

Pharmacologic Modulation during Cytokine Storm in COVID-19

Luiza Gabunia^{a*}, Shrena Khetsuriani^b, Natia Gamkrelidze^c, Levan Ratiani^d,

Natalia Filipets^e, Levan Metreveli^f, Nodar Sulashvili^g, Natia Antia^h

^{a,b,c,g,h}Tbilisi State Medical University, Georgia, Tbilisi, Georgia

^dThe First University Clinic, Georgia, Tbilisi, Georgia

^eBukovinian State Medical University, Chernivtsi, Ukraine

^fUniversity of Georgia, School of Health Sciences, Tbilisi, Georgia

^gTbilisi Open University, Tbilisi, Georgia

^hEmail: mantskavamaka@bk.ru

Abstract

The novel Coronavirus -SARS-CoV-2 activates macrophages in order to liberate cytokines into the bloodstream. Inflammatory cytokine secretion increases producing cytokine storm. In this phase significant lymphopenia occurs. Cytokine storm in response to the viral infection can result in sepsis. Ferritin is an important indicator of immune dysregulation, extreme hyperferritinemia, via direct immune-suppressive and proinflammatory effects, contributes to the cytokine storm. There are discussed some clinical evidences that indicate the importance of anti-inflammatory and cytokine-targeted therapies. These approaches are: inhibition of IL-1, IL-6, TNF- α and IFN- γ . Alternative treatments for cytokine storm - therapeutic plasma exchange and the mode of action of colchicine, glucocorticoids, and intravenous immunoglobulin - are also discussed. Convalescent plasma, anti-inflammatory treatment, together with anti-viral therapy becomes an essential part of treatment for COVID-19 infection.

Key Words: COVID-19 infection; SARS-CoV-2; cytokine storm; anti-inflammatory therapy; cytokine-targeted therapy.

1. Introduction

SARS-CoV-2 coronavirus was spread worldwide and on March 11th, 2020 the World Health Organization declared COVID-19 as pandemic disease. Special higher cases of deaths were observed in elderly patient's with concomitant diseases such as hypertension, cardiovascular disease (CVD), diabetes mellitus.

* Corresponding author.

Considering pathogenetic mechanism of the virus, it goes into the cell using angiotensin-converting enzyme-2 (ACE-2), basically through the Toll-like receptor-7 (TLR-7) expressed in endosomes.

TLR-7 activation on itself stands in need of production of TNF- α , IL-6 and IL-12, to enable production of specific cytotoxic CD8⁺ T cells. These cytokines specially target the lung cells [1].

The viruses activate macrophages in order to liberate cytokines (IL-1, IL-6, and TNF α) and chemokines (CXCL10 and CCL2) into the bloodstream. These substances result in vasodilation with consequent increased capillary permeability. Plasma leaked out into the interstitial spaces will collect around the alveoli and will cause their compression. As a result, there is a decrease in surfactant levels in Alveolar type 2 (AT2) cells. The events eventually assist alveolar collapse and impair diffusion of gases. In parallel, inflammatory cytokine secretion increases producing cytokine storm. CD4⁺ T helper (Th1) cells cause recruitment of neutrophils and macrophages by means of IL-17, IL-21, and IL-22. Later, along with disease progression, all these steps lead to shortness of breathing with cough and consequent hypoxemia [1].

Extreme and systemic release of inflammatory mediators and cytokines indicates a “cytokine storm”. Different factors are considered to be a cause of COVID-19 associated lymphopenia. One of them is the fact that lymphocytes express the ACE2 receptors on their surface. Additionally, SARS-CoV-2 may directly invade those cells and consequently lead to their degradation. Significant increased levels of interleukins (mostly IL-6, IL-2, IL-7), granulocyte colony stimulating factor, interferon- γ , inducible protein 10 and tumor necrosis factor (TNF)- α may also lead to lymphocyte apoptosis [2].

Furthermore, it is also declared that lethal outcomes by COVID-19 coincide with cytokine storm syndrome, along with it, studies suggest that disease severity in general coexists with cytokine storm syndrome [2].

Intensive care units (ICU) patients having COVID-19 infection reveal increased levels of some innate cytokines e.g. TNF α .

Serum ferritin, prognostic marker of complication of viral infection generally allies with hard up recovery of COVID-19 patients. The patients having lung tissue damage are more prone to having high levels of ferritin [2].

When intracellular iron concentration increases where iron is being stored in the form of ferritin, it is subsequently pushed out from the cell. Free iron together with hyperferritinemia due to iron dysregulation and overload in COVID-19 patients may enhance inflammatory processes by means of reactive oxygen species (ROS) induced oxidative injury of cellular biomolecules [3].

COVID-19 patients with different cardiac injury are more prone to several types of coagulation disorders compared with those without cardiac involvement. In the same time, patients having high troponin-T levels represent elevated PT, activated partial thromboplastin time, and D-dimer.

Venous thromboembolism (VTE) in hospitalized COVID-19 patients is an extremely important issue. Symptomatic VTE in hospitalized acute patients is nearly 10% [4].

Further disquiet is high figures of platelet/lymphocyte ratio designating more noticeable cytokine storm due to increased platelet activation. Prolonged immobilization during the disease, dehydration, other cardiovascular risks like obesity, hypertension, diabetes, coronary artery disease, previous history of VTE and genetic thrombophilia, e.g. Factor V Leiden mutation potentially increase VTE risk in hospitalized COVID-19 patients [4].

ACE receptor mediated endothelial cell activation and damage may additionally increase VTE risk. Inflammatory mediators and the use of hormones in severe or critically ill patients may contribute to an increased blood viscosity also. Besides, mechanical ventilation or central venous catheterization may produce vascular endothelial damage. The union of all the above may potentiate DVT (deep venous thrombosis) occurrence or even the fatal outcome development due to thrombus detachment and migration [4].

Researchers revealed that Severe COVID-19 patients often present underlying diseases such as diabetes, hypertension, smoking, chronic obstructive pulmonary disease, coronary vascular disease (CVD) [5].

Especially in elderly, there is an increased incidence of hypertension, obesity, and Diabetes Mellitus (DM). The consequences of all above mentioned may potentially increase mortality and morbidity in SARS-CoV-2 individuals.

Moreover, patients with one comorbidity, frequently have others also. According to research data, it is not completely understandable how CVD, extreme obesity, and hypertension in DM patients impact the SARS-CoV-2 infection progression. Though, the high plasma glucose levels and DM are considered to be predictive for mortality and morbidity of SARS-CoV-2 infection [5]. One possible explanation of high mortality and morbidity in DM patients with accompanying CVD, hypertension and severe obesity could be the increased viral load through ACE2 receptors in the pancreas, heart, and kidney. There could be reduced viral clearance, T-cell immune dysfunction, and hyperactivation of inflammatory signaling cascades. There is an increased expression of ACE2 in rodent models of DM. Increased ACE2 multiplies the ability of SARS-CoV-2 to enter cells. Insulin treatment reduced ACE2 expression, hypoglycemic agents (glucagon-like peptide – 1, liraglutide, and pioglitazone), anti-hypertensive agents (like ACE inhibitors), and statins heighten the ACE2 expression [5]. Cytokine storm in response to the viral infection and/or secondary infections can result in sepsis that is the cause of death in 28% of lethal COVID-19 cases. As known, hypothalamus regulates body temperature. The released IL-1, IL-6, and TNF- α trigger the release of prostaglandin, PGE₂, and causes an increase of set point and consequently, body temperature. All these abnormal inflammatory responses and symptoms can help to septic shock development. Due to pneumonia, there is hypoxia. Also vasodilation decreases peripheral resistance and circulated blood volume leading to hypotension, reduced perfusion rate of the heart. So, uncontrolled inflammatory response leads to multi-organ failure, concretely of the cardiac, hepatic and renal systems [6]. Vitamin D exhibits anti-inflammatory and immunomodulatory response together with being regulator of bone homeostasis. Hospitalized patients with COVID-19 markedly express vitamin D deficiency associated with severe/critical COVID-19 cases and increased in-hospital mortality. Different studies revealed that vitamin D and DPP-4 inhibitors prevent cytokine storm and complications of the disease in patients infected with Covid-19, especially in diabetes mellitus and arterial hypertension patients. Administration of vitamin D can be also

effective in activating viral clearance in acute respiratory distress Covid -19 patients [7]. Recent reports provide guidelines for timely identification of cytokine storm and immediate start of treatments to reduce severity are essential for the treatment of severe COVID-19. Some clinical evidences indicate the importance of anti-inflammatory therapy in severe COVID-19. Several therapeutic strategies are being used to treat the cytokine storm associated with COVID-19, expectations are immensely high for new cytokine-targeted therapies. These approaches are: inhibition of IL-1 (Anakinra, Canakinumab); inhibition of IL-6 (Tocilizumab, Sarilumab, Siltuximab); inhibition of TNF- α (Etanercept); inhibition of JAK (Baricitinib, Ruxolitinib); inhibition IFN- γ (Emapalumab); Colchicine; Glucocorticoids; Intravenous immunoglobulin; Convalescent plasma So, anti-inflammatory treatment, together with anti-viral therapy becomes an essential part of treatment for COVID-19 [8].

Inhibition of IL-1 signaling: IL-1 is an important pro-inflammatory cytokine. It has two types of ligands, IL-1 α and IL-1 β , where IL-1 β plays main role in systemic effects production. It is majorly synthesized by innate immune cells, like macrophages and monocytes. IL-1 recruits immune cells and induces secondary cytokine production resulting in acute phase reactions. Some studies declare beneficial effects with the use of anakinra in COVID-19. High-dose anakinra was used safely and improved respiratory function. In another prospective cohort study COVID-19 pneumonia patient taking anakinra demonstrated reduced need for mechanical ventilation and decreased mortality. Further validation is needed for both studies through Randomized Clinical Trials [9].

Inhibition of IL-6 signaling: IL-6 – this pro-inflammatory cytokine has multiple effects. It is triggered by infection or tissue injury and rapidly launches acute reactions. IL-6 promotes synthesis of different acute phase proteins by hepatocytes and facilitates differentiation of B and T cells. Two IL-6 inhibitors are under trial in treatment of COVID-19. In a few case series, sarilumab and tocilizumab have shown beneficial effects in decreasing severity and mortality in critically ill COVID-19 patients. A recent retrospective cohort study showed that tocilizumab significantly reduced the mechanical ventilation risk or death. In another study of 154 ICU patients requiring mechanical ventilation, tocilizumab minimized the risk of death - by 45%. Though study also revealed that tocilizumab increased the risk of superinfection significantly. Furthermore RCTs are required to validate these clinical effects [10].

Inhibition of TNF- α signaling: TNF- α - a cytokine primarily produced by activated macrophages in the acute inflammatory response. In some trials TNF- α was first to be elevated among different cytokines. Evaluating the role of TNF- α in acute inflammation, TNF- α blockade should have promising effects in various cytokine storm conditions. Another study revealed that TNF- α mediated acute lung lesion was minimized by using an aptamer targeting TNF- α . As acute lung injury is a main characteristic in COVID-19, this study should provide a possible therapeutic application in treating COVID-19 cytokine storm [11].

Inhibition of IFN- γ signaling: IFN- γ is released by macrophages, NK cells, and T cells, and it has direct stimulatory effect on main inflammatory effector cells, due to which IFN- γ is thought to be a major effector cytokine in different cytokine storm cases. So far there has been no significant trials supporting the use of IFN- γ inhibitors in COVID -19 patients. Some researchers have tried to use inhibitors of IFN in the early phase of COVID-19. As the results showed, it decreased response to SARS-CoV-2 playing an important role in the development of cytokine storm associated with COVID-19 [12].

Moreover, to cytokine blockade therapy, different anti-inflammatory approaches were applied in an attempt to treat COVID-19. This trends exhibit that anti-inflammatory medication is as much important as anti-viral treatment of Covid-19. Along with other serious cases, some clinicians currently use glucocorticoids as an empirical treatment for severe COVID-19. Unlike specific cytokine inhibitors, glucocorticoids are considered to be nonspecifically effective against cytokine

storm by inhibiting multiple inflammatory processes. Glucocorticoids not only express immunosuppressive effects, but also produce anti-inflammatory response by inhibiting the production of major inflammatory molecules, including prostaglandins and leukotrienes. Some studies report positive effects of steroids in severe COVID-19 [13]. An alternative treatment of cytokine storm associated with COVID-19 is considered to be TPE (therapeutic plasma exchange) can have clinical benefits on severe COVID-19 if started promptly after early diagnosis based on rapid clinical assessment such as serum ferritin and high-sensitivity cardiac troponin I determination. Researchers highly recommend usage of TPE with convalescent plasma together with other potentially effective options for treating severe COVID-19 [14]. Another agent suggested to be useful in this regard is the endogenously synthesized melatonin. Collective data shows melatonin to be effective in treatment for COVID-19 and supports the recommendation for its use. The anti-inflammatory and antioxidant characteristics of melatonin are playing role in protecting the lungs from injury in many experimental models. Despite, melatonin has several advantages to be cheap, non-toxic even in high dose range, has a long shelf-life and can be feasible and self-administered which is a major advantage when large numbers of individuals are involved. Thus, the use of melatonin may mitigate the COVID-19 pandemic.

2. Conclusion

The cytokine storm is a main cause of mortality and morbidity in COVID-19 disease. COVID-19-induced cytokine storm is caused by a malfunctioning innate immune response and an excessive adaptive immune response. The dimensions of pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α is importantly different between mild and severe cases of COVID-19. Through successful use of laboratory tests and hematological markers, a cytokine storm may be timely diagnosed and controlled. Several synthetic drugs and products have revealed promising results in minimizing COVID-19-induced cytokine storms, though furthermore validations of their efficacy and safety in randomized clinical trials are still required. For possible severe cases, a quick and intense administration of the potential therapeutic agents would cut down the effect of the progressive cytokine storm characteristic to COVID-19.

Acknowledgments

Society of Rheology, 405133029; Popularization of Rheology Science Program (PRSP); Project “Georgian reality: The sustainability of scientific research during the Covid-19 pandemic”.

References

- [1]. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. *Cytokine & growth factor reviews*. 2020; 1(54):62-75
- [2]. Filippo, Cattaneo Gaetano Maria, Capelli Patrizio Serum ferritin levels in inflammation: a retrospective comparative analysis between COVID-19 and emergency surgical non-COVID-19 patients. *World Journal of Emergency Surgery*. 2021; 16:9
- [3]. Cavezzi A., et al. COVID-19: hemoglobin, iron, and hypoxia beyond inflammation. A narrative review

Clin. Pract. (2020), 10.4081/cp.2020.1271

- [4]. Evangelos Terpos, Ioannis Ntanasis-Stathopoulos, Ismail Elalamy, Efstathios Kastritis et al. Hematological findings and complications of COVID-19. *Am J Hematol.* 2020; 95:834-847
- [5]. Emami A., Javanmardi F., Pirbonyeh N., Akbari A. Prevalence of underlying diseases in hospitalized patients with COVID-19: a systematic review and meta-analysis. *Arch. Acad. Emerg. Med.* 2020; 8
- [6]. Mehta P., McAuley D.F., Brown M., Sanchez E., Tattersall R.S., Manson J.J. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet (London, England).* 2020; 395:1033-1034
- [7]. Marcelo Maia Pinheiro, Andrea Fabbri, Marco Infante. Cytokine storm modulation in COVID-19: a proposed role for vitamin D and DPP-4 inhibitor combination therapy (VIDPP-4i) April 2021 *Immunotherapy* 13(7); PMC - PubMed; DOI: [10.2217/imt-2020-0349](https://doi.org/10.2217/imt-2020-0349)
- [8]. Yang L, Liu S, Liu J et al. COVID-19: immunopathogenesis and immunotherapeutics. *Signal Transduct. Target. Ther.* 2020; 5(1):128
- [9]. Cavalli G, De Luca G, Campochiaro C, Della-Torre E, Ripa M, Canetti D. et al. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol.* 2020; 2(6):e325-e31 doi: 10.1016/S2665-9913(20)30127-2
- [10]. Campochiaro C, Della-Torre E, Cavalli G, De Luca G, Ripa M, Boffini N. et al. Efficacy and safety of tocilizumab in severe COVID-19 patients: a single-centre retrospective cohort study. *Eur J Intern Med.* 2020; 76:43-9
- [11]. Lai W, Wang J, Huang B, Lin EP, Yang P. A novel TNF- α -targeting aptamer for TNF- α -mediated acute lung injury and acute liver failure. *Theranostics.* 2019; 9(6):1741-51]
- [12]. Acharya D, Liu G, Gack MU. Dysregulation of type I interferone responses in COVID-19. *Nat Rev Immunol.* 2020; 20(7):397-8
- [13]. Shang L, Zhao J, Hu Y, Du R, Cao B. On the use of corticosteroids for 2019-nCoV pneumonia. *Lancet.* 2020; 395(10225):683-470;
- [14]. Bosnak M, Erdogan S, Aktekin EH, Bay A. Therapeutic plasma exchange in primary hemophagocytic lymphohistiocytosis: Reports of two cases and a review of the literature. *Transfus Apher Sci.* 2016; 55(3):353-6