

# *Interplay of Different Parameters in COVID-19 Infection and Novel Results of Possible Prevention and Treatment (Review of the Literature)*

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**Abstract – Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the resulting coronavirus disease 2019 (Covid-19) remains a global challenge. There are intense studies for finding effective prevention and treatment options worldwide. Therefore, infection prevention, prognostic parameters of infection complication and identification of successful treatment protocols need more investigations.**

**Interpretation of laboratory parameters may support clinicians in prevention and treatment of the disease, will improve COVID 19 infection outcomes and may potentially decrease the death rate overall.**

**Study results have shown that different laboratory parameters during the disease course are significantly changed. In critically ill patients, coagulation complications and coagulation/anticoagulation imbalance are characteristic also. According to the research results, vaccination offers the best option for COVID-19 control.**

**In this review, some parameters, current therapeutic options, preventive methods of COVID 19 infection are discussed.**

**Keywords – COVID-19 infection, SARS-CoV-2 virus, laboratory parameters, prevention, treatment.**

Coronavirus disease 2019 (COVID-19) - as a new infection was firstly described in Wuhan, China in December 2019. [41]. The causative agent was SARS-CoV-2 virus similar to Severe acute respiratory syndrome coronavirus (SARS-CoV) reported before in November 2002 to June 2003 in China. [60]. SARS-CoV-2 started with local, later with epidemic outbreak and has rapidly spread all over the world reaching pandemic. Consequently, WHO declared the outbreak as a Public Health Emergency of International Concern on January 30, 2020, and a pandemic on 11 March [2,15,74].

SARS-CoV-2 invades host human cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor [31]. The spike glycoprotein (glycoprotein S) on the virion surface targeting towards ACE2 receptor is essential in the process of SARS-CoV-2 infection. Down-regulation of ACE2 receptors and systemic renin-angiotensin system imbalance occurs promoting of multi-organ failure [1,6,27,29,51].

This replication cycle of the virus has numerous steps: attachment and entry, genome transcription and replication of the virus, structural protein translation, accumulation and release of the virus [41,43].

COVID-19 is basically considered as a respiratory tract infection, but apparent data shows that it should be viewed as a polysystemic disease involving multiple systems (cardiovascular, respiratory, gastrointestinal, neurological and immune systems) [4,31,52,59]. Elderly people, especially with comorbidities are at increased risk of lethal outcome from COVID-19, though, should

be noted, that youngers without considerable underlying diseases may also develop potentially lethal complications such as fulminant myocarditis and disseminated intravascular coagulopathy (DIC) [37,58].

After approximately 5–6 days of symptoms debut, SARS-CoV-2 viral load reaches its highest point. According to statistics, disease may progress to acute respiratory distress syndrome, averagely after 8–9 days after symptom onset [62,65,69,70].

Different studies revealed numerous hematologic findings and complications of COVID-19. In the incubation period (1 to 14 days), or the early phase of the disease, with non-specific symptoms peripheral blood leukocyte and lymphocyte counts are normal or slightly reduced. Gradually, along with viremia, SARS-CoV-2 mainly attacks those tissues with high levels of ACE2 (lungs, heart and gastrointestinal tract). Mostly, 7 to 14 days from the initial symptoms, there is an efflux in the clinical manifestations of the disease [16].

The clinical manifestations of COVID-19 are various starting with mild upper respiratory tract infection, lower respiratory tract infection including non-life-threatening pneumonia, and life-threatening pneumonia with acute respiratory distress syndrome. During hospitalization COVID-19 patients mostly show the following symptoms: impaired smell and taste, fever, coughing, dyspnea, general weakness, myalgia, diarrhea, severe lymphopenia, prolonged coagulation profiles. Neutrophilia and lymphopenia may correlate with the disease severity and mortality in COVID-19 patients [58, 63,67,70].

Moreover, patients requiring intensive care units (ICU) care have increased plasma levels of several innate cytokines e.g. TNF $\alpha$  [24, 50]. The immune response to SARS-CoV-2 indicate equity between protective effects and harmful pathological reactions [4].

This is with a prominent systemic increase of inflammatory mediators and cytokines that indicates a “cytokine storm” [30]. In this stage, significant lymphopenia develops. Several factors cause COVID-19 associated lymphopenia. It has been shown that lymphocytes express the ACE2 receptor on their surface [72]; thus SARS-CoV-2 may directly invade those cells and consequently lead to their degradation. The cytokine storm is characterized by remarkable increased levels of interleukins (mostly IL-6, IL-2, IL-7, granulocyte colony stimulating factor, interferon- $\gamma$  inducible protein 10 and tumor necrosis factor (TNF)-alpha, which may promote lymphocyte apoptosis [2,32,47,54].

Another reason is that substantial cytokine activation may also be associated with atrophy of lymphoidorgans, including the spleen, and further impairs lymphocyte turnover [9]. Coexisting lactic acidosis is more apparent among cancer patients who are at increased risk for complications from COVID-19 [73] may also inhibit lymphocyte proliferation [17].

Patients with severe COVID-19 infection, in ICU exhibit higher blood plasma levels of IL-2, IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), IP-10, MCP1, macrophage inflammatory protein 1 $\alpha$  (MIP1 $\alpha$ ) and tumor necrosis factor (TNF) [24]. IL-6 levels in these patients increase along with time and are in higher figures in non-survivors rather than in survivors [78].

Previously, some correlations were found between clinical symptoms of common cold and altered zinc homeostasis. Results indicate that zinc may prevent or reduce those symptoms. Studies showed decreased symptom severity, frequency, and duration of the common cold after zinc administration [12,22,24,25] depending on dosage, zinc compound and the start time after initial symptoms [64].

Several studies reveal melatonin to be an effective antiviral agent in COVID-19 pandemic [3]. Despite of significant increase of inflammatory cytokines, COVID 19 induces reactive oxygen species, meanwhile melatonin, excellent natural antioxidant and anti-inflammatory-cytoprotector, is very low in elderly patients [57]. In severe cases of COVID-19 melatonin stimulates immunity. Therefore, as considered, the use of melatonin in proper doses can potentially avoid the development of severe symptoms in coronavirus patients, reduce the severity of their symptoms, and/or reduce the immuno-pathological consequences of infection in convalescent patients. Some studies also suggest that in addition, melatonin may help to prevent reinfections and serve as a powerful immunomodulator for future vaccines [6].

Currently, there are more and more investigations directed to the survey of Vitamin D and its possible use as treatment in COVID 19 patients. There are reported some retrospective cohort studies indicating deficient vitamin D status was associated with increased COVID-19 risk, though more randomized trials are needed to detect whether vitamin D affects COVID-19 risk [39,40].

Mentioned before, coagulation complications and coagulation/anticoagulation imbalance together with thrombocytopenia is mainly associated with the intensive symptoms of the COVID-19 disease; significant drop in platelet count was observed especially in non-survivors. [11,34,78].

Meanwhile, the D-dimer and the surveillance of its dynamics became one of the most widespread and notable indicators for thrombogenesis in covid 19 patients. Before SARS-CoV-2 outbreak, numerous data has shown D-dimer increased levels were associated with unfavorable outcomes among patients with community-acquired pneumonia [55]. In another previous studies in community-acquired pneumonia (CAP) and chronic obstructive pulmonary disease (COPD) patients have revealed that D-dimer level is increased in their severe cases and may be used as one of the prognostic biomarkers [19,47,55]. D-dimer > 1 µg/ml is mentioned to be one of the risk factors of mortality in adult inpatients with COVID-19. [74].

Novel study results showed that D-dimer and prothrombin time (PT) levels were higher on admission among COVID-19 patients requiring ICU support [24]. Patients requiring ICU treatment had significantly higher D-dimers compared with less severe cases [63,64].

Patients with several cardiac injury in the context of COVID-19 infection are more inclined to coagulation disorders compared with those without cardiac involvement. Also patients with high troponin-T levels often represent elevated PT, activated partial thromboplastin time, and D-dimer [21,53]. The risk of venous thromboembolism (VTE) in hospitalized COVID-19 patients is highly important issue. The rate of symptomatic VTE in hospitalized acute patients reaches even 10% [28]. Another important concern is a high platelet to lymphocyte ratio indicating more noticeable cytokine storm, due to enhanced platelet activation [46]. Extended immobilization during the disease, dehydration, other cardiovascular risks (ie, hypertension, diabetes, obesity, coronary artery disease, history of ischemic stroke or peripheral artery disease, previous history of VTE and classical genetic thrombophilia, such as Factor V Leiden mutation are comorbidities found in hospitalized COVID-19 patients that potentially increase VTE risk [16]. Endothelial cell activation/damage due to ACE receptor may further increase VTE risk. The release of numerous inflammatory mediators and the use of hormones and immunoglobulins in severe or critically ill patients may promote an increased blood viscosity also. Moreover, mechanical ventilation, central venous catheterization may induce vascular endothelial damage. The combination of all the above factors may lead to DVT (deep venous thrombosis) occurrence or even the possibility of lethal outcome due to thrombus detachment and migration [14]. Melatonin does not have viricidal properties; however, it has indirect antiviral effects through anti-inflammatory, antioxidant and immunity-enhancing effects [76]. Previously, melatonin was shown to suppress the features of viral infections [36].

SARS-CoV-2-ACE2 interaction has generated great interest in the development of renin-angiotensin system-based therapeutic strategies for COVID-19 treatment. In general, the renin-angiotensin system activation induces vasoconstriction, hypertension, inflammation, fibrosis, and proliferation via the ACE/angiotensin II/angiotensin II type 1 receptor (AT1R) axis and induces the opposite effects via the ACE2/angiotensin (1-7)/Mas axis function [1,51].

Melatonin is an effective inhibitor of the angiotensin II activation and presumably facilitates angiotensin (1-7) action [26,48]. Thus, the two hits to the renin-angiotensin system can both be inhibited by melatonin administration.

We can summarize that although there is no completely clear immunopathogenetic mechanisms of COVID-19 infection, several viral, and host-related parameters are pivotal and have high prognostic value of convalescence and survival of the patients. So called out-of-control immune response to the SARS-CoV-2 virus, hematologic parameters change and consequent coagulation impairment may lead not only to pulmonary tissue injury but systemic organ failure also. Due to current corona pandemic issue and rapid spread of affected people, better understanding of the interactions among host parameters and viral factors of SARS-CoV-2, safe and globally available treatment of the disease is extremely needed.

New approaches of treatment of acute and chronic infections in addition involve also management of neurological and mental manifestations, noncommunicable diseases, rehabilitation, palliative care, ethical principles [67]. The great concern is the case of absence of vaccination, the lack of medicines and specific treatment methods, therefore, the ways to solve the problem at this stage are aimed at taking defensive measures against coronavirus [35,36].

Social distancing, hand hygiene and quarantine are considered to be the main points in this regard. Increased testing and detecting more positive cases will also serve to decrease of secondary transmission throughout society [23,62].

Together with investigations of SARS-CoV-2 virology, main pathogenetic mechanisms and immunological responses underlying the clinical manifestations of COVID-19 is essential for determination of immunoregulation and rational therapies against SARS-CoV-2 [8,24,43,49].

The release of cytokines in response to the viral infection and/or secondary infections can result in a cytokine storm and symptoms of sepsis that are the cause of death in 28% of fatal COVID-19 cases [71]. Thus, uncontrolled inflammation leads to multi-organ failure, especially of the cardiac, hepatic and renal systems.

Ways to overcome the pandemic: The immune layer or the so-called "Development of herd immunity" (By infecting large numbers of the population or by vaccination) and creating a new target medication or selection from existing antiviral or antiprotozoal agents or antibiotics or their optimally effective combinations (Hydroxychloroquine, Hydroxychloroquine+Azithromycin, Remdesivir, Ritonavir / Lopinavir, Ritonavir / Lopinavir with Interferon Beta, IL-6 pathway inhibitors - Tocilizumab, Favipiravir, Interferon beta etc.) From the randomized clinical trials conducted to date, the above drugs and drug-combinations have not yet shown statistically significant efficacy in reducing both hospitalization days and mortality rates and some mediate severe toxicities and side effects, especially in patients with concomitant diseases [44,56].

A recombinant new potential drug of angiotensin converting enzyme (ACE2) lowers infection and virus growth in cell cultures and organelles; in particular, it is a trap for SARS-CoV-2 virus. Modern research has shown that a potential drug can inhibit the growth and development of the virus by inhibiting specific proteins that bind to the human angiotensin-converting enzyme (ACE2) and reach the cell [29,76].

As it turned out, hrsACE2 has a dose-dependent effect on the growth and development of the virus, and in cell cultures it has been reduced by 1000-5000 times, addition of hrsACE2 to cell cultures reduced the concentration of the virus in the organelles in multi organ damage. A copy of the angiotensin-converting enzyme hrsACE2 is a bait for the virus, in particular, the virus binds to it instead of the endogenous angiotensin-converting enzyme, which "misleads" the virus and prevents from infecting normal cells, especially in the lungs and kidneys [45,75]. These studies are limited by cell cultures and organelles. The scientists involved in the study conducted pilot clinical trials in patients infected with COVID-19 in China with a potential drug (APN001) containing hrsACE2 as the active ingredient. This potential drug has already undergone the second phase of clinical trials. The effectiveness of the drug has been studied only in the early stages of disease development, which requires further studies at later stages [13,14,38].

Other important and noteworthy issues related to the treatment of Covid-19 is monitoring of the following clinical variables: general blood test, creatinine kinase, C-reactive protein, ferritin, prothrombin time, partial thromboplastin time, D-dimer, fibrinogen, lactate dehydrogenase, troponin and electrocardiogram [5,20,27].

The following considerations should be taken into account in the treatment and management of COVID-19 infection:

- Empiric treatment for bacterial pneumonia in selected patients;
- Prevention of and evaluation for venous thromboembolism;
- Uncertainty about NSAID use;
- Avoiding nebulized medications;
- Limited role of glucocorticoids;
- Managing chronic medications: ACE inhibitors/ARBs, Statins.

Vaccination probably offers the best option for COVID-19 control. It is undoubtedly the most effective widespread opportunity of fighting against the infection. Today there are different types of vaccines and mass vaccination of the world's population has already begun. There are some vaccines already available today [66].

1) Nucleic Acid Vaccines - Such vaccines contain genetic material - DNA (deoxyribonucleic acid) or RNA (ribonucleic acid), which contains instructions for creating the virus antigen (the protein or protein component of the virus). The advantages of such vaccines are as follows: 1. They can be created relatively quickly and easily, Since the antigen is produced by a large number of cells in the human host, the immune response is strong. 2. It does not contain living components, so there is no risk of disease.

The negative side of this group of vaccines includes: 1) usually requires low temperatures for transportation and storage, 2) has never been licensed for large-scale vaccination in humans, and 3) usually requires repeated dosing (so-called "boosting").

2) RNA vaccines, given their mechanism of action, should be considered less dangerous than DNA vaccines. As DNA is a much stable molecule than RNA, transportation and storage of DNA vaccines can be more easily managed. However, in order to assess the effectiveness of the vaccine, logistical issues alone should not be taken into account, and first of all, it is vital to consider the long term consequences of the vaccine and its effects on the human body.

3) Viral vector-based (carrier) vaccines - for the synthesis of virus (in the case of corona virus) antigen. The advantages of such vaccines are as follows: 1. this technology has been elaborated already around decades, 2) triggers a strong immune response, 3) does not require special conditions for transportation and storage. The disadvantage of these vaccines is that its production is more complicated as it requires more complex technology.

4) Protein containing vaccines - such vaccines contain a purified part of the virus (subunit) that can trigger an immune response in the body but does not contain the whole virus and therefore cannot cause an infectious process. This so-called non-cellular or acellular type vaccines have already been used in medical practice for long time (e.g., hepatitis B vaccine, pertussis and pneumococcal vaccines). The advantages of this group of vaccines are: 1) extensive experience in their use (including for large scale vaccination), 2) many years of experience in the safety of such vaccines for immunocompromised patients, 3) due to their non-cellular nature, inability to cause infectious processes, 4) relative stability, which facilitates logistical problems. Disadvantages of this group of vaccines: 1) complexity of its production - therefore, less flexibility in terms of production, 2) requires repeated vaccination (boosting) and the use of adjuvants (immune response enhancer molecules), 3) antigen combinations identification requires time and resources and may become an obstacle in terms of synchronizing with the emergence of new genetic variants of the virus.

5) Vaccines containing whole particles of the virus. Such vaccines can be divided into two subgroups: 1. Live-attenuated and 2. Vaccines containing inactivated viral particles. The advantages of these vaccines are: 1) Extensive experience in their application (including for largescale vaccination), 2) Ability to elicit a strong immune response, 3) Relative ease in production, 4) Relative stability, which alleviates logistical problems. The disadvantages of this group of vaccines are: 1) live vaccines are not designated for immunocompromised patients, 2) in rare cases, they may aggravate the existing infectious process.

From the above mentioned vaccines the 3rd phase of the study have reached: Nucleic Acid Vaccines (Pfizer-BioNTech, Moderna – approved by US (FDA) and EU ), Viral vector-based (carrier) vaccines (Oxford-AstraZeneca – approved by UK and India, Gamaleya – approved by Russia, CanSino – approved by China), Protein containing vaccines (Novavax, Vector Institute – approved by Russia), Vaccines containing whole particles of the virus (Sinopharm, Sinovac, Sinopharm-Wuhan, Bharat Biotech – approved by china) [42,77].

Based on the latest research, scientists say that there may be mutated variants of the corona virus against which existing vaccines will be ineffective and will need to be updated [7,33]. The COVID-19 vaccine may spell the end of the pandemic. But until large scale vaccination is not available, it is vital to stop community transmission. In early tests on animals, the new antiviral drug MK-4482 / EIDD-2801 or Molnupiravir, has managed "complete" suppression of the coronavirus transmission in just 24 hours. Molnupiravir was originally designed to treat the flu and prevent the virus from making copies of itself, creating errors during viral RNA replication. Reduction in the amount of COVID 19 particles was observed during the trials [10].

As it is known, acute COVID-19 patients with associated systemic inflammatory response may lead to multisystem damage. The use of corticosteroids (glucocorticoids) according to modern guidelines has been shown to be highly anti-inflammatory. Based on the results of randomized studies, it was shown that the mortality rate was significantly reduced in patients receiving dexamethasone compared with patients receiving COV-19 standard treatment. In particular, the benefits of glucocorticoid use were observed in patients who were on artificial respiration (i.e., artificially supplied with oxygen), while the benefits of dexamethasone were not observed in patients who did not require artificial respiration [18,61,68].

In conclusion, COVID-19 disease has manifestations from the hematopoietic system associated with a major blood hypercoagulability. Evaluation of laboratory parameters during the disease course may support clinicians in providing spot on treatment and intensive care to critically ill patients especially. Thromboprophylaxis and timely detection of life threatening complications including DIC in will improve COVID 19 infection outcomes, and may potentially decrease the death rate overall and among infected patients without significant comorbidities.

Studies have shown that levels of lactate dehydrogenase, aspartate aminotransferase, C-reactive protein, creatine kinase, D-dimer, procalcitonin, creatinine and some other parameters increases. Decreases in hemoglobin, lymphocyte count, eosinophil count, and serum albumin have been detected in COVID-19 patients. Neutrophilia and lymphopenia, coagulation complications and coagulation/anticoagulation imbalance together with thrombocytopenia are characteristic also, especially in severe clinical cases.

Currently treatment and management of COVID-19 patients during all phases of their disease has been expanded to meet the needs of front-line clinicians and promotes a multi-disciplinary approach to care for patients with COVID-19, including those with mild, moderate, severe, and critical disease.

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