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Consideration of Severe Coronavirus Disease 2019 As Viral Sepsis and Potential Use of Immune Checkpoint Inhibitors

Zekaver Odabasi, MD[1]; Ismail Cinel, MD, PhD[2]

Abstract: Taking into consideration the multisystemic clinical and autopsy findings in "severe" coronavirus disease 2019 patients, viral sepsis would be a more accurate term to describe the whole clinical picture. The most significant pathophysiological components of this picture are intense cytokine release, prolonged inflammation, immunosuppression with T cell exhaustion, and the development of organ dysfunctions. Currently, the optimal treatment for severe coronavirus disease 2019 is uncertain. Supportive treatment and immunomodulators have a critical place in the treatment of severe patients until effective antivirals are developed. Interleukin-6 antagonists, one of the immunomodulating agents, appears to be effective in the treatment of cytokine storm, but some patients continue to have severe lymphopenia and immunosuppression. We believe it can be useful as immunomodulator therapy in critical coronavirus disease 2019 patients because of the benefits of immune checkpoint inhibitors in cancer and sepsis patients.

Key Words: acute respiratory distress syndrome; coronavirus disease 2019; lymphopenia; NKG2A; programmed cell death protein 1; sepsis

SEVERE CORONAVIRUS DISEASE 2019 AND VIRAL SEPSIS

Although the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to spread rapidly around the world, research is being actively conducted on the pathogenesis and treatment options for coronavirus disease 2019 (COVID-19), but there are still many unsolved aspects. Most of the patients infected with SARS-CoV-2 usually have a mild to moderate illness, but depending on the viral load, immune system, underlying comorbid diseases, or currently unknown factors, approximately 5% of patients develop the critical disease with respiratory failure and organ dysfunctions (1). Pneumonia is the most frequent serious manifestation of infection, while acute respiratory distress syndrome (ARDS) is the major complication in patients with severe illness. Other complications include as follows: coagulopathy, microvascular thrombosis (such as myocardial infarction and stroke), arrhythmias, acute cardiac injury, liver injury, acute kidney injury, and shock (1–5). In a series of 21 severely ill patients admitted to the ICU in the United States, ARDS was observed in most patients, and one-third developed cardiomyopathy (4). Human angiotensin-converting enzyme 2 (ACE2) is a functional receptor attacked by SARS-CoV-2 for cell entry, similar to SARS-CoV (6). ACE2 is broadly expressed in the nasal mucosa, bronchus, lung, heart, esophagus, kidney, stomach, bladder, and ileum, and these human organs are all vulnerable to SARS-CoV-2, especially in severe cases with viremia (7). Presumably, viral replication of SARS-CoV-2 in target organs, as well as resulting cellular damage, causes a systemic inflammatory response, cytokine storm, mainly ARDS, and multiple organ damage. Some autopsy studies support these findings. In an autopsy study of 21 severe COVID-19 patients, the primary cause of death was respiratory failure with exudative diffuse alveolar damage with massive capillary congestion accompanied by microthrombi (8). In another postmortem study conducted on 12 patients, a high concentration of SARS-CoV-2 RNA was detected in the lung tissue of all the patients and around half of them had high titers of viral RNA in the liver, kidney, or heart, in addition to viremia (9).

According to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), sepsis is defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection" (10). Considering the multisystemic clinical and autopsy findings in "severe COVID-19 patients," viral sepsis would be a more accurate term to describe the whole clinical picture (**Fig. 1**). To put it simply, if mild to moderate COVID-19 is considered

[1]Department of Infectious Diseases, School of Medicine, Marmara University, Istanbul, Turkey.

[2]Department of Anesthesiology and Intensive Care, School of Medicine, Marmara University, Istanbul, Turkey.

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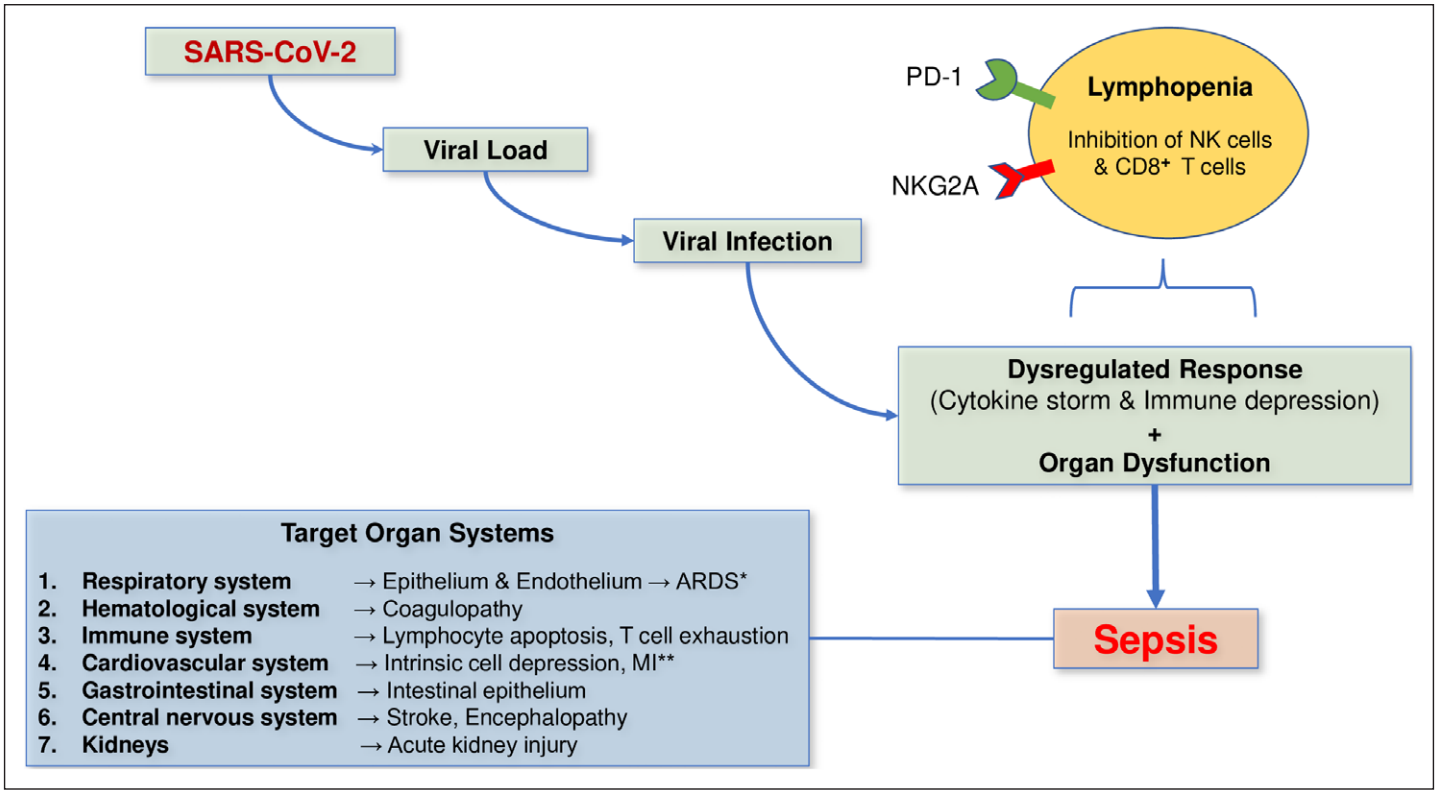

Figure 1. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, sepsis, and multisystemic effects. Severe SARS-CoV-2 infection due to high viral load causes immune dysregulation, suppression in CD8+ T cells and Natural Killer (NK) cells, and multisystemic effects due to viral sepsis. *ARDS = acute respiratory distress syndrome, **MI = myocardial infarction, PD-1 = programmed cell death protein 1.

"bad infection," then severe COVID-19 is "viral sepsis" or "bad viral sepsis." Viral sepsis can be caused by a variety of viruses such as herpes simplex virus, influenza, enteroviruses, human parechoviruses, ebola, and dengue virus (11). Common features of viral sepsis are intense cytokine release, prolonged inflammation, and consequently, immunosuppression, T cell exhaustion, the development of multiple organ damage, and increased susceptibility to secondary bacterial infections. A combination of these concepts has led to a new approach to severe COVID-19 patients as "a subset of sepsis," in which particularly profound cellular, immunologic, and metabolic abnormalities are associated with a higher risk of mortality than with infection alone.

THERAPEUTIC APPROACHES FOR SEVERE COVID-19 AND CYTOKINE STORM

Currently, the optimal approach to the treatment for COVID-19 is uncertain. We can classify treatment approaches under three main headings: potential anti-infectives, supportive treatments for specific target organ systems, and immunomodulators (**Fig. 2**). Understanding the mechanism of viral sepsis and associated immunosuppression in COVID-19 is vitally important. In this way, it will be possible to develop effective treatment approaches (12).

The identification of the toll-like receptors (TLRs) and the associated concept of innate immunity based upon pathogen-associated molecular pattern molecules allowed for significant advances in our understanding of the molecular biology and pathophysiology of sepsis (13). Previous studies revealed that lung epithelial cells, macrophages, and dendritic cells all express cytokines to some extent during influenza infection via the activation of pattern recognition receptors, including TLR3, TLR7, and TLR8, a retinoic acid-inducible gene I, and the nucleotide-binding oligomerization domain-like receptor family members (14). Cytokine storm, which is a key factor in the rapid progression of COVID-19, induces not only inflammation but also apoptosis-related immunosuppression (13, 15, 16). The primary features of the cytokine storm are fever, progressive dyspnea, tachypnea, elevated interleukin (IL)-6, C-reactive protein, ferritin, and other inflammatory markers (17, 18). The treatment of cytokine storm is of vital importance as it is associated with severe and mortal disease. Currently, there are no specific therapeutic agents for SARS-CoV-2 and cytokine storm induced by COVID-19. However, IL-6 receptor antibodies, as an immunotherapeutic agent, have been under compassionate use for cytokine storm treatment in COVID-19 patients.

IL-6 is a multifunctional cytokine produced by different cells such as immune cells, mesenchymal cells, endothelial cells, and fibroblasts in response to infections and tissue damage. Betacoronavirus infection of monocytes, macrophages, and dendritic cells results in their activation and the secretion of IL-6 and other inflammatory cytokines that result in cytokine release syndrome (CRS). IL-6 can signal through different pathways: classic signaling, trans-signaling, and trans-presentation (18). In classical signaling, IL-6 binds to the membrane-bound IL-6 receptor (mIL-6R, found mainly on macrophages, neutrophils, T cells, etc.) and shows pleiotropic effects on the acquired and innate immune

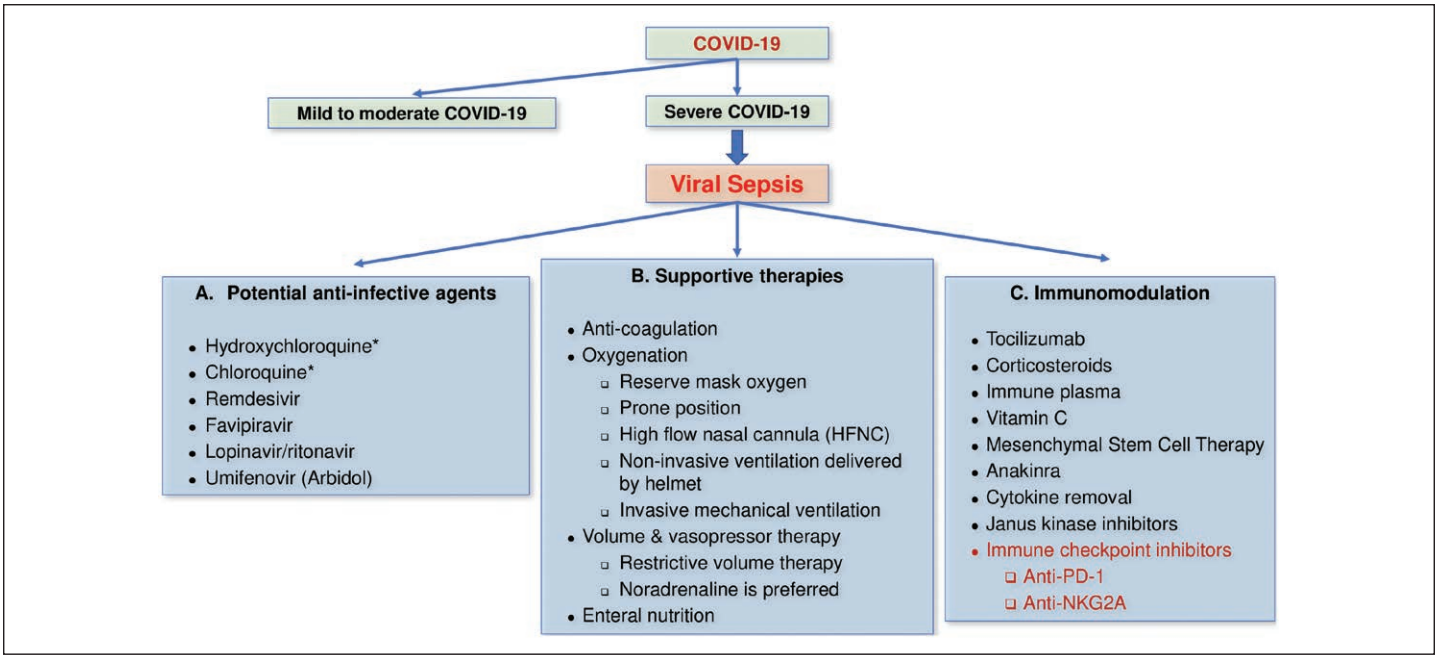

Figure 2. Potential therapeutic approaches that can be used in severe coronavirus disease 2019 (COVID-19) patients. *Hydroxychloroquine and chloroquine also have immunomodulatory effects. PD-1 = programmed cell death protein 1.

system. On the other hand, in the trans-signaling pathway, IL-6 forms a complex with soluble IL-6 receptor (sIL-6R) and can activate virtually all cells of the body, especially the endothelial cells, and develop inflammatory effects (19). Both classic- and trans-signals contribute to the development of cytokine storm, pulmonary dysfunction, and ARDS. CRS may also be the contributing factor to T cell exhaustion, apoptosis, and lymphopenia. Tocilizumab is a humanized anti-IL-6R monoclonal antibody that binds both mIL-6R and sIL-6R and then inhibits classical and trans-signals. A small clinical trial in China examined the effectiveness of tocilizumab in patients who met the criteria for severe COVID-19. After a few days of tocilizumab treatment, symptoms such as fever, pulmonary infiltrates, and oxygenation improved, and also the lymphocyte counts returned to normal (20). Unfortunately, despite the use of tocilizumab and antivirals, some severe COVID-19 patients still have lymphopenia, and the prognosis in these patients is poor. At this point, we need alternative therapeutic agents that will boost the immune system and correct the lymphopenia in severe COVID-19 patients.

T CELL EXHAUSTION AND IMMUNE CHECKPOINT INHIBITORS AS A THERAPEUTIC OPTION

CD8+ cytotoxic T cells and Natural Killer (NK) cells play a critical role in the protection and control of viral infections. Lymphopenia is commonly seen in patients with severe COVID-19 disease and may be a sign of poor prognosis (21). Immunopathologically, in severe COVID-19 patients, there is a marked increase in inflammatory cytokines (IL-2, IL-6) combined with lymphopenia (22). There was also an unexpected increase in anti-inflammatory cytokines such as IL-10 and IL-4, which is an uncommon phenomenon for acute-phase viral infection (17). As the severity of the disease progresses in patients with COVID-19, a concomitant rise in inflammatory cytokine levels may lead to the depletion and exhaustion of T cell populations. As a result, the adaptive immune response cannot be effectively initiated because of the substantial reduction and dysfunction of lymphocytes. The uncontrolled virus infection leads to more macrophage infiltration and a further worsening organ injury. The degree of lymphopenia has been shown to correlate with the severity of COVID-19 and the mortality of septic shock (21, 23). A recent study conducted by Zheng et al (24) showed that the total number of NK cells and CD8+ T lymphocytes had decreased significantly in severe COVID-19 patients. Furthermore, it showed that NKG2A expression had significantly increased in immune cells whose functions were suppressed, a picture consistent with T cell exhaustion. Furthermore, the number of NK and CD8+ T cells had increased in recovering patients, while the expression of NKG2A had decreased.

Similarly, a previous study has shown that NKG2A receptors on cytotoxic T lymphocytes are over-upregulated during acute polyomavirus infection. Consequently, there was a noticeable decrease in the cytotoxic cellular immune response to prevent viral clearance and viral oncogenesis (25). T cell exhaustion and similar findings were also seen in HIV, chronic hepatitis B, and hepatitis C infections (26). It is well known that patients with sepsis have an increased checkpoint molecule expression—cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death protein 1 (PD-1)—and this condition causes lymphopenia (27–29). In another study, which involved patients with *Candida* bloodstream infection, circulating immune effector cells displayed an immunophenotype consistent with immunosuppression, as evidenced by T cell exhaustion and the number of PD-1 positive CD8+ T cells that had significantly increased (30). Taken together, these observations point to a common cascade of events during viral and/

or bacterial sepsis which leads to increased checkpoint molecule expression and T cell exhaustion.

Developing an efficient immunotherapeutic approach to restoring cell-mediated immunity may play an essential role in overcoming severe COVID-19. When we act on the logic of immune checkpoint inhibitors applied in cancer treatments, we believe that the inhibition of NKG2A receptors, which are upregulated in COVID-19, will boost the antiviral activity of cytotoxic T cells and NK cells. The immune checkpoint molecules, CTLA-4, and PD-1 are potent immunomodulators with their inhibitory effects on T cell activation. Cancer cells and presumably cells infected with viruses produce ligands that stimulate inhibitory checkpoints and inhibit the activity of T cells. When these checkpoints are blocked, T cells are able to kill cancer cells and virally infected cells more strongly. Currently, many monoclonal antibodies are targeting these immune checkpoints that have been used in cancer treatment (31). Immune checkpoint inhibitors may also increase absolute lymphocyte count in cancer patients, and this finding is a good prognostic factor and sign of response to treatment (32). In this regard, upon extensive literature search, monalizumab caught our attention as a novel immune checkpoint inhibitor developed against NKG2A receptors (33). Monalizumab is a humanized anti-NKG2A monoclonal antibody that can increase the degranulation of NK cells and hence the production of interferon-gamma that is a vital cytokine for natural and adaptive immunity against viral infections (34).

We herein propose that a combination of NKG2A inhibitor as an immune system booster with IL-6 receptor antibody as an anti-inflammatory agent may be beneficial in severe COVID-19 cases. Inhibition of PD-1 and programmed cell death ligand 1 (PD-L1) has been shown to improve pathogen clearance in viral infection models (35). Hotchkiss et al (36) hypothesized that by blocking PD-1 or PD-L1, antibody-mediated immunotherapy can reverse T cell depletion-mediated immunosuppression in critically ill patients with sepsis. In their clinical evaluation of PD-1/PD-L1 pathway inhibition in sepsis, monoclonal antibodies against PD-1/PD-L1 were well tolerated, with no evidence of drug-induced hypercytokinemia or cytokine storm, and at higher doses, some indication of restored immune status.

Currently, there are a few clinical studies registered to clinicaltrials.gov that are aimed at evaluating the efficacy of antibodies against PD-1 receptors in COVID-19. We urgently need to consider the use of proven immunomodulatory agents in the treatment of severe COVID-19 sepsis until effective vaccines and antiviral drugs are developed.

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For information regarding this article, E-mail: zekaver@marmara.edu.tr

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