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Three critical clinicobiological phases of the human SARS-associated coronavirus infections

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Abstract. – OBJECTIVE: COVID-19 immune syndrome is a multi-systemic disorder induced by the COVID-19 infection. Pathobiological transitions and clinical stages of the COVID-19 syndrome following the attack of SARS-CoV-2 on the human body have not been fully explored. The aim of this review is to outline the three critical prominent phase regarding the clinicogenomics course of the COVID-19 immune syndrome.

MATERIALS AND METHODS: In the clinical setting, the COVID-19 process presents as "asymptomatic/pre-symptomatic phase", "respiratory phase with mild/moderate/severe symptoms" and "multi-systemic clinical syndrome with impaired/disproportionate and/or defective immunity". The corresponding three genomic phases include the "ACE2, ANPEP transcripts in the initial phase", "EGFR and IGF2R transcripts in the propagating phase" and the "immune system related critical gene involvements of the complicating phase".

RESULTS: The separation of the phases is important since the genomic features of each phase are different from each other and these different mechanisms lead to distinct clinical multi-systemic features. Comprehensive genomic profiling with next generation sequencing may play an important role in defining and clarifying these three unique separate phases for COVID-19. From our point of view, it is important to understand these unique phases of the syndrome in order to approach a COVID-19 patient bedside.

CONCLUSIONS: This three-phase approach may be useful for future studies which will focus on the clinical management and development of the vaccines and/or specific drugs targeting the COVID-19 processes. ANPEP gene pathway may have a potential for the vaccine development. Regarding the specific disease treatments, MAS agonists, TXA127, Angiotensin (1-7) and soluble ACE2 could have therapeutic potential for the COVID-19 course. Moreover, future CRISPR technology can be utilized for the genomic editing and future management of the clinical course of the syndrome.

Key Words:
Coronavirus, COVID-19, SARS-CoV-2.

Introduction

Coronaviruses family pathogens can affect both humankind and animals. A Novel Coronavirus (nCoV) was recently identified which leading to severe pneumonia cases in the Wuhan city of China, at about the end of the year 2019. After leading to an epidemic all over China, with its very rapid spread, it turned to a global challenging pandemic. In February 2020, the World Health Organization (WHO) named the viral disease as COVID-19 (i.e., Coronavirus disease 2019)[1]. The virus which leads to the COVID-19 infection is nominated as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) replacing the former name of 2019-nCoV. The aim of this review is to outline the three-phase clinicogenomic course of COVID-19 immune syndrome as indicated in our recently published bioinformatics study[2].

The incubation period of the COVID-19 infection was shown to be within 14 days following the critical contact[3]. In a recent study with 1099 patients with COVID-19, median incubation duration was four days[4]. The clinical severity and mortality rates of the infection could vary among the affected cases. Mainly there are there different clinical courses of COVID-19. Approximately 81% of the COVID-19 cases classified as mild disease since those patients with SARS-CoV-2 are either asymptomatic or have mild pneumonia[5]. Nearly 14% of the patients are classified as severe clinical disease since those patients can present with dyspnea, hypoxia, or >50 percent

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lung involvement on the imaging within 24 to 48 hours. The remaining 5% of the patients are considered at the critical disease state since they have quite poor clinical course, such as respiratory failure, shock, or multi-organ dysfunction[5]. The great majority of the patients with mortality had advanced age or medical co-morbidities. The estimated overall mortality rate in noncritical COVID-19 cases is 2.3%. On the other hand, a recent study with 2634 patients who had been hospitalized for COVID-19 disclosed that 14% of the patients were treated in the intensive care unit (ICU) and 12% received invasive mechanical ventilation, whereas the mortality rate of the patient subpopulation who was receiving mechanical ventilatory support was 88%[6].

In the published literature, several risk factors were defined for the description of severe illness in COVID-19. Those criteria include advanced age (above 65 years), male gender, black race, co-morbidities, like cardiovascular disease, diabetes mellitus, hypertension, pre-existing pulmonary disease, chronic lung disease, cancer, chronic kidney disease, obesity (BMI≥30), immunocompromising conditions (including the history of transplant), liver disease, use of anti-biologic agents (e.g., TNF inhibitors, interleukin inhibitors, anti-B cell agents), human immunodeficiency virus (HIV), CD4 cell count <200 cells/microL or unknown CD4 count[4,5,7-11]. A recent cohort study[12] from United Kingdom (UK) concluded that asthma, lower socio-economic background and Asian origin were associated with higher risk for severe COVID-19. There are also other laboratory risk factors for severe COVID-19, such as lymphopenia, increments in the liver enzymes, lactate dehydrogenase, inflammatory markers (CRP, ferritin), D-dimer (>1 mcg/mL), prothrombin time, troponin, creatine phosphokinase and serum markers of acute kidney injury[8,13,14].

In a recent study[15] from USA, the clinical snapshot of COVID-19 patients who were admitted to hospitals was described. According to this US study the mean age of the patients was 62 years and there was a male predominance in gender (61%). 36% of the patients had obesity. The most frequent presentation of the patients in emergency departments was cough (79%), fever (77%), dyspnea (57%), myalgia (24%), diarrhea (24%), nausea/vomiting (20%), respectively. In laboratory examination, lymphopenia (90%) and thrombocytopenia (27%) were observed among the patients. During the clinical course of these patients, 13% needed renal replacement therapy, 19% had arrhythmias, 33% required invasive mechanical ventilation, and 95% needed vasopressors. The patients who had required mechanical ventilation were more likely to be male, have obesity, have elevated liver functions tests and have elevated inflammatory markers (ferritin, d-dimer, CRP and procalcitonin). In the outcome of those patients, 66% had discharged however unfortunately 10% had died.

Numerous articles are now being published regarding the COVID-19 infection; however, the clinical course of COVID-19 based on the genomic basis is not yet fully understood. Herein, we suggest that the COVID-19 infection may be considered as the three critical clinicobiological steps; which comprised of the initial, propagating and complicating phases. We propose that each phase has unique features in relation with the different clinicogenomic mechanisms which especially include the renin-angiotensin-aldosterone system[2]. In the clinical setting of these three phases present as "asymptomatic/pre-symptomatic phase", "respiratory phase with mild/moderate/severe symptoms" and "multi-systemic clinical syndrome with impaired/disproportionate and/or defective immunity" (Figure 1). The corresponding three genomic phases include the "ACE2, ANPEP transcripts in the initial phase", "EGFR and IGF2R transcripts in the propagating phase" and the "immune system related critical genes in complicating phase" (Figure 2). We propose that the third phase may be stated as "COVID-19 Syndrome" rather than "infection" since in this terminal phase the clinical course is much more than a usual infection complication; it includes complex immunological and biological damaging mechanisms to a wide variety of organ systems.

Materials and Methods

Initial Phase of the COVID-19 Immune Syndrome (Asymptomatic/ Pre-Symptomatic Disease)

Asymptomatic Disease of COVID-19

The asymptomatic disease form of COVID-19 was quite well established. In a recent study[17], a COVID-19 outbreak on a crusie ship was reported as about half of the 619 confirmed COVID-19 cases were asymptomatic at the time of diagnosis. In another study[18], 56% of the patients who had a positive screening test were asymptomatic at the time of diagnosis in a nursing facility. There are

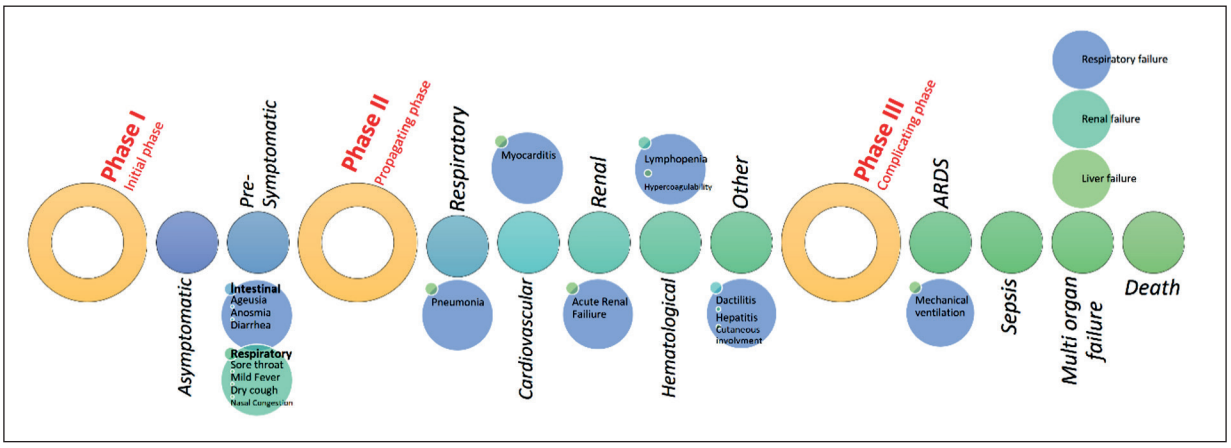

Figure 1. The depiction of the three critical clinical phases, symptoms and signs of the COVID-19 infection/immune syndrome. (ARDS: acute respiratory distress syndrome).

even higher proportions of reported asymptomatic cases. Eighty eight percent of pregnant women were reported to be asymptomatic on presentation in a study from USA[19]. Thus, it can be concluded that approximately among 50-80% of the COVID-19 infected patients had no symptoms.

Pre-Symptomatic Disease of COVID-19

The symptoms of COVID-19 infection occur after an incubation period of approximately 5.2 days. In this phase, the COVID-19 patients mainly have the symptoms of two organ systems which are called as the respiratory and gastrointestinal systems. The COVID-19 patients could either have "respiratory form of COVID-19 infection" or "intestinal form of COVID-19 infection" or both. In some cases, there can be a fever with no accompanying signs[20].

Respiratory form of pre-symptomatic COVID-19 disease: respiratory form of COVID-19 infection mainly involves firstly upper respiratory system and then procedes to the lower respiratory system. The authors of the Chinese Center for Disease Control and Prevention report divided the clinical manifestations of the disease by there severity and they have reported that the mild disease with non-pneumonia and mild pneumonia occurred in 81% of cases[5]. In this mild disease form, the patients generally admitted to the hospital with the symptoms of an upper respiratory tract viral infection, including mild fever, cough (dry), sore throat, oropharyngeal mucositis, nasal congestion, malaise, headache, muscle pain. In this initial phase of the COVID-19 infection, the signs and symptoms of a more serious severe disease, such as dyspnea, are not present[21].

Intestinal form of pre-symptomatic COVID-19 disease: in the intestinal form of COVID-19 in-

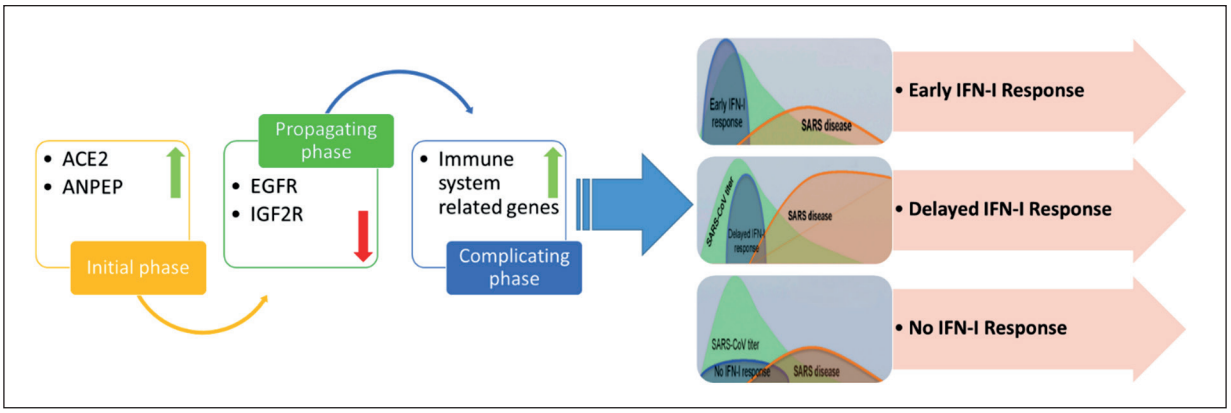

Figure 2. The depiction of the three critical genomic phases of the COVID-19 infection/immune syndrome (data driven by[2,16] adopted with permission).

fection, smell and taste disorders (e.g., anosmia and dysgeusia) have been reported[22,23] as the common symptoms. In a study[23] with 59 COVID-19 patients, 34% self-reported either a smell or taste aberration and 19% reported both. In another study[24] with 202 mild COVID-19 outpatients, 64% showed alterations in smell or taste, and 24% described very severe alterations; smell or taste changes were reported as the only symptom in 3% overall and preceded symptoms in another 12%. Although it is unclear that those findings are a distinguishing feature of COVID-19, it is important that those symptoms emerge in the pre-symptomatic mild COVID-19 disease; therefore, those symptoms could be the onset of more severe gastrointestinal system involvement. The other gastrointestinal symptoms (e.g., nausea and diarrhea) have also been described in previous studies; these gastrointestinal symptoms are generally present at the presymptomatic phase. In a study[25] which reports gastrointestinal symptoms in the patients with confirmed COVID-19, the prevalence was 18% in overall, with diarrhea, nausea/vomiting, or abdominal pain reported in 13, 10, and 9%, respectively. In a recent study[26], it has been reported that intestinal epithelium supports SARS-CoV-2 replication. The SARS-CoV-2 receptor angiotensin-converting enzyme 2 (ACE2) is highly expressed on differentiated enterocytes. In human small intestinal organoids, enterocytes were involved by SARS-CoV and SARS-CoV-2 as confirmed by confocal-microscopy and electron-microscopy. Consequently, substantial titers of infectious viral particles were detected in the study. Also, mRNA expression analysis in this study showed strong induction of a generic viral response.

SARS-Coronavirus Family, ACE2 and Spike Protein

The attachment of the pathogen virus to the host cell is a very important step for the initiation and formation of viral infections. The SARS-CoV-2 virus binds to host cell's ACE2 receptor through its spike proteins present in the envelope part of the virus. Watanabe et al[27] reveal the glycan structures on a recombinant SARS-CoV-2 antigen. The development of a vaccine against the virus by preventing this sticking has been the subject of many studies. However, a completely successful vaccine design has not been made yet, except for limited success[28]. In cell lines, angiotensin-converting enzyme 2 (ACE2) has been defined as a potential SARS-CoV receptor. The first genetic evidence that ACE2 is a crucial SARS-CoV receptor *in vivo* was proposed by Kuba et al[29]. Likewise, it has been showed that SARS-CoV infections and the Spike protein of the SARS-CoV reduce ACE2 expression. The addition of SARS-CoV Spike into mice worsens acute lung failure *in vivo* that can be decreased by blocking the renin-angiotensin pathway. In 2005, Kuba et al[29] suggested a molecular explanation why SARS-CoV infections cause severe lung failure and proposed a treatment for the SARS and possibly other respiratory disease viruses.

From 2002 SARS-CoV outbreak till now, widespread structural studies have shown key atomic-level relations between the SARS-CoV spike protein receptor-binding domain (RBD) and its host receptor ACE2 that control both the cross-species and human-to-human transmissions of SARS-CoV. Wan et al[30] analyzed the potential receptor usage by 2019-nCoV. They found that the sequence of 2019-nCoV RBD, with its receptor-binding motif (RBM) which directly links ACE2, is alike to that of SARS-CoV, proposing that 2019-nCoV uses ACE2 as its receptor. Moreover, numerous residues in 2019-nCoV RBM (particularly Gln493) provide favorable relations with human ACE2. Furthermore, numerous other residues in 2019-nCoV RBM (particularly Asn501) are matched with, but not ideal for, binding human ACE2, proposing that 2019-nCoV has developed some ability for human-to-human transmission. Furthermore, while phylogenetic studies proposing a bat origin of 2019-nCoV, 2019-nCoV besides potentially identifies ACE2 from a variety of animal species (except mice and rats), linking these animal species as possible intermediate hosts or animal models for 2019-nCoV infections.

Pulmonary ACE2 Activity and Neutrophil Infiltration

ACE2 cuts single-terminal residues from several bioactive peptides, such as angiotensin II and it has an important role in pathogenesis of inflammatory lung diseases. In 2018, in order to clarify the mechanism underlying the role of ACE2 in inflammatory lung disease Sodhi et al[31] aimed to identify biological targets of ACE2 in the lung. They investigated the ACE2 effects on des-Arg9 bradykinin (DABK) in airway epithelial depending on the hypothesis that DABK is a biological substrate of ACE2 and ACE2 has an important role in the pathogenesis of acute lung injury by controlling DABK/bradykinin receptor B1 (BK-B1R) axis signaling. They have concluded that

loss of ACE2 role in mouse lung in the setting of endotoxin inhalation causes stimulation of the DABK/BKB1R axis, secretion of proinflammatory chemokines such as C-X-C motif chemokine 5 (CXCL5), macrophage inflammatory protein-2 (MIP2), C-X-C motif chemokine 1 (KC), and TNF-αfrom airway epithelia, increased neutrophil infiltration, and worsened lung inflammation and injury[31]. It has been proposed that a decrease in pulmonary ACE2 action leads to lung inflammation, because of a reduced ability to inhibit DABK/BKB1R axis-related signaling, leading to a quicker onset of neutrophil infiltration and increased inflammation in the lung. Therefore, a biological substrate of ACE2 in the airways was identified as a novel therapeutic target.

Hypertensive Patients Taking an ACE Inhibitor or ARB Had a Lower Mortality at 28 Days Compared With Those Treated With Alternative Antihypertensive Agents

One of the main problems for clinicians treating COVID-19 in patients with hypertension is the usage of ACEIs and ARBs. Zhang et al[32] aimed to clarify the relationship between in-hospital use of ACEI/ARB and all-cause mortality in COVID-19 patients with hypertension. They retrospectively analyzed 1128 adult patients with hypertension diagnosed with COVID-19, including 188 taking ACEI/ARB and 940 without using ACEI/ARB. They have concluded that among hospitalized COVID-19 patients with hypertension, inpatient use of ACEI/ARB was related with lower risk of all-cause mortality compared with ACEI/ARB non-users. Although this study is needed to be confirmed by prospective randomized studies, at least it seems unlikely that in-hospital use of ACEI/ARB was related with an increased mortality risk.

The Initial Thoughts on ACEi/ARB Is Linked With the Negative Effects on COVID-19 Since These Agents Upregulate the ACE2 Expression

Selective inhibition of either Ang II synthesis or activity increases in cardiac ACE2 gene expression and cardiac ACE2 activity. However, the combination of losartan and lisinopril was related with higher cardiac ACE2 activity but not cardiac ACE2 mRNA[33]. While the main outcome of ACE inhibition may result from the combined effect of decreased Ang II formation and Ang-(1-7) metabolism, the antihypertensive effect of AT1 antagonists may in part be due to increased Ang II metabolism by ACE2. Therefore, when the outbreak emerged with COVID-19, clinicians are concerned with the patients who were using ACEi/ARB. However, the recent studies showed that ACEIs/ARBs are not related with the severity or mortality of COVID-19 in patients with hypertension hospitalized for COVID-19 infection[34].

Genomic Features of Initial Phase of COVID-19

ACE2

Strains of the Coronavirus family, SARS-CoV, HCoV-NL63 and SARS-CoV-2, use ACE2 as a mediator to entry into the cell at the initial stage of cell adhesion and penetration[35]. In our previous *in silico* study we found that lung epithelial cells treated with SARS-CoV showed high expression in the ACE2 and Aminopeptidase N (ANPEP) genes at the initial phase of the infection (12-24 hours)[2]. Consequently, Angiotensin II (Ang II) is cleaved to Ang-(1-7) by ACE2, which protecting cell from intracellular damages such as vasoconstriction, hypertrophy, oxidative stress and increased reactive oxygen species (ROS) caused by binding to AT1R[36]. Thus, Ang-(1-7) binds to its G-protein-coupled MASR receptor and antagonizes and counteracts AngII's cardiovascular effects by performing protective functions, such as vasodilation, antiproliferative effect, antigrowth, diuresis, natriuresis and reduced ROS[37].

Complementary to our findings; Kuba et al[29] showed a decrease in ACE2 protein level in the lungs of SARS-CoV-infected mice after 48 hours. This decrease causes the loss of the catalytic effect of the receptors in the outer part of the membrane. Many studies[29,38,39] support that COVID patients differ in ACE2 expressions with varying degrees if they have a secondary disease (diabetes, hypertension, cardiovascular disease, etc.) that can directly affect the RAS system.

ANPEP

ACE2 has been found to be an important receptor for SARS-CoV-2 in the initial phase. However, besides to the ACE2, ANPEP has been shown to act as the co-receptor in the first phase and has been suggested to be very important for the virus to develop the infection. Cell surface Aminopeptidase N, bound to the cell membrane, is an enzyme that is expressed in many different tissues in the human body, such as kidney and lung, and

provides hydrolysis of proteins and peptides[40]. ANPEP, which regulates many cell functions, has also been reported to act as a receptor for human coronavirus 229E/HCoV-229E and cytomegalovirus previously. In this way, it constitutes a major region upon entry into the cell[41].

Santiago et al[42] demonstrated that ANPEP has 3 different structures on its cell surface, named closed, intermediate and open form with different functions. The structure recognized by Coronaviruses is the open form and drugs that bind to this form of ANPEP can prevent from coronavirus infection[42]. Previously, we demonstrated that in the initial phase of the SARS-CoV-2 infection of the virus in lung cell lines, ANPEP, along with ACE2, was also highly expressed.

In parallel with our findings, ANPEP, DPP4 (receptor for MERS-CoV virus) and ENPEP have been found[43] to show the most similar expression feature with ACE2 in 13 human tissues. DPP4 is another known receptor for Coronaviruses. It is reported that two other important receptors of Coronavirus, ANPEP and DPP4, are highly expressed in proximal and distal enterocytes[43]. Those high expressions are associated with gastrointestinal symptoms, such as, diarrhea as a result of Coronavirus infection. Lu et al[44] suggested that in COVID-19 infection, CD26 is another peptidase that acts as a receptor, such as ANPEP and ACE2.

The Coronavirus family consists of 4 genera alpha, beta, gamma, and delta. SARS-CoV-2 is in beta Coronavirus genera and studies show that the importance of ANPEP in Coronavirus infection is not limited only to Betacoronavirues. It has been shown that in neonatal pigs ANPEP is essential to develop a transmissible gastroenteritis virus (TGEV) infection from the alphacoronaviruses family[45]. TGEV shows high mortality and morbidity caused by diarrhea and dehydration due to necrosis of enterocytes[45].

The interaction of ACE and ANPEP with the virus seems to be they do not only function with the attachment of the virus and its entry into the cell. But also save time for the viruses to make their replication. Considering the relationship between the renin-angiotensin system and coronavirus family besides SARS-CoV-2, the drugs or molecules of Ang-(1-7), MAS agonists, TXA127 (which is a pharmaceutical formulation of the naturally occurring peptide), soluble ACE2 target directly themselves or their functions of the important receptors of ACE and ANPEP, which may have critical roles in the treatment or prevention of SARS-CoV-2[46].

Proposals for the Initial Phase of COVID-19

Initial phase of the COVID-19 has unique clinical and genomic features. We suggest that ACE2 is related with the respiratory form of the initial phase, whereas ANPEP is related with the intestinal form[47,48]. Vaccine studies are ongoing worldwide and we suggest that targeting ANPEP with vaccines in the initial phase may help us to prevent the disease from spreading further.

Moreover, since the genomic profile of the disease is related with ACE pathways at initial phase, herein we suggest that MAS receptor agonists and TXA127, which is a pharmaceutical formulation of the naturally occurring peptide, Angiotensin (1-7)[49]. Moreover, in this initial phase of the disease soluble ACE2 could be a potential therapy since it can block the COVID-19 spread mechanisms at the initial phase of the disease.

The other exciting mechanism that could have a potential for a treatment for COVID is the possible relationship between ACE2-HMGB1 and ANPEP-HMGB1. There is a need for future studies in order to confirm the strong possibility for the relationship between these genes. If this can be confirmed, Ankaferd hemostat could be another potential treatment agent in the initial phase of COVID-19 patients who had sore throat and oropharyngeal mucositis[50].

Propagating Phase of the COVID-19 Immune Syndrome (Mild/Moderate/ Severe Symptoms)

Clinical Features of Propagating Phase of COVID-19

Respiratory involvement in the propagating phase. In this second phase, COVID-19 infection proceeds to the lower respiratory system, myocardium and other organ systems. Respiratory system involvement presents as either moderate or severe pneumonia. In moderate pneumonia respiratory symptoms, such as cough and shortness of breath (or tachypnea in children) are present without signs of severe pneumonia. The patients who have severe pneumonia has fever and it is related with severe dyspnea, respiratory distress, tachypnea (> 30 breaths/min), and hypoxia (SpO_2 < 90% on room air). Nevertheless, the fever symptom

must be evaluated cautiously as even in severe forms of the disease, it can be moderate or even absent. Cyanosis can happen in children[21].

Cardiovascular system involvement in the propagating phase. COVID-19 could affect the cardiovascular system and the heart itself. The proposed mechanisms of the cardiovascular injury have been defined and include direct myocardial injury from hemodynamic derangement or hypoxemia, inflammatory myocarditis, stress cardiomyopathy, microvascular dysfunction or thrombosis due to hypercoagulability, or systemic inflammation (cytokine storm), which may also destabilize coronary artery plaques[51]. Myocardial involvement in COVID-19 is also reported in the literature. Myocardial injury is defined as the presence of at least one cardiac troponin value above the 99th percentile upper reference limit[52]. Most patients with COVID-19 with myocardial injury present with the typical symptoms and signs of SARS-CoV-2 infection, such as fever, cough, dyspnea, and bilateral infiltrates on chest imaging. Myocardial involvement may or may not be accompanied by prior or concurrent symptoms of respiratory infection[53].

Hematopoietic system involvement in the propagating phase. Lymphopenia may be considered as the main laboratory finding, with prognostic potential. Also, it is reported that neutrophil/lymphocyte ratio and peak platelet/lymphocyte ratio may be also helpful as a prognostic marker[54]. On the other hand, individuals with COVID-19 may have a number of coagulation abnormalities. There is evidence of direct invasion of endothelial cells by the SARS-CoV-2 virus, potentially leading to endothelial cell injury[55]. Immobilization in hospitalized COVID-19 patients can cause stasis of blood flow regardless of whether they have COVID-19. Also, alterations of prothrombotic factors have been reported in patients with COVID-19 such as elevated factor VII, elevated fibrinogen, circulating prothrombotic microparticles, neutrophil extracellular traps (NETs)[47,56]. All elements of Virchow's triad present in COVID-19 patients leading to hypercoagulability. In a recent study it was shown that systemic antiocoagulation may be associated with improved outcomes among patients hospitalized with COVID-19. The potential benefits of systemic anticoagulation, however, should be weighed against the risk of bleeding particularly for the thrombocytopenic patients and therefore should be individualized[57].

Renal system involvement in the propagating phase. Renal abnormalities present in the majority of patients with COVID-19 pneumonia. Kidney disease among patients with COVID-19 can manifest as acute kidney injury (AKI), hematuria, or proteinuria[58]. It remains unclear if AKI is largely due to hemodynamic changes and cytokine release or if the virus also results in direct cytotoxicity. Although proteinuria, hematuria, and AKI treated in these patients within 3 weeks after the onset of symptoms, renal complications in COVID-19 were related with higher mortality[59]. In a study, kidney histopathology was examined in an autopsy series of 26 patients who died of respiratory failure secondary to COVID-19[58]. All cases had acute tubular injury (of varying severity); a range of other histopathology findings, such as erythrocyte clusters and pigmented casts were also present. Of the nine samples examined for intracellular virus, particles resembling coronaviruses were detected in seven. Of those seven cases, immunostaining was positive for SARS-CoV nucleoprotein antibody in three.

Other organ system involvement in the propagating phase. COVID-19 is a dynamic disease and it can involve nearly every organ system. Conjunctivitis has also been described in COVID-19[60]. Guillain-Barré syndrome has also been reported, with onset 5 to 10 days after initial symptoms[61]. There are other published data suggesting COVID-19 invades the central nervous system. Anecdotal reports from specialists consistently report hyposmia or anosmia to be common, predominantly in the early stage of infection. It is possible that COVID-19 invades the central nervous system by the olfactory receptors of cranial nerve I in the nasal cavity cell membrane[62].

Genomic Features of Propagating Phase of COVID-19

EGFR

In addition to the expression change in ACE2 and ANPEP genes, which serve as two important

receptor and co-receptor in the initial phase of COVID-19, a decrement in the RNA level is observed in EGFR and IGF2R genes (propagating phase). This intermediate phase is closely related to the initial phase and the complicating phase, which contains with crucial roles in determination of the severity of the infection.

Epithelial response has an important and functional role in the immune response against respiratory infection diseases. The epithelial response occurs via signaling cascades that activate the EGF receptor (EGFR). Toll-like receptors (TLRs) are important for the recognition of pathogens that enter the body by inhalation on the surface of the epithelium. Toll like receptors with EGFR is important for generating an innate immune response[63,64]. Therefore, EGFR downregulation can prevent an early immune response against the infection. This can lead to the progression and spread of viral infection. The role of EGFR has been shown to be important in influenza virus infection and its role could vary for different types of clinical influenza (lethal, nonlethal infection)[63,64]. The same situation may be true for SARS, which can cause serious infection in the respitory system. The low expression of EGFR can further increase the severity of infection. However, this is not a general rule and has been shown to be among the factors determining the severity of the disease and genes belonging to other pathways in certain infections.

IGF2R

Besides the EGFR transcript, another low expressed gene due to viral infection is IGF2R in the propagating phase. IGF2R encodes a receptor for insulin-like growth factor 2 and mannose 6-phosphate. There are binding sites for each ligand on different segments of the protein. This receptor has several functions, including intracellular transport of lysosomal enzymes, transforming growth factor beta activation, and degredation of insulin-like growth factor 2. Normal IGF2R functions of virus-infected cells may be impaired and affect the cell's apoptosis. On the other hand, it has been shown that IGF2R with Atp6v0c can block the entry of reovirus and several other viruses to the cell via endocytosis pathways. Low expression of IGF2R is probably an important facilitating factor for entry of the virus into the cell by endocytosis[65]. In addition, IGF2 enhances Treg cell suppression functions[66]. Therefore, the downregulation of IGF2R plays an important role in the development of Treg cell suppression functions. As a conclusion, low expression of these 2 genes EGFR and IGF2R facilitates entry of the virus into the cell, while harder the formation of the immune response to the viral infection.

Proposals for the Propagating Phase of COVID-19

The propagating phase has different clinical and genomic features from the initial phase of COVID-19 infection. In the propating phase COVID-19 gets ready for spread all over the body. We suggest that EGFR and IGF2R play significant role for the organ involvement during the disease course of COVID-19. In the literature it is well known that COVID-19 severe disease is associated with several risk factors. We suggest that the EGFR and IGF2R gene pathways are the key genomic elements for the virus spread throughout the body and also EGFR and IGF2R may explain why the virus tends to spread more in patients who had risk factors, such as hypertension and diabetes mellitus.

The Complicating Phase of the COVID-19 Immune Syndrome (impaired/disproportionate and/or defective immunity)

Clinical Features of Complicating Phase of COVID-19

Dynamic profile of Covid-19. In a study the dynamic profile of laboratory findings in patients with novel coronavirus (2019-nCoV)-infected pneumonia[9]. During hospitalization, most cases had evident lymphopenia, and nonsurvivors developed deeper lymphopenia over time. Leucocyte counts and neutrophil counts were higher in nonsurvivors than those in survivors. The level of D-dimer was higher in nonsurvivors than in survivors. As the disease progressed and clinical status worsened, the levels of blood urea and creatinine increased before death. Therefore, it can be suggested that during the clinical course of COVID-19, the disease spreads all over the body, including several organ systems, such as respiratory system, cardiovascular system, gastrointestinal system, bone marrow and hematopoietic system, renal system, liver and other systems.

ARDS in the complicating phase of COVID-19. In the complicating phase of COVID-19 disease Acute Respiratory Distress Syndrome (ARDS) may occur in patients. ARDS diagnosis needs clinical and ventilatory criteria. ARDS seen in severe COVID-19 is defined as difficulty in breathing and low blood oxygen level[67]. This syndrome is indicative of a serious new-onset respiratory failure or for deteriorating of an already known respiratory picture. Some patients may succumb to secondary bacterial and fungal infections[68]. ARDS may lead directly to respiratory failure, which is the cause of death in 70% of fatal COVID-19 cases[69]. Different forms of ARDS are notable depending on the grade of hypoxia. The main parameter for clinician is the PaO_2/FiO_2 ratio and the classification is based on this ratio. In the mild ARDS picture the ratio is between 200 mmHg $< PaO_2/FiO_2 \leq 300$ mmHg. In noderate ARDS picture it is between 100 mmHg $< PaO_2/FiO_2 \leq 200$ mmHg and the severe ARDS form is defined as $PaO_2/FiO_2 \leq 100$ mmHg. The imaging test reveales bilateral lung opacities which are not described by effusions, lobar, or lung collapse[21].

Sepsis in the complicating phase of COVID-19. The second clinical picture in this phase could be sepsis. Sepsis characterizes a lethal organ failure triggered by a dysregulated host response to infection, with organ dysfunction. The vast secretion of cytokines by the immune system in answer to the viral infection and/or secondary infections can lead to a cytokine storm and symptoms of sepsis that are the cause of death in 28% of fatal COVID-19 cases[69].

Multi-organ failure and septic shock. Interestingly, in the complicating phase of the COVID-19 infection there is a multi-organ involvement. In these cases, uncontrolled inflammation inflicts multi-organ damage leading to organ failure, especially of the cardiac, hepatic and renal systems. The involvement in respiratory system manifest as severe dyspnea and hypoxemia, in the renal system as reduced urine output, in the cardiovascular system as tachycardia, in neurological system as altered mental status. The laboratory tests show hyperbilirubinemia, acidosis, high lactate, coagulopathy, and thrombocytopenia in the complicating phase of COVID-19 syndrome. Most patients with SARS- CoV infection who progressed to renal failure eventually died[69]. If this poor clinical course progressively worsens, septic shock can occur. Septic shock is related with increased mortality, circulatory, and cellular/metabolic abnormalities such as serum lactate level greater than 2 mmol/L[21].

Drug targets for the disease management. In a recent study it was stated that screening a subset of preclinical compounds which targets human proteins or host factors in multiple viral assays identified two sets of pharmacological agents that displayed antiviral activity: inhibitors of mRNA translation and predicted regulators of the Sigma1 and Sigma2 receptors[70].

Genomic Features of the Complicating Phase of COVID-19

IFIT2, IFIT3, IFITM and IFN

The amount and timing of the IFN-based immune response has been shown to be important in most other viral infections, as well as COVID-19[29]. The innate immune response against virus infected cells occurs and maintained by the production of cytokines, chemokines and Interferon-induced gene (ISGs). Inflammation, which develops as a result of an innate immune response against viruses, has a significant effect on the course and severity of the infection. Cytokines, chemokines and ISGs, which have important roles in the innate immune response against viruses, have been shown to be over expressed in the complicating phase.

The interferon type I (IFNI) signalling pathway contains important genes that inhibit various viruses and viral infections, including corona virus infections. Among those genes stimulated by IFN with antiviral activity, there are two genetically and functionally different families. These are: IFIT (the IFN-induced protein with tetratricopeptide repeats) family and IFITM (the IFN-induced transmembrane protein) family. IFIT proteins induced after type I interferon (IFN) or IRF3 show their antiviral effects by binding to initiation factor 3 (eIF3) translation initiation complex and inhibiting protein translation[71,72].

On the other hand, IFITM proteins stop replication of enveloped viruses, including SARS-CoV before they enter the cytosol. These two important genes have been shown to be upregulated

in both SARS-CoV and SARS-CoV-2. IFIT1, the first discovered and important ISG of the human IFIT family, recognizes unmethylated 2′O RNA and shows its antiviral effect by changing the effective translation of the uncapped viral mRNA. SARS-CoV and other Coronavirus RNAs are protected from IFIT recognition because they encode the nsp16 protein, which shows 2′-O-methyltransferase (2-OMT) activity[72,73].

Interferon could play an important role in an effective treatment of COVID-19. Strengthening interferon therapy may be possible when functional nsp16 methyltransferase activity is reduced[74]. The human IFIT family consists of 4 members. These are IFIT1 (ISG56), IFIT2 (ISG54), IFIT3 (ISG60 or IFIT4), and IFIT5 (ISG58)[75]. Apart from IFIT, other members also have significant antiviral effects via the innate immune responses of IFN1. IFN1 and IFN3, which are generally produced in the process of viral or bacterial infections, lead to an increase in the expression of normally low expressed IFIT genes. We also found a significant increase in IFIT2, IFIT3 and IFITM1 in lung epithelial cells infected with SARS-CoV in the complicating phase in our previous study. In parallel, Huang et al[7] demonstrated that IFIT1 is upregulated in SARS-CoV-2 infection.

RSAD2

Interferon stimulate genes (RSAD2, IFIT1, and CXCL10) also have important regulatory and functions alongside antiviral IFN (IFN alpha and IFN beta), proinflamatory cytokines and chemokines (IL-6, TNF, IFN-γ, and CCL5) in the formation of an effective immune response in SARS-CoV infection. Danesh et al[76] detected a significant upregulation in the RSAD2 gene in ferrets infected with SARS-CoV after 48 hours post infection. Similarly, we detected an increase of RSAD2 in lung epithelial cells infected with SARS-CoV[76,77].

CXCL10 and CXCL11

C-X-C motif chemokine ligand family is molecules that have different functions in the immune response to various viral infections, including stimulation of monocytes, natural killer and T cell migration and modulation of adhesion molecule expression. There is an increase in both gene and protein levels in the gene expression of different members of this family (CXCL5, CXCL8, CXCL10, and CXCL11) in corona virus infections[78-80].

OAS2

Another gene that we detected a significant increase in expression is OAS2 gene in complicating phase after lung epithelial cells are infected with SARS-CoV. OAS2 encodes essential proteins involved in the innate immune response to viral infection. Cinatl Jr et al[81] demonstrated that an increase in CXC chemokines and OAS2 gene occured when there is no increase in IFN alpha and beta genes in the first 24 hours after infection in intestinal cell lines with SARS CoV. Likewise, we found that there was an increase in CXCL10, CXCL11 and OAS2 between 24 hours and 48 hours after infection, but no significant change in IFN genes. Probably the increase in IFN occurs in the coming days of infection with an increase in the stimulation of functional proteins that these genes are responsible.

SAMD9

This gene encodes protein containing a sterile alpha motif domain. The encoded protein settles in the cytoplasm and can play a role in regulating cell proliferation and apoptosis. Increased expression of this gene has been shown in both SARS-CoV and SARS-CoV-2 infections[82,83].

Proposals for the Propagating Phase of COVID-19

The last terminal phase of the COVID-19 immune syndrome is associated with poor clinical outcome. In this third phase especially, immune system-related genes, such as IFN gene and other genes play important role. IFN functions are altered and therefore in this phase, immune system is either non-functioning or dysfunctioning. The inflammatory monocyte and macrophages could lead to severe cytokine storm. The chaos and loss of control in the immune system leads to severe damage in organs. The treatment in this phase may include targeting of IFN genes. Conventional supportive options, such as mechanical ventilation, are also needed in this phase COVID-19.

Results

Herein, we propose a three-phase clinicobiological approach to the COVID-19 infection/immune syndrome. The separation of the phases is important since the genomic features of each phase are different from each other and different mechanisms lead to distinct clinical features. From our point of view, it is important to under-

Table I. Critical molecules located at the crossroads of clinicobiological pathways that should be focused on the future management of COVID-19.

Molecule	Genomic evidence	Possible clinical reflections	Future perspective
ACE2	Receptor of some coronavirus family members	Initial phase (respiratory form)	A good candidate for vaccine
ANPEP	Co-receptor of some coronavirus family members	Initial phase (intestinal form)	A good candidate for vaccine
HMGB1	Generates SARS induced pulmonary damages	Initial phase, inhibited by ABS (Ankaferd hemostat)	Trials are warranted with Glycyrrhizin and topical ABS (Ankaferd hemostat)
EGFR and IGF2R	Regulates virus penetration & replication as well as immune response against viral infection	Propagating phase, related with risk factors (hypertension and diabetes Mellitus) for severe diseases	Prognostic marker for severe COVID-19 patients
IFNI and other immune genes	Involved in various key innate and adaptive immune responses	Complicating phase, macrophage associated syndrom and cytokine storm	Treatment in severe COVID-19 patients

stand the unique phases of disease to approach a COVID-19 patient bedside. We suggest that ANPEP gene pathway may have a potential for vaccine development. For the specific treatment; MAS agonists, TXA127, [which is a pharmaceutical formulation of the naturally occurring peptide, Angiotensin (1-7)] and soluble ACE2 have potential for COVID-19 patients. Moreover, ABS (ankaferd hemostat) can block HMGB1 gene pathway with its content of Glycyrrhiza glabra and ABS may be topically used in the patients who are in the initial phase of disease and have sore throat- oropharyngeal mucositis based on further controlled clinical trials (Table I).

We suggest that the ideal treatment for COVID-19 should be in the early phases. Our 3-phase approach may be used for future studies which will focus on clinical management and vaccines-specific drugs. Comprehensive genomic profiling with next generation sequencing may play an important role in defining and clarifying these three unique separate phases for COVID-19. Moreover, in future CRISPR technology can be utilized for genomic editing and clinical course of the disease may be altered by this technique[84]. COVID-19 is a global disaster which has no proven beneficial treatment is developed yet. It is hoped that future clinical and biological scientific efforts (Table I) shed further light on the better management of the patients with COVID-19 Worldwide.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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