COVID-19; Immunology, pathology, severity and immunosuppressants

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Abstract: As most COVID-19 patients show mild to moderate symptoms; 15% suffer from severe respiratory symptoms, and 5% develop acute respiratory distress syndrome which may be associated with multiple organ failure. The highly virulent SARS-CoV-2 affects organs, other than lungs, such as brain, kidney, cardiovascular system and gastrointestinal tract. This review highlights the relation between SARS-CoV-2 and organ involvement. SARS-CoV-2 causes the cytokine storm and damage of the lung cells with activation of NF-κB, hence the damage may reach other organs that leads to death in some cases. The current review discusses the factors affecting COVID-19 severity including the immune response to SARS-CoV-2 with the associated cytokine storm, smoking, gender and blood group. This review discusses also genetic factors associated with severe COVID-19 cases, alongside with comorbid diseases such as diabetes, cardiovascular diseases and obesity. Also, this review focuses on the effect of immunosuppressants and antirheumatic drugs on the protection and treatment of severe COVID-19 cases.

Keywords: COVID-19; SARS-COV-2; pathology; immunology; severity; immunosuppressants.

Highlights:
• About 15% of COVID-19 patients suffer from severe respiratory symptoms and 5% develop acute respiratory distress syndrome (ARDS), septic shock and may be associated with multiple organ failure.
• SARS-CoV-2 is a multi-systemic highly virulent virus which, in addition to lungs, affects other organs such as brain, kidney and cardiovascular system.
• Cytokine storm leads to the damage of the lung cells which can cause activation of NF-κB, hence the damage may reach other organs that leads to death in some cases.
• Smoking and gender are correlated with increased expression with ACE2 and TMPRSS2 receptors.
• Genetic and comorbidity factors are associated with severe COVID-19.
• Strong clinical studies are needed to determine whether patients receiving immunosuppressive drugs are protected from getting COVID-19 than normal population.

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1. INTRODUCTION
The global pandemic by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have emerged from Wuhan, Hubei province of China in December 2019 and rapidly spread worldwide. On 11th March 2020, the World Health Organization (WHO) announced that COVID-19 (Coronavirus Disease 2019) a pandemic1. As of 21 December 2020, WHO reports more than 7,547,471 confirmed cases of COVID-19, including 1,686,267 deaths2.

2. Impacts of Covid-19 on human body
Coronaviruses are transmitted within respiratory droplets of coughing and sneezing. SARS-CoV-2 enters the nasal system by breathing and starts replication3. SARS-CoV-2 binds with receptor angiotensin converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) for host cell entry. After entry to the cell cytoplasm, ribonucleic acid (RNA); the couple large open reading frames (ORFs) lab are translated into a protein viral transcriptase complex (RNA-dependent RNA polymerase (RdRp), phosphatase activity and helicase). Replication of the genome comprises the synthesis of a full-length negative-strand RNA and serves as a template for full-length genomic RNA. After translation, fundamental proteins are restricted to the Golgi intracelluar membranes, the endoplasmic reticulum Golgi intermediate compartment (ERGIC) which is called the site of budding. New virions that are assembled full genome RNA are released from the cell4-5.

3. Symptoms of COVID-19 and organ involvement
About 80% of COVID-19 patients have no symptoms or show mild to moderate symptoms; which are mainly fever, fatigue, cough, sore throat and may be associated with loss of smell and taste, about 15% suffer from severe respiratory symptoms (pneumonia) and finally about only 5% of the patients develop acute respiratory distress syndrome (ARDS), septic shock which may be associated with multiple organ failure6SARS-CoV-2 is a multi-systemic highly virulent virus which, in addition to lungs, affects other organs such as brain, kidney and gastrointestinal tract6.

3.1. COVID-19 and kidney involvement
The clinical data reported high incidence of acute kidney injury (AKI) in patients infected with SARS-CoV-2. In a study in Wuhan, about 29% of critically ill patients had AKI7. There are two theories explaining the injury of the kidney. The first one suggests that AKI results from sepsis associated ischemia while the second one proposes that it may be due to direct viral induced acute tubular necrosis. The latter claim rests on the detection of viral particles by polymerase chain reaction (PCR) in the urine8,9, the peculiar expression pattern of angiotensin converting enzyme 2 (ACE2) (a major receptor for the virus) which is limited to the proximal tubules9,10 which are the sites of affection in the kidneys of SARS-CoV2 infected patients2 and the correlation of the time of viral detection in the urine with the onset of AKI.

3.2. COVID-19 and cardiovascular involvement
About 20-30% of hospitalized SARS-CoV2 patients experience cardiac complications11. In a recent study on 671 critically ill patients, these complications were collectively identified as death related in about 50% of the patients12. In addition, although cardiac injury occurs more in patients with preexisting cardiac conditions13, there is reported de novo cardiac involvement in infected patients ranging from elevated troponin14, arrhythmias to acute myocardial injury, acute heart failure with or without cardiogenic shock15, cardiac arrest, pericardial effusion with or without tamponade and hypercoagulable state16-23.

The mortality rate jumps very high if these complications happened11. This may be due to higher expression of ACE2 in these conditions compared to healthy population24-26, this enables direct injury to the heart and the endothelial tissue27. Other two causes lead to indirect injury either due to cytokine storm associated coagulopathy or acute respiratory distress syndrome (ARDS) - induced hypoxia20,21.

3.3. COVID-19 and nervous system involvement
Neurological affection by SARS-CoV-2 is evident clinically in many reported cases ranging from temporary anosmia, strokes to severe neurological deficits up to death28-32. There have been three possible explanations for these findings.

First, they could be a result of the systemic inflammation in the body with subsequent multi-organ dysfunction that may result in encephalopathy or even stroke.

Also, this affection can be caused by post infection immune mediated responses like those causing Guillain–Barré syndrome (GBS), acute disseminated encephalomyelitis (ADEM) or encephalitis.
Third assumption that should be put in consideration is that the virus is supposed to be capable of invading the central nervous system. This is based on the presence of ACE 2 receptors in the brain and the fact that this novel virion has very similar structure and behavior to the animal coronaviridae that cause neurological manifestations in their hosts\(^3\). This has been supposed after detection of the virus in the cerebrospinal fluid (CSF) by RT-PCR\(^{34,38}\). In the real situation, the majority of SARS-CoV-2 patients who had neurological insult in the course of the infection had their CSF PCR negative\(^{36-38}\).

So, it is sample contamination in positive cases and hence it is false positive which is unlikely in experienced settings\(^39\) or the organism is not in the CSF but mainly intracellular as detected in autopsies\(^{37,40}\) although not confirmed\(^{38,41}\) or it is not in the brain at all and these symptoms are a result of immune system activation especially with a picture of immune response in the CSF.

Until our recent time, although we have a great number of studies, we don't know much about the behavior of the virus, the cause behind presence of different clinical phenotypes, why the immune system act differently and what are the long-term effects on the nervous system. We are in a need for a more focused research to be able to create guidelines for appropriate management of the cases and prevention of this disease.

4. Immune response to COVID-19 and disease severity

The entrance of COVID-19 is responsible for the activation of both innate and adaptive immune systems. Rapid and well-coordinated immune response grants the first line of defense against viral infection, excessive inflammatory response, the dysregulated adaptive host immune defense may create harmful tissue destruction at both at the site of virus entry and at systemic level\(^42\). The imbalance between the innate inflammatory and adaptive immune responses by excessive action of innate and impaired adaptive immunity resulted in decreased circulating pro-inflammatory cytokine levels (e.g., TNF\(_\alpha\), IL-6, IL-8, IL-10, etc.) with lymphopenia in CD4\(^+\) (which directs the overall adaptive immunity) and CD8\(^+\) T cells known as “cytotoxic T-cells” (that clear and destroy the viral infected cells). These low T-cells numbers are associated with clinical markers of inflammations as ferritin, D-dimer, and hsCRP as well as reduction in B cells, natural killer cells (NK), monocytes, eosinophil and basophil, resulted in the hyper inflammation which is known as “cytokine storm” that is suggested to be associated with severe COVID-19 (Fig. 1).\(^{43-45}\) Cytokine storm leads to the damage of the lung cells “apoptosis or necrosis” which can cause activation of NF-\(\kappa\)B; hence the damage may reach other organs that leads to death in some cases\(^{45,46}\).

The Lancet has issued a report on cytokine storm characteristics as being one of the reasons for COVID-19 severity. This report is supported up by the data on one of the major constituents of death due to SARS-CoV-2 infection, which is severe respiratory distress syndrome (ARDS)\(^7\). According to a study done in Wuhan, China, 88 (41.1%) patients were with severe symptoms and126 (58.9%) patients were of mild and moderate symptoms. Severe patients commonly had neurologic symptoms confirmed as acute cerebrovascular disorders, consciousness impairment and skeletal muscle symptoms\(^48\). Bourboulis et al.\(^1\) studied the immune responses of 54 COVID-19 patients, 28 of whom had severe respiratory failure. All patients with severe respiratory failure revealed either macrophage activation symptoms (MAS) or very low human leukocyte antigen D related (HLA-DR) appearance accompanied by profound deficiency of CD4 lymphocytes, CD19 lymphocytes, and natural killer (NK) cells. Thus, the immune dysregulation in severe COVID-19 is characterized by low expression of HLA-DR (MHC class II cell surface receptors on CD4 T-lymphocytes) which is mediated by increased IL-6 secretion. This low expression has a negative impact on the ability of the T4 lymphocyte to recognize SARS-CoV-2 antigens in antigen-presenting cells. The immune dysregulation in severe COVID-19 is also characterized by lymphopenia, correlated with continued cytokine production and hyper-inflammation, which leads to cytokine storm, then necrosis of cells \(^49,50\).

The deficiency of interferons (INFs) cause irregular secretion of immune cells. In a research on people with different degrees of infection, it was found that genes encoding proteins that involved in INF1 and INF2 responses were high in mild and moderate patients where they were low in more severe patients\(^55,49\). The early production of antibodies may cause viral integration inside immune cells and more viral replication and resulting in severe disease even in young people\(^50\).
The researchers found that the main entry point of COVID-19 is ACE2 receptor via SARS-CoV-2 Spike of S protein at the surface of envelope, ACE2 receptors are present in the alveolar cells of the lung as well as blood vessels, heart, ilium, esophagus, tubules of kidney\cite{1,51} and T-cells of the adaptive immune system. The presence of ACE2 in the lymphocyte cells may lead to their death by the virus or even direct damage of the lymphocytic organs as they also have ACE2 receptors, however no evidence of lymphocytic organ damage till now which will need more investigations\cite{1,44}. It was found that cigarette smoking enhances expression of ACE2 receptors in the lung\cite{52}. Females found to have stronger innate and adaptive immune system with more resistance to viral infections than men as females have two X chromosomes which contain high number of immunological genes\cite{53}, females also have high level of estrogen which enhances adaptive immune cells as T-cells, unlike androgen hormone in men which increase neutrophils number and pro-inflammatory mediators as cytokines\cite{53,54}. They also found that the host transmembrane serine protease TMPRSS2 (a key gene in prostate cancer, also, it is expressed in the bronchial epithelial cells)\cite{55} plays a vital role in the severity of the disease as it modifies S proteins in SARS-CoV-2 enhancing the viral entry and pathogenesis, this could be one reason for the severity of the disease in men more than in women as androgen hormone activates and induces the expression of TMPRSS2 and ACE2 co-expression in males' pneumocytes than female cells, which may interpret the higher susceptibility of men for severe COVID-19 infection\cite{55}.

5.2. Genetic factors are associated with severe COVID-19

In a study by examining the genome of 81,000 people, it was found that there was a deleterious variant in both ACE2 and TMPRSS2. The deleterious variants occur more in both African/African-American and Non-Finnish European, there were less deleterious variant in East Asian, Latino American and Finnish populations, while there was no deleterious variant in both Amish and Ashkenazi Jewish populations\cite{56,57}.

It was found that human variants (K26R, S16P, T27A, K31R, H34R, E35K, E37K, D38V, N51S, N64K, K68E, F72V, T92l, Q102P, G326E, G352V, D355N, H378R, Q388L, and D509Y) increase S protein binding, so, increase susceptibility and severity, while ACE2 variants (K31R, E35K, E37K, D38V, N33I, H34R, Q388L and Y83H) increase the resistance to infection\cite{58}.

It’s found that booster and inhibitors variants are present in males more than females, this can also explain the gender bias in severity as booster variants are present more than inhibitor variants\cite{59}. In another study, it was found that (N720D) variant which improve TMPRSS2 activation are increased in Europeans, Iranians, Kuwaitis and Qatars respectively, all of which increase the severity\cite{60}. An Egyptian study on 61 COVID-19 patients showed that 204 variation in the genomic Egyptian sample, most of these variations were like that in USA, Australia, France, Sweden and Saudi Arabia. The most common and important variation is the one in D614 to D614G that presents in the S1 domain of S glycoprotein, that facilitates the COVID-19 entry into the host cells by binding to ACE2 receptors, resulting in increased infection and transmission.\cite{61} S-protein mutation (D614G) may enhance SARS-CoV-2 transmission, but further studied are needed to decide the impact of this mutation on the severity of COVID-19\cite{62}. An Egyptian study confirmed that mutation in SARS-COV-2 receptors isn’t the reason for mild symptoms and low death rate among Egyptian patients. They recommend conducting further studies on large sample sizes\cite{63}.

It has been found that HLA system (Human leukocyte antigen) which includes three genotypes A, B or C is correlated to SARS-CoV-2 infection. It is found that HLA-B*46:01 type has the least number of peptide binding sites to COVID-19, so...
people with HLA-B*46:01 are more susceptible to the infection. Unlike that, HLA-B*15:03 is correlated with higher protection against SARS-COV-2 through T- immune cells.

Cross-species transmission due to presence of allelic, genetic or structural variation in SARS-COV2 proteins or allelic or genetic variation in ACE2 receptor in host cells (including rs73635825 (S19P) and rs143936283 (E329G)) might be associated with some pathological consequences with ultimate susceptibility and resistance to COVID-19 infection.

The ABO system of blood groups is related to the severity of the disease. It was found that people who have blood group A were more susceptible than others, while those have blood group O were less susceptible to the disease. People with group O blood was found to be quite resistant to COVID-19 infection, as the infection rate are reduced in those people. In a recent study conducted on 14,112 individuals, increased infection rates among non-O blood types were observed. It was found that people with group A have less risks for intubation and death than other non-O groups, while group B have higher risks for intubation and death than others. Also, negative Rh factor was found to be more protective against infection, intubation and death with SARS-COV-2.

It is suspected that the presence of A or B antigens in non-O group is the reason for this susceptibility to SARS-COV-2, as these antigens present not only in blood cells but also in other organs and they are expressed in both glycoproteins and glycosphingolipids, which may form complex with S proteins in SARS-COV-2. Another theory suggested that, the presence of anti-blood groups’ antibodies is the reason, but all these theories need more investigations.

5.3. Comorbidity factors are associated with severe COVID-19

It was investigated that hypertension, diabetes, cardiovascular diseases and obesity are the most relevant factors for severe COVID-19 along with old age, male gender and smoking.

Hypertensive patients were found to be more susceptible to COVID-19 infection, due to the dysregulation in their renin-aldosterone-angiotensin system and most of them use ACE2 inhibitors which experimentally increase the receptor expression, as well as hypertension is related to immune dysfunction which increase the rate of severe disease.

In diabetic patients several factors affect the severity of the disease, such as low innate and adaptive immune response. Diabetic patients have down regulation in ACE2 receptors than normal, although this may decrease viral entry to the cells, ACE2 has some anti-inflammatory effects, so it is important to help in preventing the inflammation in the lung.

The presence of cardiovascular (CV) complication in patients with COVID-19 increase the rate of comorbidities and mortality, especially in those with severe manifestations.

Several mechanisms may be related to the severity of COVID-19 disease in CV patient with, especially in acute cardiac injury due to the increase in high-sensitivity cardiac troponin I (hs-cTnl) above 99. These mechanisms include direct myocardial involvement by COVID-19 using ACE2 in the heart. Other suggested mechanism of COVID-19 severity is cytokine storm due to irregular response of T-helper cells and systemic inflammatory activation or hypoxia that may induce excessive intracellular calcium causing myocardial cells apoptosis.

Obesity, which is responsible for a lot of metabolic disorder, which are directly or indirectly responsible for severe COVID-19 infection. Obesity enhances the development of cardiovascular disease. Formation of excess ectopic fat leads to insulin resistance which may develop type 2 diabetes mellitus (A risk factor for severe infection). It reduces pancreatic B-cells function through interaction of SARS-CoV-2 with ACE2 on these cells. Obesity also reduces the lung function, enhances chronic lung diseases such as asthma and increases rate of thrombosis; all these consequences are considered as comorbidity factors of COVID-19. ACE2 is highly expressed in adipose tissues especially visceral adipose tissues. The direct effect of obesity is through the adipose tissues that secret cytokines, chemokines, adipokines and leptin. In obese people, Treg cells produce low level of anti-inflammatory cytokines and more pro-inflammatory cytokines which keep low grade of inflammatory effect (higher than non-obese and lower than infected and traumatic patients). The increase of leptin is responsible for decrease of Treg cells level, leading to higher inflammatory effect. TNF-α, IL1 beta and IL-6 are present in high levels in obese patients.
6. Effect of immunosuppressants and antirheumatic drugs on the protection and treatment of severe COVID-19 cases

In view of the huge number of cytokines induced by SARS-CoV\textsuperscript{77}, MERS-CoV, and 2019-CoV infections, corticosteroids were used regularly for the treatment of patients with severe illness, for possible benefit by diminishing inflammatory-induced lung injury. But, modern evidence in patients with SARS and MERS intimates that taking corticosteroids did not have an influence on loss of the virus, but rather slowed viral clearance\textsuperscript{42,77}. In an observational study, patients who are hospitalized with severe symptoms of pneumonia and ARDs show improvement in the case and reduce the risk factor of death after administering low to moderate dose of methylprednisolone for short period of time\textsuperscript{31,75}. Corticosteroids are not associated with increased mortality of rheumatic patients with COVID-19 infection\textsuperscript{78}.

As the cytokine storm and excessive inflammatory and immune response are the reason for the severe disease, corticosteroids which are considered as potent anti-inflammatory should be with cautions as wrong use of corticosteroids may worsen the case and cause excessive immune response\textsuperscript{79}. Russell and his colleagues stated that the use of glucocorticoids is not preferred in viral infections causing lung inflammation\textsuperscript{79}. Autoimmune or rheumatic patients who require glucocorticoids in their treatment protocols, should not stop glucocorticoids during COVID-19 infection, lower or moderate doses are required for short courses\textsuperscript{80,81}. Until evidence-based guidelines are issued, case-to-case decisions should be taken for every patient\textsuperscript{80}.

The question is are patients receiving immunosuppressive drug protected from getting COVID-19 than normal population? Strong clinical studies are needed to answer this question. In Italy, Ferri and his colleagues (2020) reported higher prevalence of COVID-19 among patients with autoimmune disease e.g. rheumatic patients\textsuperscript{82}.

Other studies stated that there is no increased risk of SARS-CoV-2 infections in patients with autoimmune diseases when compared to normal people\textsuperscript{83}. In an Italian cohort study, they found that presence of comorbid diseases and older age are associated with the poor outcome rather than the type of rheumatic disease or administrated immunosuppressants\textsuperscript{84}.

In spite higher prevalence of COVID-19 in patients with autoimmune disease, many studies revealed that anti-rheumatic drugs such as hydroxyl-chloroquine and methotrexate may play protective role against harmful manifestation of COVID-19\textsuperscript{82,85}. Although patients who were receiving immunosuppressants as kidney transplant recipients are more vulnerable to severe COVID-19, most of them are recovered and achieved good prognosis\textsuperscript{86}. To reach the accurate fact regarding the outcome of COVID-19 in patients of autoimmune diseases who are receiving immunosuppressants, more evidence-based controlled trials should be done\textsuperscript{87}.

7. CONCLUSIONS

SARS-CoV-2 is a multisystemic highly virulent virus. In addition to lungs, SARS-CoV-2 affects other organs such as kidney, cardiovascular system and nervous system. SARS-CoV-2 causes imbalance between the innate and adaptive immune responses, which leads to the cytokine storm with its subsequent damage of lung cell and other organs which ends with death in some cases. COVID-19 severity is affected by genetic factors and presence of comorbid disease e.g. diabetes, cardiovascular disease, and obesity. Disease severity is also increased with smoking and male gender, as they are correlated with increased expression of ACE2 and TMPRSS2 receptors.

Strong clinical studies are needed to determine whether patients receiving immunosuppressive drugs are protected from getting COVID-19 than normal population, as the current available data is conflicting.

Conflict of interest
The authors declare that they have no conflict of interests regarding this study.

Author contribution
All authors have contributed in writing the manuscript. HFF & OKR participated in conceptualization. OKR was responsible for revising and editing the manuscript.

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**List of abbreviations:**

ACE2: angiotensin converting enzyme 2  
ADEM: encephalomyelitis  
AKI: acute kidney injury  
ARDS: acute respiratory distress syndrome  
COVID-19: Coronavirus Disease 2019  
CSF: cerebrospinal fluid  
ERGIC: endoplasmic reticulum Golgi intermediate compartment  
GBS: Guillain-Barré syndrome  
HLA-DR: human leukocyte antigen D related  
hs-cTnI: high-sensitivity cardiac troponin I  
INFs: interferons  
MAS: macrophage activation symptoms  
NK: natural killer cells  
ORFs: open reading frames  
PCR: polymerase chain reaction  
RdRp: RNA-dependent RNA polymerase  
RNA: ribonucleic acid  
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2  
TMPRSS2: transmembrane protease serine 2  

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