AN OVERVIEW OF SARS-COV-2: COVID-19 EPIDEMIOLOGY, ORIGIN, AND PATHOLOGY

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Following SARS (years 2002-2003) and MERS (year 2012) outbreaks, humans have faced a new highly contagious virus, SARS-COV-2, which began late 2019 and resulted in a pandemic (COVID-19 pandemic). The disease has been spread in at least 216 countries, areas, or territories, and infected over 22 million people. Although, the virus is categorized as a member of coronaviruses and is closely related to SARS-CoV (the virus which cause SARS), many aspects of the virus and its pathogenesis are still unknown. Considering the lack of any approved treatment or vaccine, gaining fundamental knowledge about the virus might pave the way toward discovering a safe and effective medicine or vaccine. Thus, in this review, we have collected the most recent information about the novel coronavirus epidemiology, origin, structure, and replication cycle. Additionally, COVID-19 immunopathology, pathology, and pathogenesis are discussed.

ACKNOWLEDGMENTS

First and foremost, I wish to thank my wife, Dr. Tayebeh Anajafi, who has stood by me and has given me all her love, support, and help.

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DEDICATION

To My Dear Wife

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1. INTRODUCTION

Coronaviruses (CoVs), discovered in 1960 [1], are enveloped, non-segmented, positive-sense RNA viruses, which belong to the Nidovirales order. Nidovirales consist of three families of viruses, which are different in type, number, and size of structural proteins. The family members are Coronaviridae, Arteriviridae, and Roniviridae. Among these families, Coronaviridae has the largest genome (about 30 kilobase) and is divided into two sub-families: Torovirinae and Coronavirinae. Four genera of viruses are included in the Coronavirinae sub-family including alpha, beta, gamma, and delta coronaviruses[2].

Coronaviruses can cause a variety of diseases in various animals such as pigs, chickens, cows, dogs, and cats[2]. Gamma and Delta coronaviruses tend to infect birds; for instance, infectious bronchitis virus (IBV) can infect birds such as chickens. The virus replicates in chicken's upper and lower respiratory tract, reproductive system, and kidneys and causes flu like symptoms (such as watery eyes, snicking, rales and nasal discharge) in the birds[3-5].

While Alpha and Beta coronaviruses mostly infect humans causing illnesses with different severities. For instance, HCoV-HKU1 (Beta CoV), HCoV-OC43(Beta CoV), HCoV-NL63(Alpha CoV), and HCoV-229E (Alpha CoV) are associated with mild respiratory diseases in humans[1, 6]. However, the Severe Acute Respiratory Syndrome (SARS) outbreak caused by SARS-CoV (Beta CoV) in 2002-2003 with 9% mortality, and the Middle East Respiratory Syndrome (MERS) outbreak caused by MERS-CoV (Beta CoV) in 2012 with 40% fatality showed that these viruses can also cause severe respiratory diseases in humans. These outbreaks and the current novel coronavirus pandemic show that these viruses could spread from zoonotic sources and cause serious diseases in humans[2].

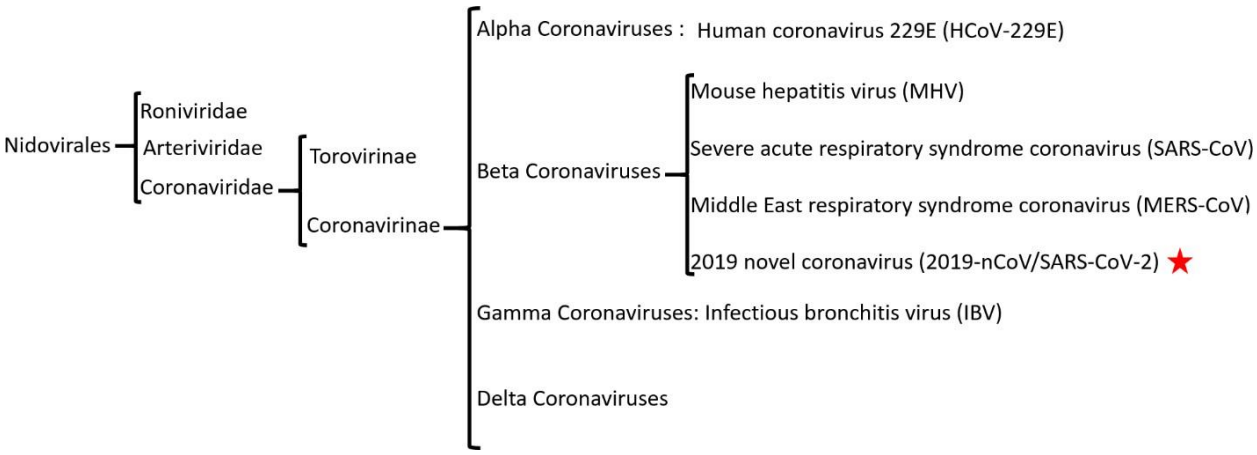

Figure 1. Coronaviruses Classification. Novel coronavirus (SARS-CoV-2) belongs to Beta Coronaviruses genus is marked by the red star. Note: The full phylogenic tree is much more complex with many members, however, only few members are shown here.

In December 2019, a new, highly contagious, severe respiratory disease was reported in Wuhan, China. Metagenomic RNA sequencing of a sample from bronchoalveolar lavage fluid of hospitalized patient revealed that a new RNA virus (SARS-CoV-2, also known as 2019 novel coronavirus, or 2019 nCoV) was the causing agent. Phylogenetic analysis of the SARS-CoV-2 genome (29,903 nucleotides) suggests that the virus belongs to Beta coronaviruses with 89.1% nucleotide similarity[7]. Case fatality rate of COVID-19(~4.3%), the disease which is caused by SARS-CoV-2, is believed to be less than that of SARS and MERS, but higher than the Influenza fatality rate, which is 0.1%. Due to lack of enough testing kits and presence of asymptomatic infected individuals, knowing the exact number of patients is impossible. Therefore, the accurate estimation of the case fatality rate of COVID-19 is not possible yet[8].

Herein, we have collected the most recent information about the novel coronavirus epidemiology, origin, structure, and replication cycle. Besides, COVID-19 immunopathology, pathology, and pathogenesis are discussed.

2. COVID-19: EPIDEMIOLOGY AND ORIGIN

On 12 December 2019, the first patient was hospitalized in Wuhan, China for severe respiratory complications. Following this observation, more patients with similar symptoms (fever, cough, shortness of breath, etc.) were admitted to hospitals in Wuhan[7]. Epidemiological investigations revealed that many of the first cohort of the patients were connected to a wholesale sea food market in Wuhan [7, 9-11]. The disease spread quickly to other parts of China by 13 January 2020, and epidemiological studies showed that person to person transmission of the disease was happening. On the same day, the first case of the disease was reported outside of China in Thailand from a person who traveled to Wuhan. By 23 January 2020 the virus was spread to six countries other than China, and the number of infected patients increased by 20 times. At that time, China implemented a quarantine in Wuhan. On 30 January 2020, the number of confirmed cases increased by 240 times (from 41 to 9826 cases)[12] and the World Health Organization (WHO) announced that the novel coronavirus epidemic is a public health emergency of international concern. On 11 February 2020, 441 positive cases in 24 countries outside of China, and the first mortality in Philippine were reported[12]. On the same day, the WHO officially named this illness Coronavirus disease 2019 (COVID-19), and named the new coronavirus SARS-CoV-2 (also known as novel coronavirus: nCoV). Later, on 03 March 2020, the WHO reported this outbreak a pandemic. According to a WHO report, as of 18 August 2020 at 11:15 pm (Central US time), this virus had infected 22,309,795 individuals in 216 countries, and it had killed 789,380 people.

Basic reproductive number (R0), the number of cases infected directly by one patient, was originally calculated to be 2.2-2.7 in the initial phase of the epidemy in China. However, a recent epidemiologic study has shown that the median is 5.7 (3.8–8.9;95% confidence interval)[13]. This value might be subjected to change due to the dynamic nature of the disease, socio-behavioral and

environmental changes during the outbreak [14]. Also, it is noteworthy that this number is much higher than that of the SARS-CoV (2.3–3.7 without intervention and <1 with intervention)[15-19] and MERS (0.5-0.92)[20]. The other complexity associated with SARS-CoV-2 is the 1-14 day incubation period (asymptomatic, but contagious).[21, 22]. These factors demonstrate why SARS-CoV-2 has spread in such a short time all over the world and is known as a highly contagious virus in comparison with other coronaviruses in this family.

Considering the width of the global health risks, the economic burden that the virus has had so far, and to avoid or diminish future risks in similar scenarios, it is important to recognize the origin of the virus. There are several theories around this subject and further research might help in finding the origin of this virus. Since many of the first group of patients were connected to a sea food market, where some live animals (such as snake, frog, birds and hedgehogs) and carcasses were available for sale, zoonotic transfer might be a strong theory. Genetic analysis of SARS-CoV-2 taken from patients, and the coronavirus taken from bats, snakes, and pangolins has shown great similarity[23-25]. This strengthens the hypothesis of the presence of an animal host (such as snake or pangolin) intermediate before human transmission. The same scenario exists for MERS-CoV and SARS-CoV. Another possible theory is that zoonotic transfer has happened directly to humans, and the virus has adapted while human to human transfer has happened. Other proposed theories include possible escape of the virus from a research laboratory, where it has gone through some mutations during passages, and intentional engineering of the new virus. However, the WHO has announced that none of these theories are confirmed yet[24] (Figure 2).

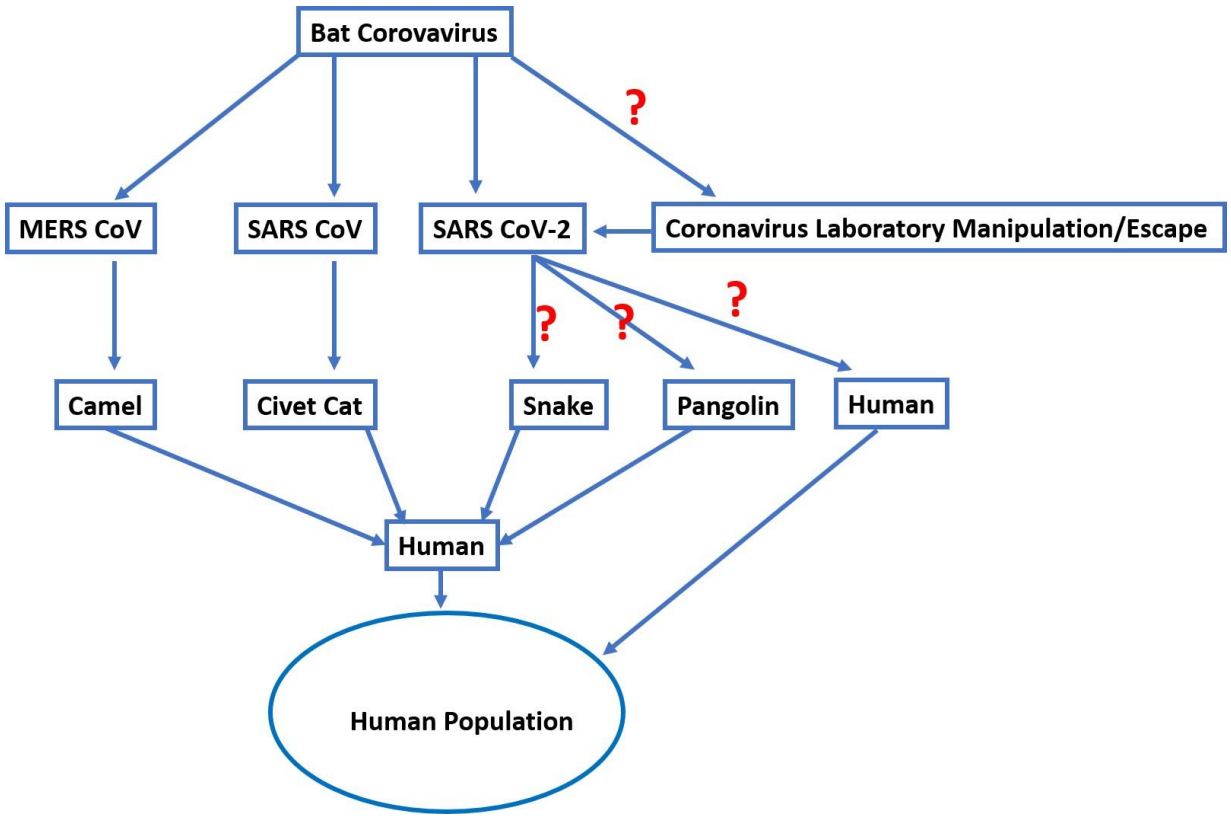

Figure 2**.** Schematic representation of theories hypothesizing the Coronaviruses origin. Note: WHO has not confirmed any of these theories.

3. CORONAVIRUSES STRUCTURE

Cryo-electron microscopy and cryo-electron tomography of Coronaviruses have revealed that these viruses are about 125 nm in size and have a spherical structure[26, 27]. Since the surface of these viruses is decorated with club-shaped spike proteins, which resemble crowns, they are called Coronaviruses. Other than the Spike proteins, these viruses contain three additional main structural proteins including Membrane, Nucleocapsid, and Envelope[2] (Figure 3).

The Spike protein is a trimeric, class I fusion glycoprotein, which facilitates the host receptor binding, and following a cascade of events, membrane fusion and entrance[2, 28]. Hence, this protein is one of the main targets for designing vaccines and therapeutic neutralizing antibodies (Figure 4)[28, 29]. Each monomer of the protein consists of two subunits: S1, which is essential for attachment of the virus to its host, and S2, which mediates membrane fusion. The S1 protein consists of N-terminal domain and C-terminal domain. Depending on the type of coronavirus, one of the two domains acts as the receptor binding domain. For instance, both SARS-CoV and MERS-CoV use the S1 C-terminal domain as the receptor binding domain. However, in mouse hepatitis virus, the N-terminal domain is associated with the receptor binding activity of S1 protein[28]. Recent studies have shown that SARS-CoV and SARS-CoV-2 receptor binding domains have about 74% similarity. Thus, there are some structural differences between these domains[28, 30]. Although both viruses use human angiotensin-converting enzyme 2 (hACE2) as their human cell receptor, SARS-CoV-2 binding to this receptor is stronger than that of SARS-CoV[28-31]. The S1 subunit binds to the cell receptor, and the S2 subunit anchors the spike protein to the virus. Since S protein is a fusion protein, cleavage by proteases is essential for its activation. One cleavage (known as priming) happens between the S1 and S2 subunits, and the other cleavage happens within the S2 subunit (known as S2´ cleavage site). In different viruses, this cleavage is

mediated by various enzymes such as furin, transmembrane protease serine protease-2 (TMPRSS-2), transmembrane protease serine protease-4 (TMPRSS-4), trypsin, human airway trypsin-like protease, and cathepsins[28]. Due to the presence of a furin cleavage site between the S1 and S2 domains of SARS-CoV-2, it is hypothesized that this cleavage happens by furin in this virus[32]. However, in a recent study it was shown that transmembrane protease serine protease-2 primes the virus entry and a known inhibitor of this serine protease (camostat mesylate) can block the viral entrance[33]. Hence, the priming enzyme in the SARS-CoV-2 virus remains controversial[28]

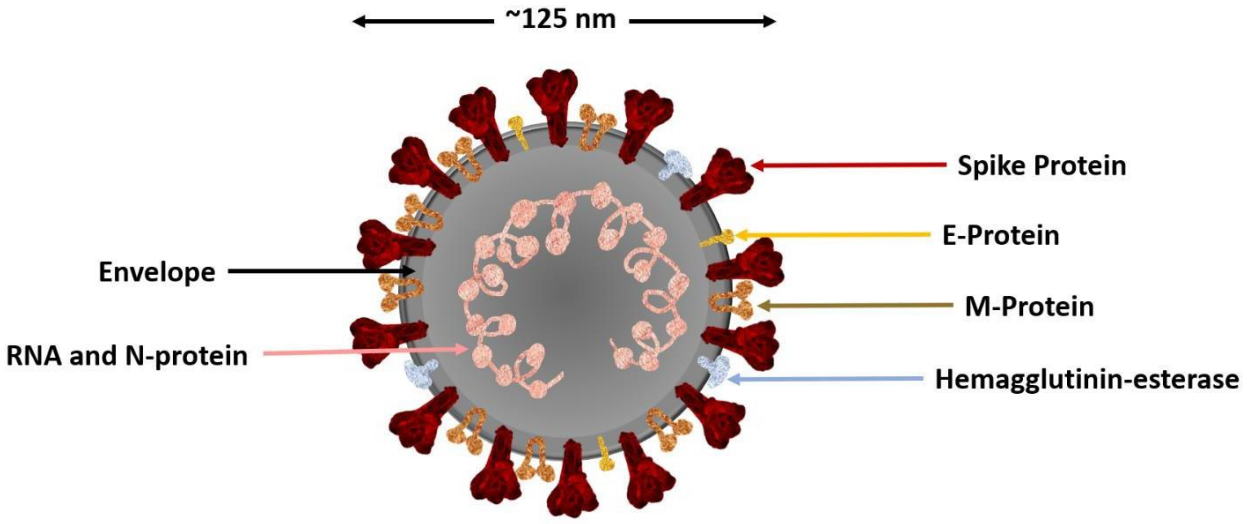

Figure 3. Schematic representation of Coronaviruses structure.

M (Membrane) protein is a small (~25–30 kDa) transmembrane glycoprotein, which is the most abundant structural protein in coronaviruses. This protein plays an important role in assembly and formation of the virus particles[2, 34].

E (Envelope) protein (~25–30 kDa) is another structural protein in coronaviruses, which is associated with the viral assembly, release, and pathogenesis. This protein also acts as an ion channel in some of the known coronaviruses[2].

N-protein (Nucleocapsid) is a highly phosphorylated protein which has high affinity toward viral RNA and encapsulate it. This protein also interacts with M-protein to help in viral genome assembly[2, 34].

Hemagglutinin-esterase (HE) protein is another structural protein in beta-coronaviruses. This protein has acetyl-esterase activity and is believed to collaborate with S protein in host cell entrance[2].

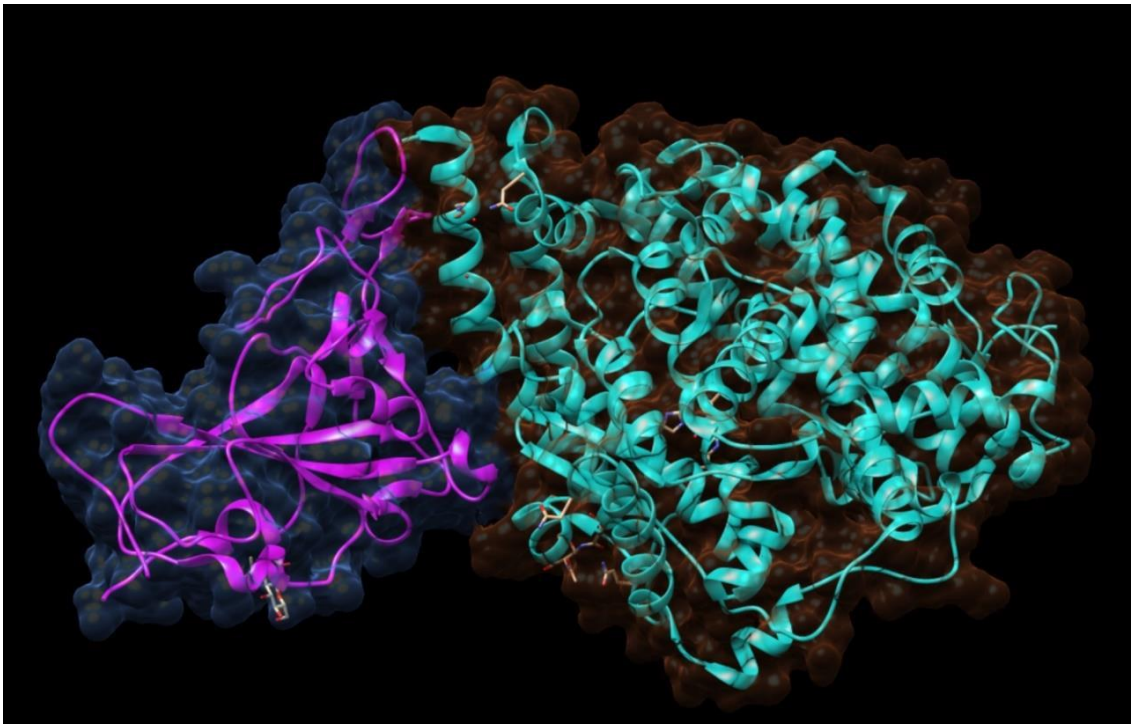

Figure 4. Schematic structure of SARS-CoV-2 spike receptor binding domain (purple) complexed with ACE2 receptor (cyan). PDB, ID: 6LZG.

4. SARS-CoV-2 REPLICATION CYCLE

SARS-CoV-2 uses hACE2 receptor to enter its host, and whether other receptors can be used by this virus for cell entrance is not known yet. This receptor is expressed by Type II alveolar cells in the lungs[21]. It should be noted that it is controversial whether hACE2 is solely expressed by Type II alveolar cells, or if these cells have the highest expression level among lung cells[35, 36]. Upon binding of the virus to the host cell receptor, via S protein, viral entry takes place via endocytosis or viral-cell membrane fusion. Following virus entrance, viral RNA releases into the cell's cytoplasm. Then, non-structural viral proteins, which are essential for RNA synthesis, such as RNA replicase–transcriptase complex, get translated. This step is followed by RNA replication and translation of structural proteins. Translation of nucleocapsid proteins occurs in the cytoplasm; however, S, M, and E-proteins get translated by the ribosomes, which reside on the endoplasmic reticulum. Nucleocapsid proteins, which are associated with the viral RNA in the cytoplasm, fuse with the virion precursor and then the mature virus gets transferred to the host cell surface by the Golgi Apparatus. Finally, the virus releases from the cell by exocytosis and infects a new host[37] (Figure 5).

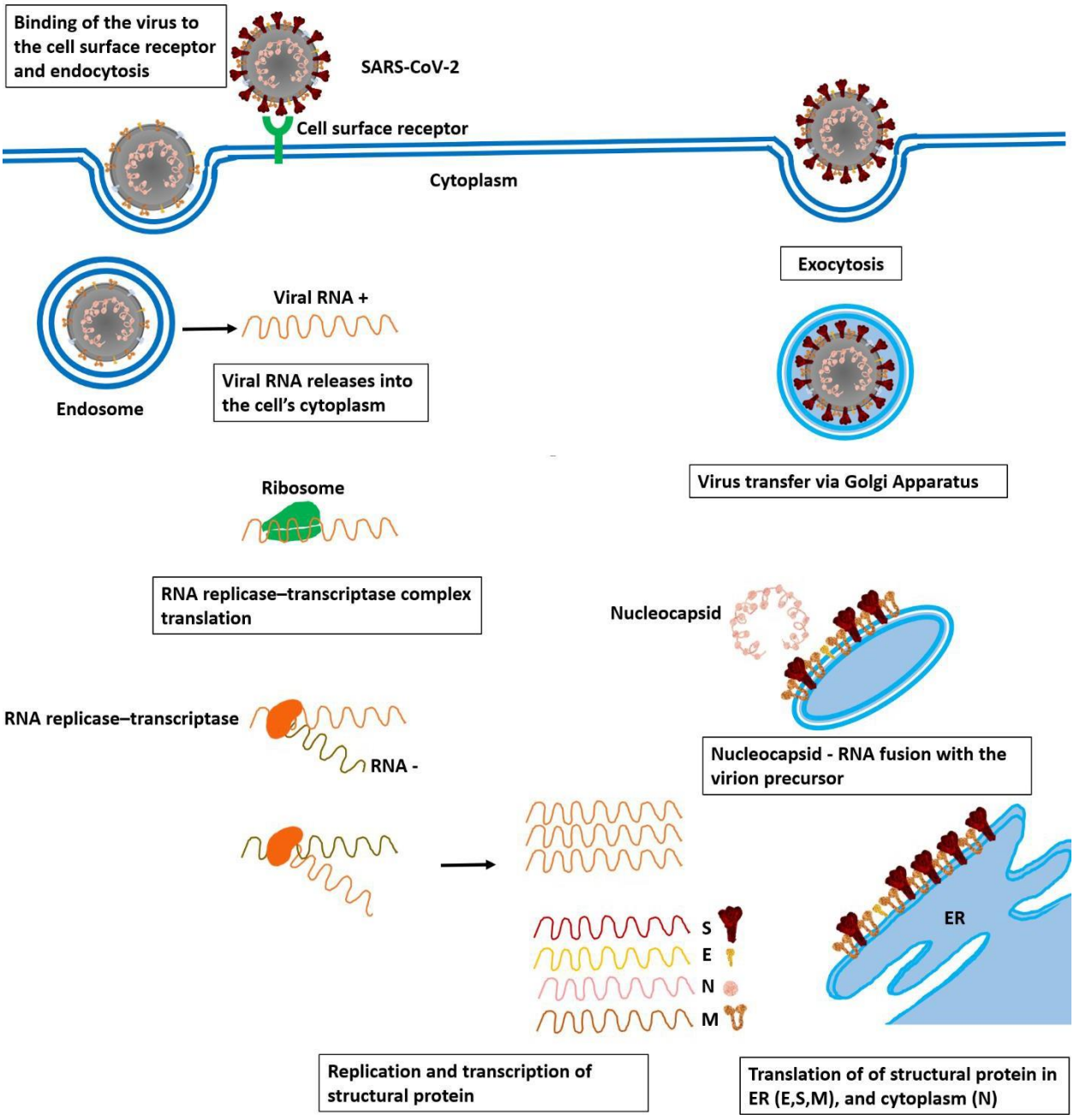

Figure 5. Schematic representation of SARS-CoV-2 replication cycle. ER: Endoplasmic Reticulum, E: Envelope protein, S: Spike protein, M: Membrane protein, N: Nucleocapsid.

5. COVID-19 PATHOLOGY

In COVID-19, the primary route of infection is thought to be close contact with infected individuals through their respiratory droplets while they talk, sneeze, or cough[21, 22]. As respiratory droplets and aerosols are considered the main route of transmission, this topic and use of personal protective equipment (e.g. mask) to mitigate the spread of the virus is discussed in a separate section (Using PPE and Practicing Social Distancing in Response to the Current Pandemic) of this article. Detection of SARS-CoV-2 in fecal, urine, saliva, and blood samples of some patients confirms the possibility of other routes of disease transmission, such as fecal-oral transmission[22, 38-41]. For example, in one study, 29% of fecal specimens taken from COVID-19 patients showed positive results for presence of live virus, while 93% of bronchoalveolar lavage fluid specimens were positive for the presence of live virus in the same study [42]. In a separate study, 52% of fecal samples taken from patients with gastrointestinal symptoms, and 39% of fecal samples taken from patients without gastrointestinal symptoms were positive for the presence of the live virus [43]. Considering the COVID-19 pathogenesis (discussed in later sections), presence of the live virus in fecal specimens might be related to whether the virus has had the chance to get to the gastrointestinal tract, or it has been removed by the immune system before it reaches this organ.

Most common symptoms are fever, myalgia, fatigue, cough, sore throat, shortness of breath, chills, repeated shaking with chills, and new loss of taste or smell (anosmia). Other common symptoms in COVID-19 patients are lymphopenia, pneumonia with abnormal chest CT, and dyspnea. Furthermore, in severe cases, higher levels of cytokines (such as IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, and TNFα) are observed. Less common symptoms include sputum production, hemoptysis, gastrointestinal track symptoms like diarrhea, headache, and

ischemic changes in toes[21, 39, 44-46]. It is estimated that about 5% of patients have severe respiratory symptoms with lung injuries, which might lead to multiorgan failure and even death [47]. A healthy immune system is essential for fighting pathogens, thus immunocompromised people (such as HIV patients, cancer patients under certain cancer therapeutics, individuals with organ transplantation), people with weakened immune systems (such as elderly, people with diabetes or obesity), are at higher risk of developing a severe form of the disease. Besides, people who already have lung diseases might suffer from lung inflammation, which can be worsened by SARS-CoV-2 infection. In COVID-19 patients with cardiovascular complications, fever (which can increase the heart rate) coupled with low oxygen levels and higher possibility of clot formation can bring an extra burden on these patient's heart and cause serious illness. It has also been declared by the CDC (Center of Disease Control and Prevention) that older adults, immunocompromised patients, people with underlying diseases such as heart and lung complications, diabetes and obesity are more prone to developing a more severe form of COVID-19.

6. COVID-19 IMMUNOPATHOLOGY

6.1. Innate Immune System

Innate immune system is the first line of defense against new pathogens including coronaviruses. In SARS-COV infection, it is suggested that Toll-like receptor (TLR)-2 might detect the virus S protein. Since SARS-COV-2 S protein is structurally similar to that of SARS-COV, then TLR-2 might also detect SARS-COV-2 S protein[48]. Coronaviruses which are close to SARS, usually enter the host cells through receptor mediated endocytosis. Thus, their RNA will be released inside the cell's cytoplasm. The RNA then will be detected by cytosolic RNA sensors such as retinoic acid-inducible gene I (RIG-I)-like receptor and melanoma differentiation-associated gene 5 (MDA5). Thus, S protein and viral RNA might be the most significant pathogen associated molecular patterns (PAMP) in SARS-related coronaviruses[48]. Following this recognition, transcriptional factors like NF-κB and IRF3 get activated and translocated to the cell nucleus to mediate transcription of proinflammatory cytokines such as type I interferon (IFN). Upon interaction of type I IFN with its receptor (interferon-α/β receptor: IFNAR) downstream signaling activates the JAK-STAT pathway which leads to phosphorylation of STAT1 and STAT2 by JAK1 and TYK2 kinases. Finally, a protein complex of STAT1-STAT2 -IRF9 translocates to the cell nucleus and induces INF-stimulated genes (ISGs) transcription (Figure 6). Transcription of ISG genes results in production of antiviral, antiproliferative, and immunomodulatory proteins[49]. In early stages of viral infections, the mentioned innate immune response is usually effective in viral replication suppression and induction of adaptive immune response[21].

In some coronaviruses-derived diseases such as SARS-CoV and MERS, the innate immune response (discussed above) is suppressed by either structural or accessory viral proteins. The viral modulatory actions might affect various points of immune response such as interfering with PAMP

recognition, IFN transcriptional factors nuclear translocation, IFN signaling, etc. Since SARS-CoV-2 is closely related to SARS-CoV and MERS-CoV, it is speculated that this virus also uses similar strategies to dampen the innate immune response[21, 50]. However, in a study which was performed on 24 critically ill COVID-19 patients, overly suppressed innate immune response was not detected[51]. Thus, this topic is controversial at this point, and more research can shed light on the possible innate immune evasion strategies of SARS-CoV-2.

In COVID-19 patients, elevated levels of neutrophils, and cytokines like IL-6, c-reactive protein, IP-10, MCP-1, MIP-1A, and TNFα are reported. Furthermore, an increased ratio of

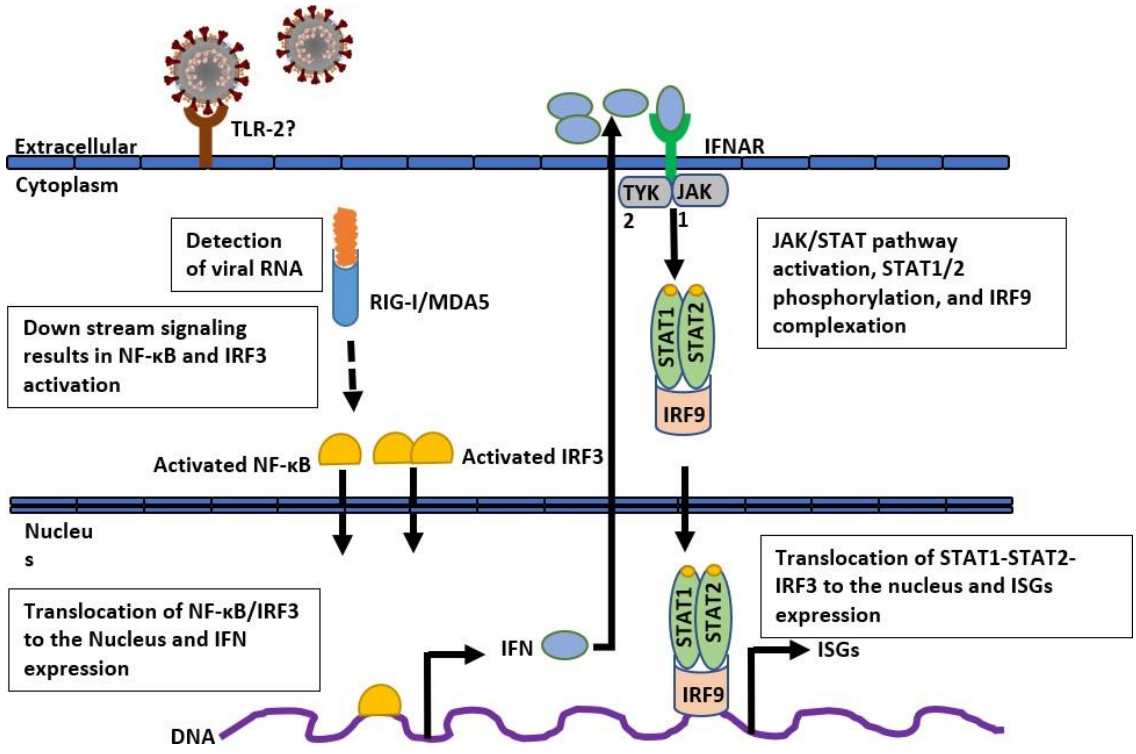

Figure 6. Schematic representation of IFN and ISGs transcription in coronavirus infected cells. Upon receptor mediated endocytosis of virus (maybe by TLR-2), presence of the virus is detected by cytosolic sensors. Downstream signaling results in activation and translocation of transcriptional factors (NFκB/IRF3) needed for IFN transcription. The secreted IFN interacts with IFNAR, and JAK/STAT pathway gets activated and ISGs transcriptional factor (STAT1-STAT2-IRF9 complex) translocate to the nucleus and transcribes the ISGs.

neutrophils to lymphocytes is related with the severity of the disease[7, 9, 44]. In critically ill SARS and MERS patients, it is suggested that a delayed and dysregulated type I IFN response promotes an influx of neutrophils and monocyte-macrophages to the lungs leading to acute respiratory distress syndrome. Similarly, the 1-14 asymptomatic days (incubation period) in some COVID-19 patients, while they are contagious, might be due to the delayed type I IFN response. Moreover, the fact that people with underlying diseases and elderly are more prone to developing the sever form of the disease, might be an indication of their weaker innate immune system, which fails to contain the virus in the early stages of the disease[21]. Similar to SARS and MERS patients, the presence of high levels of proinflammatory cytokines in COVID-19 patient's systems, might be an indication of a type of cytokine storm occurrence in severely ill COVID-19 patients[21, 52].

6.2. Cytokine Storm

In severely ill Influenza patients, the virus is detected by pattern recognition receptors of lung epithelial cells, macrophages, and dendritic cells, which leads to expression of (therefore these cells express) large amounts of cytokines. Similarly, it is believed that cytokine storm might be a major player in pathogenesis of SARS-COV-2, but it is not clear where the cytokine storm starts[47].

Secreted proinflammatory cytokines lead to inflammation in the primary site of infection and spread to other parts of body through systemic circulation. Local inflammation in the affected organ can lead to organ dysfunction by increasing the blood flow and subsequently increasing the population of leukocytes and other blood proteins in the extracellular environment. This can lead to edema and a decrease in the tissue perfusion[49]. Following cytokine storm, a healing mechanism, which is accomplished by fibrosis[53], may lead to organ damage[49]. Similar

pathogenesis might occur in some patients infected by SARS-CoV or Influenza, where the lung damage might lead to acute respiratory distress syndrome[49].

As mentioned, the inflammation might spread throughout the patient's body by systemic circulation. These patients usually suffer from systemic sepsis complications such as hypotension (low blood pressure), hyper- or hypothermia, leukopenia, and thrombocytopenia (low levels of platelets)[49, 54]. In a similar fashion, recent studies on severely ill COVID-19 patients has revealed that these patients show symptoms of septic shock ("viral sepsis") including cold extremities, weak peripheral pulses, microcirculation dysfunction, sever lung injury, and complications associated with the liver and kidneys[55]. Besides, lung tissue damage such as formation of hyaline membranes and thickening of the alveolar walls, as well as influx of mononuclear and macrophages in the lung extracellular environments in COVID-19 patients are other symptoms indicating that a cytokine storm has happened in the patient's body[47].

6.3. Adaptive Immune System

The innate immune responses to viruses, such as cytokine's secretion and recruitment of macrophages, initiate the adaptive immune responses. Analysis of lung biopsy samples taken from a severely ill COVID-19 patient have shown that mononuclear cells (mostly lymphocytes) infiltrate interstitial spaces in the lungs. Besides, Flow cytometry of patient's peripheral blood has shown that number of CD4+ and CD8+ T cells count are reduced[56]. Thus, it has been suggested that one of the reasons for the lymphopenia in most of COVID-19 patients is that the lymphocytes have been recruited to the lung tissue in the war against the virus[57]. In SARS it has been shown that other than lung epithelial cells, the virus is able to infect immune cells such as T lymphocytes, macrophages, and monocyte- derived dendritic cells[58-62]. Therefore, lymphocyte cell death has been related to the lymphopenia in the SARS-CoV patients[57, 58]. Not many of the immune cells

in the lungs express the ACE2 receptor[63]. Although SARS-CoV is genetically the closest human virus to the SARS-CoV-2, the ability of the novel virus to infect the immune cells remains unclear at this time[21] (Figure 7).

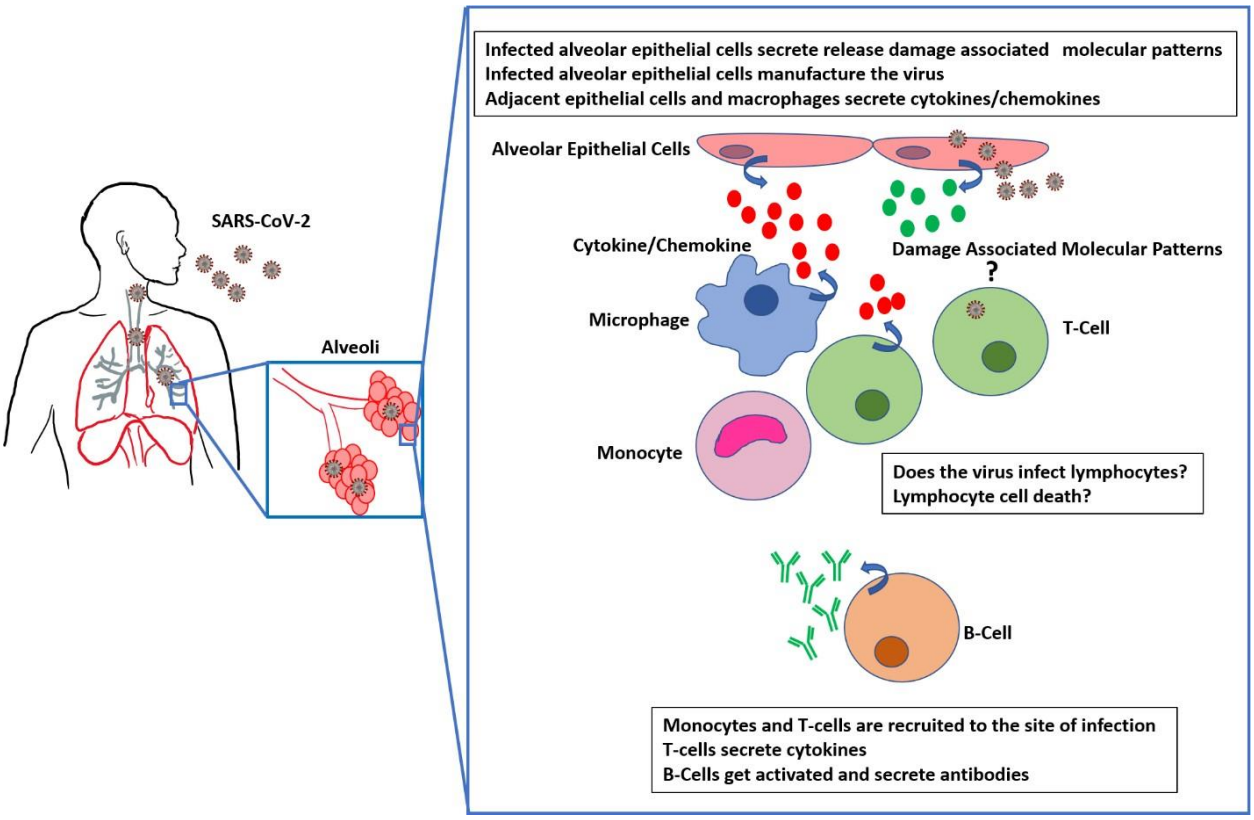

Figure 7. Immune response to SARS-CoV-2 infection. SARS-CoV-2 infects alveolar epithelial cells (Type II alveolar cells). The infected cells are highjacked by the viral RNA and manufacture the virus. The infected cell secrete damage associated molecular patterns which is detected by adjacent epithelial cells. The epithelial cells and alveolar resident macrophages secrete proinflammatory cytokines/chemokines. These molecules attract macrophages, monocytes and T-cells to the site of infection. Also, T-cells secrete cytokines, and this increases the inflammation. It is not clear whether the virus can infect the T-cells causing T-cells death and lymphopenia or not. Helper T-cells initiate B-Cell activation and the B-cells finally would produce virus specific antibodies.

In SARS and MERS patients, seroconversion occurs 4-14 days and 2-3 weeks after infection, respectively[64, 65]. Besides, it has been shown that anti-SARS-CoV IgG antibodies

and neutralizing antibodies peak 4 months after disease initiation, and they are detectable in SARS patient's serum at least 16 months after onset of the disease. However, MERS-CoV specific antibodies titer decline more quickly in MERS patients serum and persist for at least one year after infection[65]. In most of COVID-19 patients' serum, anti-SARS-CoV-2 IgM (about 80% of cases) and IgG (about 90% of cases) antibodies are detected. [9, 66, 67]. Since IgM antibodies are the first antibodies which are secreted By B cells after acute infection (first humoral defense mechanism), thus it is expected that these antibodies are detectable in patient's serum shortly after infection. However, it is expected that IgG antibodies which are normally produced after IgM, and have more affinity to the antigen, be detected later during the infection. The level of IgM and IgG antibodies has been analyzed in COVID-19 patients[66]. The analysis revealed that on average IgM is detected three days after infection and its levels increase slowly during the first week of infection, peaks after two weeks, and decline subsequently. As expected, the IgG levels start to increase after IgM production, where on average, it will be detected after first week of infection, peaks after three weeks and will remain in high level at least for 48 days (course of the study). Thus, in case of COVID-19 infection, detection of IgM antibodies in individuals might report presence of recent infection, while background levels of IgM and presence of IgG antibodies indicates that the infection has started awhile back, or the person is recovered. Whether antibodytiter is correlated to severity of the disease[68] in COVID-19 patients is controversial. For example in an study on 338 COVID-19 patients, it has been shown that in mild COVID-19 infections, usually IgG levels remain high and IgM levels decreases over time, whereas in critically ill patients (probably because of immunodeficiency) the IgM levels are higher, and IgG levels are lower than that of mild infections[66]. However, in another study it is shown that these antibody levels are not predictors of COVID-19 severity[69]. The reason for this discrepancy might be

because of the differences in methods that have been used for measuring the antibodies. In the former study chemiluminescence immunoassay was used for measuring the antibody levels, while in the latter one proteome array method was used for the assessment.

It is noteworthy that presence of antibodies in recovered patient's blood does not necessarily confer immunity to these individuals[70]. Immune system should be able to destroy (e.g. employing the natural killer cells, and/or T-cells) or neutralize (by neutralizing antibodies: antibodies which can bind to the pathogen and inactivate it) a pathogen in order to be able to control infection. Furthermore, the level of the antibodies and T-cells is an important factor in containing the pathogen. Another important factor is half-life of the antibodies which varies among different isotypes. For instant IgM has a half-life of 5-6 days[71], whereas IgG's half-life is longer, due to recycling by FcRn receptor[72], and can be up to three weeks[73]. A significant feature of immune system is its ability to maintain the memory of the infecting pathogen using memory B and T cells which can mount a quick immune response upon a recurring infection[67, 74]. In MERS and SARS patients presence of memory T-cells have been demonstrated post infection[75]. However, in case of COVID-19 more time is needed to investigate whether long-term humoral immunity (memory B and T cells), against SARS-CoV-2 can be developed upon first infection, and how long this immunity will persist in the patient's system.

7. COVID-19 PATHOGENESIS

Based on the collected information from both recovered and deceased COVID-19 patients, there are some hypotheses around the disease pathogenesis in various stages of the disease. It is hypothesized that after infection of type II alveolar lung epithelial cells, SARS-CoV-2 can enter the blood circulation, and thus get the opportunity to infect other organs that express ACE2 receptors, such as the kidneys, heart, and gastrointestinal tract. This might be the reason for presence of the virus in patient's urine and fecal samples[45] and multi-organ failure. It is also speculated that in severely ill patients, defected epithelial-endothelial barriers in lungs might help the virus to reach capillary endothelial cells. Thus, the virus might attack the endothelial cells and access the peripheral blood. This might be a reason for the abnormal coagulation, which has been repeatedly reported in critically ill patients[47]. However, in patients with strong immune systems, the virus would be contained in the lungs and get cleared by the immune system in initial stages of the disease.

8. COVID-19 SYMPTOMS PATHOPHYSIOLOGY

Hyposmia (decrease or loss of sense of smell) is reported to be associated with olfactory bulb involvement after SARS-CoV-2 infection. Furthermore, in a recent study (pre-print) it has been shown that ACE-2 receptor is expressed in Hypothalamus[76], and another study suggests that MRI data from two COVID-19 patients might be indicative of Hypothalamus involvement after SARS-CoV-2 infection[77]. Thus, following nasal infection and olfactory bulb involvement, the virus might be able to travel to the Hypothalamus. Although the fever associated with COVID-19 might be a systemic inflammatory response to the infection, this symptom and other reported symptoms such as hypo/hyperthermia might be associated with neurological complications and Hypothalamus involvement[78].

It has been shown that skeletal muscle cells express ACE-2[79]. Moreover, another study reports that lactate level is increasing in some COVID-19 patients[80]. Thus, it is hypothesized that skeletal muscle cells damage due to SARS-CoV-2 infection leads to an increase in lactate dehydrogenase and subsequently lactate level. This increased lactate level may be the reason for Myalgia in COVID-19 patients[81].

There have been some hypotheses about possible mechanism of thrombocytopenia reported in some cases of COVID-19. 1) SARS-CoV-2 might be able to infect bone marrow cells and thus interfere with production of platelets. 2) Cytokine storm associated with COVID-19 might interrupt platelet synthesis by damaging the bone marrow cells. 3) Production of autoantibodies following infection and clearance of platelets by the immune system[82].

Systemic inflammatory response associated with sever COVID 19 may cause vasodilation, capillary leak and hypotension[83]. Other symptoms such as lymphopenia, and lung damage (following cytokine storm which causes shortness of breath) are discussed earlier.

9. USING PPE AND PRACTICING SOCIAL DISTANCING IN RESPONSE TO CURRENT PANDEMIC

Although aerosol-generating procedures (AGPs) such as intubation, ventilation, airway suctioning and cardiopulmonary resuscitation (CPR)[84] are possible COVID-19 rout of transition especially in health care facilities, it is believed that respiratory droplets and direct (kissing, hand shaking, etc.) or indirect (fomites) contact are the most significant cause of the disease transmission[85]. Aerosols are infectious particles with diameter of <5-10 μm which can travel a long distance (~8 m but varies by environmental factors such as air turbulence), remain alive in air for some time (SARS-CoV-2 can remain viable for ~3 hours in aerosols) and can reach the lower respiratory tract. While droplets are generally bigger particles which can travel a shorter distance (<2 m), settle down quicker, and contaminate the proximity of the infection source (for instance SARS-CoV-2 can survive on plastic surfaces up to 3 days). Thus, in case of COVID-19 transmission, often close contact is defined as being close (< 2m distance) to an infection person for > 15 minutes. But this definition can be argued by studies which have shown that aerosols can even be produced by talking, singing, sneezing and coughing. These activities can produce particles with different sizes (0.01-500 μm). Some of the bigger droplets can evaporate in the air and form “droplet nuclei” which are vehicles for airborne transmission of the pathogen. For instance, it has been shown that sneezing and coughing can produce a cloud of particles with various sizes which can travel for 7-8 meter[86] (Figure 8).

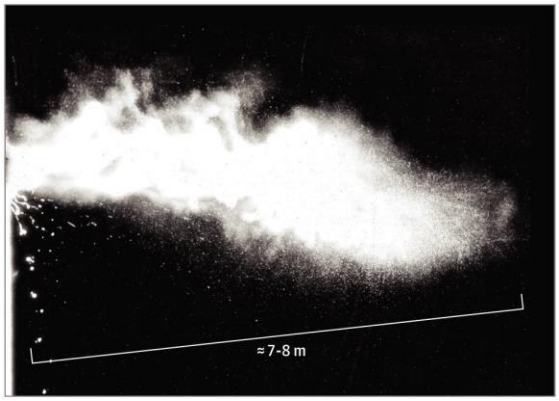

Figure 8. Sneezing and coughing can produce multi-size particles which can travel for 7-8 meters. Picture is taken from: Antimicrob Resist Infect Control, 2020. **9**(1): p. 100 [86].

Based on the current information, discussed above, to decrease rate of COVID-19 transition, practicing social distancing, wearing face masks, frequent thorough hand washing (for at least 20 second), and sanitizing common surfaces is recommended by CDC. The idea is to avoid virus carrying droplets, and droplet nuclei to reach susceptible individuals. CDC recommends keeping at least 2-meter distance with others whenever it is necessary to be present in public settings. Furthermore, using face mask is a physical barrier which can prevent respiratory droplets from traveling outside (Figure 9). Due to the presence of asymptomatic (people who are infected but will not show any symptoms), and pre-symptomatic (people who are infected with the virus but will show symptoms later) people in the society, all people over 2 years old (except those who have trouble in breezing or the ones without autonomy) are recommended (by CDC) to wear face mask when are in public settings. N95 respirators and surgical masks are considered as personal protective equipment. They can protect the wearer from pathogens. However, N95 respirators and surgical masks are not recommended to be worn publicly, because these are essential PPEs which are needed for protection of healthcare workers. Although masks with exhalation valves or vents can protect the wearer, but respiratory droplets can pass through the vent and transmit the disease. Thus, these masks are not recommended in coronavirus source control[85-89].

Figure 9. Face mask can prevent coronavirus containing droplets to travel out. Picture is taken from: https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/cloth-face-cover-guidance.html [90].

10. COVID-19 VACCINE DEVELOPMENT

Since there is not any specific treatment available for COVID-19, some scientists believe that a safe and effective vaccine might be a key to get control over COVID-19. Hence, vaccine development against SARS-CoV-2 is a hot debate all over the world. Based on “Faster Cure” (a center in Milken institute) vaccine tracker information[91], as of 18 Aug 2020 there are 202 vaccines under development all over the world. Various strategies are being used for vaccine development (Figure 10). The most significant strategies are summarized below.

10.1. DNA-Based Vaccines

DNA- based vaccine is a fairly new strategy for vaccine development. In this strategy an engineered plasmid is used to deliver immunogenic antigen gene(s) to the individual’s tissues. The idea is institute production of antigens following inoculation. Benefits of using this method includes absence of an infectious agent, ease of large-scale manufacturing and high stability of the product[87, 92]. Although no DNA vaccine is commercialized for human immunization, this method is evolving quickly, and several companies are using this strategy to develop a COVID-19 vaccine. For instance, Inovio has already started a Phase I clinical trial on its DNA-based potential COVID-19 vaccine[91].

10.2. Inactivated Virus Vaccines

Inactivated viral vaccines use a killed virus (killed by heat or chemical treatment)[93]. These vaccines would not produce an immune response as strong as that of live viruses, thus these vaccines usually need booster shots in order to keep immunity against the pathogen[94]. These vaccines can be used in immunocompromised patients who cannot receive an attenuated vaccine[91]. Several inactivated virus vaccines are in market against viral diseases including, Flu, Hepatitis A, Polio and Rabies[95]. Several companies are in different phases of an inactivated viral

vaccine against COVID-19. Among these, Sinovac and Sinopharm are in the late stage of clinical trials[91].

10.3. Live Attenuated Virus Vaccines

Live attenuated virus vaccines use a weakened live virus to stimulated immune system. The weakened viruses cannot replicate enough to cause disease in the vaccine recipients. Since the attenuated virus is similar to the real pathogen, immune response to these vaccines is often strong, long lasting, and may need only one or two shots[96]. Several highly effective attenuated virus vaccines are available in market against viral diseases such as MMR combined vaccine (Measles, Mumps, Rubella), Rotavirus, Smallpox, Chickenpox, and Yellow fever[95]. Currently four companies are conducting pre-clinical studies which are aimed to use this strategy to develop a vaccine against COVID-19[91].

10.4. Replicating & Non-Replicating Viral Vectors

In the Replicating/Non-replicating viral vector vaccines, a non-pathogenic virus (such as adenovirus) will be used to deliver gene(s), encoding immunogenic protein(s), of the pathogen of interest[97, 98]. Currently, there is no Non-replicating viral vector vaccine available in market[91], however two Replicating viral vector vaccines are approved recently against Ebola and Dengue[91, 99, 100]. Currently, several companies are in pre-clinical phase of vaccine development using Replicating/Non-replicating viral vectors. Of note, University of Oxford has already started phase III clinical trial of a Non-replicating viral vector vaccine against COVID-19, and Cansino Biologics is planning to start a Phase III clinical trial on its vaccine candidate on August 2020 using similar strategy against COVID-19[91].

10.5. Protein Subunit Vaccines

As it appears from their name, in protein subunit vaccines the most immunogenic subunit(s) of concerned virus is identified and will be used to introduce immune response in vaccine recipient. Since no viable microorganism will be introduced to the recipient's body, these vaccines are considered very safe[101]. Hepatitis B vaccine is an example of protein subunit vaccines [95]. Currently many companies are in various phases of developing COVID-19 vaccine using this strategy. Among these companies Novavax is planning to start a phase III clinical trial on its vaccine candidate against COVID19[91].

10.6. RNA-Based Vaccines

RNA-based vaccines are very similar to DNA-based vaccines. However, these vaccines use RNA encoding immunogenic protein(s) to stimulate the immune response. Moreover, these vaccines are considered safer in compare with DNA viruses because there is no risk of host cell's DNA sequence disruption[102, 103]. No RNA-based vaccine has been introduced into market so far, however several vaccine candidates are under development against COVID-19. For example, Moderna and Pfizer has already started phase III clinical trials of their RNA-based vaccine candidates recently[91].

10.7. Virus-Like Particle Vaccines

The basis of this technology is that viral structural proteins can self-assemble and form a virus-like particle similar to the respected virus[104]. These virus-like particles can trigger the immune system, and as they do not contain any viable pathogen, they are considered as safe vaccines[91]. There are two vaccines, available in market, against Human papillomavirus (HPV) in which this strategy has used for vaccine development. Several companies are using virus-like

particle strategy to develop a vaccine against COVID19. Among these companies, Medicago Inc. has planned to start a phase II/III clinical trial by October 2020[91].

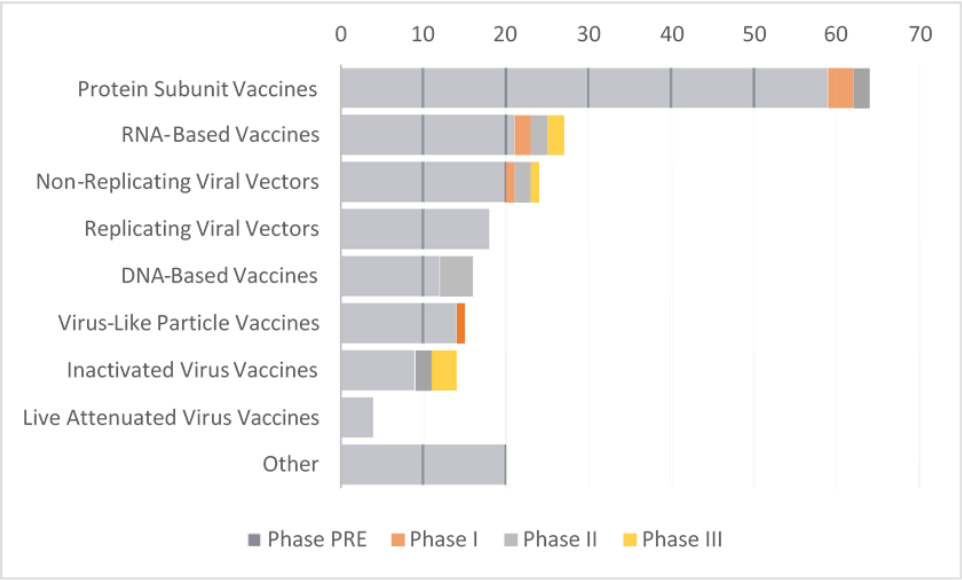

Figure 10. COVID-19 vaccine development strategies. Based on “Faster Cure” (a center in Milken institute) vaccine tracker information as of 18 Aug 2020 there are 202 vaccines under development all over the world[91].

11. CONCLUSION

Many questions remain to be answered about SARS-CoV-2. Is the hACE2 the only receptor mediating host cell entrance? Does the virus only infect type II alveolar cells in the lungs, or can it infect other cells expressing ACE2 such as endothelial cells? What is the mechanism behind lymphopenia in the patient's peripheral blood? Does the virus directly attack patients' organs other than the lungs? More research is needed to shed light on the unknown aspects of pathogenesis of the novel coronavirus.

Emergence of SARS-CoV-2 shows that more novel pathogens might spill from animal species in the future and infect humans. Thus, governments all over the world might need to be more prepared should a new disease appear in human individuals. For instance, securing enough personal protective equipment (PPE), sanitizing materials, and budget for similar scenarios might be a wise precaution. Furthermore, in an effort to contain a newly evolved disease in the originated location, emergence of the disease should be communicated all over the world immediately.

Based on a WHO report, there is no evidence that any of the current medicines can prevent or cure COVID-19. However, it has been shown that some western traditional or home remedies might be helpful for alleviating the patient's symptoms. Up until 20 May 2020, many potential treatments (219 treatments including antibodies, antivirals, cell-based therapies, RNA-based treatments, etc.) and vaccine (140 vaccines including DNA based, inactivated virus, non-replicating viral vector, RNA-based, protein subunit, replicating viral vector, virus like particle, etc.) candidates are under investigation in pre-clinical/clinical trials all over the world. Until an effective treatment or vaccine is discovered, using personal protective equipment, frequent hand washing, and social distancing is suggested by the WHO to mitigate consequences of the pandemic.

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