibits their ability of hemoglobin hydrolysis, and blocks their replication cycle (Fox, 1993). It also reduces the inflammatory response of immune cells by interfering with the dimerization of a and ß chain of MHC II complexes. It is likely that Hydroxychloroquine blocks fusion and entry of SARS-CoV and SARS-CoV-2 by raising the pH of endosomes (Vincent et al., 2005). It also altered the glycosylation pattern of ACE2, thereby it may reduce the binding affinity of SARS-CoV-2 with ACE-2, the primary receptor of viral cell entry (Wang et al., 2020b). Most of the other drugs that are at different stages of the trial are antiviral with the established mechanism of action against different RNA viruses, or are immunomodulatory agents mainly used to suppress the cytokine storm observed in most of the critically ill patients. Pulmonary edema due to inflammatory exudation is a joint presentation of critically ill patients of COVID-19 (Zhang et al., 2020b). Vascular endothelial growth factors (VEGFs) are a potent inducer of vascular permeability (Bates, 2010). Bevacizumab, a monoclonal antibody binds to VEGF to prevent angiogenesis. To prevent Pulmonary edema in severally ill patients, Bevacizumab may be a viable option and is considered in clinical trials. Tetrandrine isolated from traditional herbs from China has antiviral effects against Human Coronavirus OC43 (Kim et al., 2019), and is used in trials to treat COVID-19

patients.

Table 1: List of therapeutic interventions that are in use to fight against COVID-19 (as listed in https://clinicaltrials.gov as of April 10, 2020.) This table includes all ongoing trials related to therapeutic intervention against COVID-19 that have completed recruiting, are currently recruiting, or have yet to start recruiting patients.

Sl. #	Name of the drug	Mode of action
1	Hydroxychloroquine sulfate	Anti-parasitic drug
2	Chloroquine	Anti-parasitic drug
3	Azithromycin	Antibiotic/anti-inflammatory
4	Remdesivir	Adenosine nucleotide analogue prodrug having broad-spectrum antiviral
5	Lopinavir/ritonavir	Protease inhibitors
6	Ribavirin	A purine nucleoside analog antiviral drug
7	Camostat Mesilate	Protease inhibitor
8	Favipiravir	Broad spectrum RNA-dependent RNA polymerase (RdRp) inhibitor of R
9	DAS181	It cleaves sialic receptors from the airway epithelium that acts as entry r
10	Umifenovir	antiviral
11	Oseltamivir	Antiviral that inhibits viral neuraminidase enzyme
12	rhACE2	Block viral entry and decrease viral replication; anti-inflammatory
13	Losartan	Angiotensin II receptor blocker
14	Methylprednisolone	Glucocorticoid having anti-inflammatory and immunomodulatory effect
15	Ciclesonide	Glucocorticoid having anti-inflammatory and immunomodulatory effect
16	Bevacizumab	A VEGF inhibitor
17	Baricitinib	Janus kinase (JAK1 and JAK2 inhibitor) inhibitor; anti-inflammatory
18	Ruxolitinib	JAK1/2 inhibitor used to treat myelofibrosis
19	Sargramostim	Sargramostim binds to GM-CSF, that stimulates JAK2 STAT1/STAT3 s
20	Tocilizumab	A mAb competitive inhibitor of IL-6 & IL-6R binding
21	Sarilumab	A humanized mAb that blocks interleukin-6 (IL-6) receptor
22	Anakinra	Competitively binds with IL-1RI to inhibit elevated IL-1 mediated immu
23	Thalidomide	Immunomodulator, suppress TNF-α production
24	Mavrilimumab	A humanized mAb that blocks GM-CSF-R, developed to treat rheumato
25	Meplazumab	Humanized IgG2 mAb against CD147, have antiviral activity against SA
26	Leronlimab	A humanized IgG4 mAb that antagonist CCR5
27	Nivolumab	Check point inhibitor, a humanized IgG4 anti-PD1-R mAb
28	Interferon Beta-1B	Immunomodulator: Reduce production of pro-inflammatory cytokines, E
29	CD24Fc	Check point inhibitor with anti-inflammatory activity
30	Piclidenoson	anti-inflammatory
31	Colchicine	anti-mitotic drug with anti-inflammatory property
32	Tetrandrine	Ca channel blocker and anti-inflammatory
33	Anti-SARS-CoV-2 convalescent plasma	Infuse neutralizing antibodies from SARS-CoV-2 infected persons recover
34	BCG Vaccine	The trained immunity
35	Fingolimod	Binds to binds to sphingosine-1 phosphate receptor and blocks egress of T
36	Naproxen	Nonsteroidal anti-inflammatory drug (NSAID)
37	Anluohuaxian	Used to block pulmonary fibrosis and improving lung function
38	Peginterferon Lambda-1a	Not known
39	Tranexamic acid	Lysine analog inhibits activation of plasminogen. Used to prevent excessi

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Figure 1: Age and gender distribution of people died due to coronavirus disease 2019 (COVID-19) in United States as of April 20, 2020. The data is calculated based on total number of deaths (15251) as of April 20, 2020 that was received and coded by National Center for Health Statistics. (This do not represent all deaths as there is a lag between when death occur, and death certificate issued). Data source: CenSters for Disease Control and Prevention: National Center for Health Statistics. https://www.cdc.gov/nchs/nvss/vsrr/COVID19/.