ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Review Article / JAPR / Sec. B
Hassan, 2021, 5 (4), 377-386

Journal of Advanced Pharmacy Research

Section B: Pharmaceutical Analytical & Organic Chemistry, Medicinal & Biochemistry

Effect of COVID-19 on Hemoglobin: Theories and Recommended Drugs

Zeinab A. Hassan*; Mohamed H. Ahmed; Hend H. Sanad; Ahmed S. Ali

Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt

***Corresponding author:** Zeinab. A. Hassan, Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt. Tel. (+2)01284427620*
Email address: Zeinab_hassan@pharm.helwan.edu.eg

Submitted on: 08-06-2021; Revised on: 27-07-2021; Accepted on: 19-08-2021

To cite this article: Hassan, Z. A.; Ahmed, M. H.; Sanad, H. H.; Ali, A. S. Effect of COVID-19 on Hemoglobin: Theories and recommended Drugs. *J. Adv. Pharm. Res.* **2021**, *5* (4), 377-386. DOI: 10.21608/aprh.2021.79803.1134

ABSTRACT

COVID-19 has affected over 110 million patients worldwide till February 2021. Scientists seek to understand the pathophysiology and proposed several theories. COVID-19 severe clinical phenomena occurred due to cytokine storms that cause hypochlorous acid (HOCl) production. HOCl has several harmful effects on hemoglobin, like heme degradation and iron overload toxicity. There are two theories about the attack of COVID-19 on Hemoglobin. One widely shared hypothesis based on the docking model, recommended that SARS-CoV-2 proteins ORF1ab and ORF3a bind to hemoglobin protein, changing heme to ORF10, resulting in the breakdown of the heme into iron and Protoporphyrin IX (PPIX), causing the loss of hemoglobin function and iron toxicity. While other experimental study of patients with COVID-19 and acute respiratory distress syndrome (ARDS) patients is compared in the objection theory. The results showed that COVID-19 patients were not showing any hemolytic anemia or altering the hemoglobin-oxygen dissociation curve and hence, COVID-19 has no role in the removal of iron and oxygen delivery. In addition, there is a hypothesis that hemoglobin structure plays a role in the physiopathology of COVID- 19 disease. In children a high fetal hemoglobin levels leading to a decrease in the mortality rate with COVID-19. Another hypothesis linked between the blood grouping and the risk of developing COVID-19 infection. O group persons have a lower chance for infection. From all the previous studies, many drugs formulated like fetal hemoglobin inducers, Iron chelators, and Lactoferrin represents options for the treatment of COVID-19. The present review is an attempt to describe some hypothesizes related to the Hemoglobin theory. In addition, there is information on several novel treatments for COVID-19 therapy.

Keywords: *Hemoglobin; COVID-19; Iron chelators*

INTRODUCTION

COVID-19 has shaken the world for the last months; An acute respiratory syndrome caused by Coronavirus SARS-CoV-2 spread fast and leading to rapid deterioration in the respiratory functions and may lead to death. In addition to the negative impacts on the economy and people's social life [1]. A specific hallmark involving COVID-19, the rapid health deterioration due to multi-system injuries. The hypothesis assumed that the generation of excess reactive oxygen species leads to hypoxemia, further cell stress, and heme destruction [2]. Oxygen supplementation fails in critical cases due to the severe hypoxia caused by the virus. The prognosis and pathophysiology of COVID-19 are not the same as the rest of the Coronavirus family and is still poorly understood[3]. About 80% of COVID-19 cases suffer from mild to moderate symptoms, while 20% with severe

symptoms. The mortality rate in COVID-19 patients is up to 50 percentage, due to accompanied risk factors as aging and non-communicable diseases. Diagnosis of COVID-19 is related to the decreased Hemoglobin, leukocytes-neutrophiles ratio, elevated D-dimer, and ferritin enzyme. The violent immune response of the human body against the COVID-19 virus causes the release of "cytokines storm", resulting in ROS production's cascade leading to hypoxia [4,5].

Various hypotheses about the relation between the COVID-19 and the 1-beta Hemoglobin chain were assault. Theoretical research relies on the ability of COVID-19 proteins with porphyrin to form a conserved domain and release free iron leading to a drop in the affinity of Hemoglobin binding to oxygen and interfere with the Hemoglobin anabolism. [6]

The experimental research argues the link between COVID-19 and Hemoglobin clinical laboratory results from 21 patients positive to COVID-19 to 21 patients with acute respiratory distress syndrome (ARDS) but without COVID-19.[7] Many studies and clinical trials linked the presence of Fetal Hemoglobin (HF), change of blood grouping, iron toxicity, mortality, and morbidity rate of COVID-19 [8], which lead to hypotheses created to approve these theories using HF inducer and Iron chelator for treatment.

The present review is an effort to describe some hypothesizes and pathophysiology of COVID -19 related to the Hemoglobin theory. In addition, there is information on several novel treatments for COVID-19 therapy.

Genomic organization of COVID-19

Coronaviruses consist of two types of protein, which are structural and nonstructural. Structural proteins constitute a significant part consists of four types of a Spike (S), an envelope (E), a membrane (M), and a nucleocapsid (N). Nonstructural proteins (nsp) are minor parts occupied two-thirds of the genome, consist of two open reading frames ORF1a and ORF1b encoded into 16 types (nsp1-nsp16), each protein of them plays a critical role in viral metabolism and interaction with the host machinery **Figure 1.** [9]

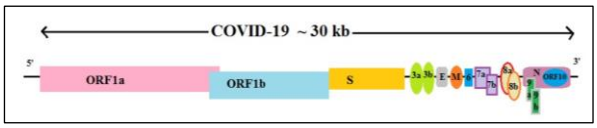

Figure 1. Genomic organization of COVID-19.

Pathophysiology of COVID-19

SARS-CoV-2 is a positive sense RNA strand, nucleocapsid, and spikes protein. The virus enters the host cell by spike protein through angiotensin-converting enzyme two receptors (ACE2), located on the lower respiratory epithelium, releasing the nucleocapsid for mRNA transcription and the hijacking of the host cell producing viral proteins[10].

In a mild to moderate viral infection, WBCs, especially neutrophils, produce myeloperoxidase enzymes releasing inflammatory cytokines, generating Reactive Oxygen Species (ROS) like (H_2O_2), ($O_2^{\bullet-}$), and (HOCl) as scavengers for viral proteins [11,12]. However, in severe cases, HOCl is heavily produced due to the cytokine storm, beating oxygen at the heme-binding site causing Hemoglobin degradation and Iron (Fe^{2+}) release; free iron undergoes Fenton reaction producing another reactive hydroxyl (•OH) [13].

Another critical regulator is nitric oxide; upon depletion, vasoconstriction occurs, leading to a negative impact on the pulmonary and peripheral circulations. Producing excess ROS followed by an increase in hemoglobin degradation rate leads to hypoxia [14].

Linking these mechanisms (ROS, cytokine storm, myeloperoxidase, nitric oxide depletion) might beat the hypoxia cascade that occurs by COVID-19 [2] as in **Figure 2**.

In the early stage of COVID-19 infection, infected cells produce cytokines to influence leukocytes, neutrophils, and macrophages. Neutrophils produce myeloperoxidase enzymes to generate the **reactive oxygen species** (•O, •OH, H_2O_2, HOCl) act as a scavenger against the viral protein[15]. As a result, the overproduction of reactive oxygen molecules leads to oxidative stress, activation of caspase-3 inducing apoptosis; and reactive oxygen species are converting to more stable H_2O_2 [16].

The antimicrobial activity of Neutrophils is through ***Myeloperoxidase enzymes***, followed by HOCl production to fight the virus. However, in severe cases, hemeprotein destruction and free iron ions are released by interaction with HOCl and O- radical. [17]

Decrease in peripheral oxygen saturation is one of the hallmarks of COVID-19, with a reduction of about 50-70% without symptoms due to Hemoglobin's alteration at the alveolar membrane by the ***HOCl-MPO*** [18].

Hemoglobin *i*s a tetramer of four heme groups attached to globin subunits, and it carries O_2/Co_2. Hb exists in two forms, reduced oxygenated (Hb-oxy) and deoxygenated. HOCl binds to Hb-oxy, leading to Hemoglobin disruption and the release of free iron, and the formation of metHemoglobin and Ferryl Hb ($Hb\text{-}Fe_4{=}O$). The oxygen affinity will decrease, eventually, tissue hypoxia. Early oxygen supplementation might help in Hb reoxygenation, but in late cases, due to increased ROS, oxygen supplement may make it worth.[19,20]

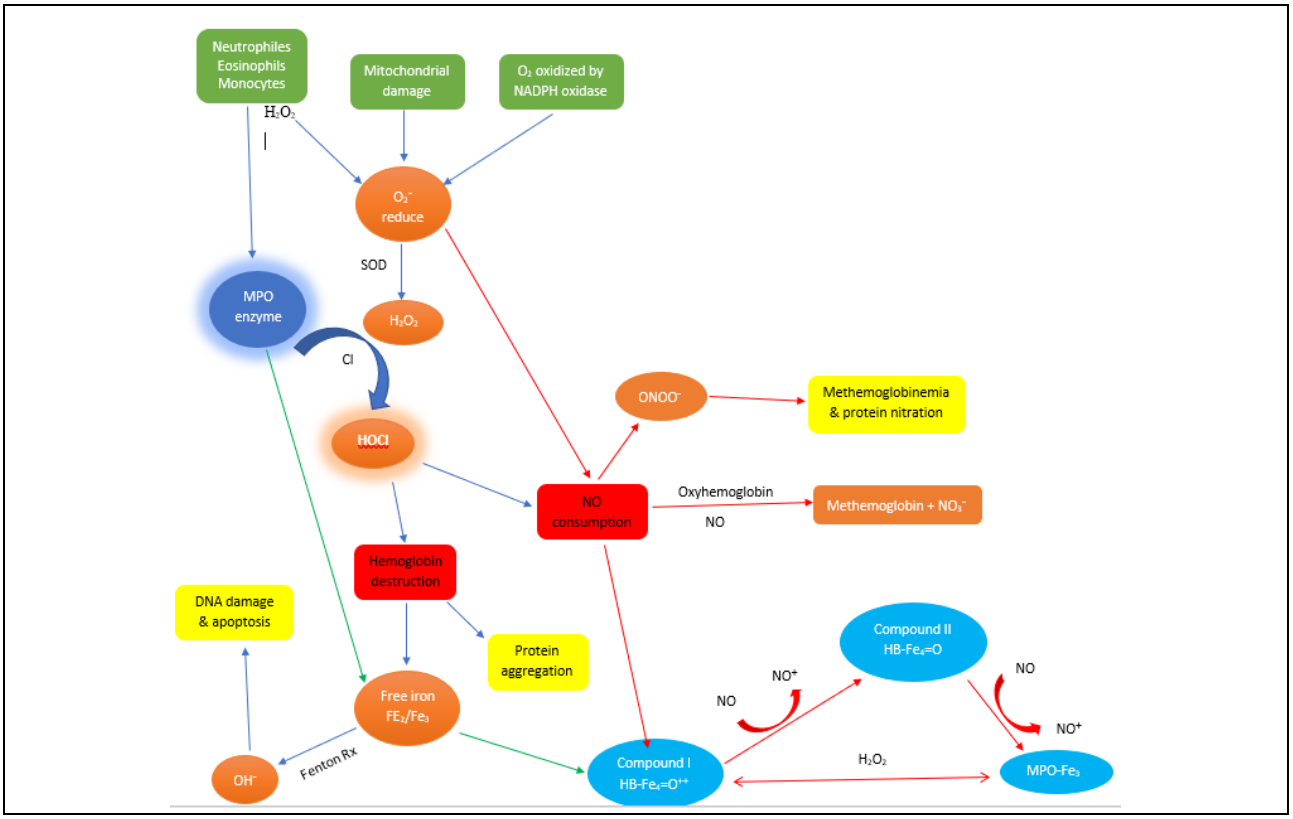

Figure 2. Neutrophil MPO -generated oxidants and their potential role in COVID-19. At normal condition, neutrophils produce MPO enzyme & ROS particles as reduced O_2^- which converted by superoxide dismutase to generate H_2O_2 as a scavenger for antimicrobials. A secondary more potent scavenger HOCl is produced by myeloperoxidase from H_2O_2 with chloride ions from blood. In sever COVID-19 cases, MPO is produced in excess forming a lot of HOCl particles. HOCl cause oxidative stress leasing to heme destruction, vasoconstriction & hypoxia. **In blue arrows** heme destruction hypothesis, which result in formation of Free iron & OH^- leads to hypoxia, blood clotting & DNA damage. **In red arrows** nitric oxide consumption hypothesis, which result in vasoconstriction and low oxygen saturation levels due to methemoglobinemia. **In green arrows** another hypothesis of nitric oxide consumption by formation of compound 1 & 2 by MPO and free iron result in toxic NO^+ metabolite.

Iron is an essential element for many metabolic processes like DNA synthesis, repairing, transcription of DNA, energy production, and drug detoxification. The excessive amount of free unbound Fe2+ leads to increased toxic reactive oxygen species (ROS) formation by the Haber-Weiss and Fenton reactions. ROS formation alters lipid, nucleic acid, and protein functions, activation of acute/chronic inflammatory response leading to many complications [21] (**Figure 3**).

Clinically, severe hypoxemia COVID-19 patients have lower levels of serum iron in association with lymphocyte counts. The significantly low serum iron may contribute to possible T-cell dysfunction.[22] Shah et al. found non-significant differences in transferrin saturation or serum ferritin levels between the non-severe and severe hypoxemia patient groups. Zhao and colleagues have reported that "the severity and mortality of the disease was closely correlated with serum iron levels". [23] The common presence of digestive symptoms in COVID-19 patients was explained in part by a link to hepcidin. [24] In Bardon et al. pooled analysis, elevated LDH values were associated with a 6-fold increased odds of severe COVID-19 disease and >16-fold increase in odds of mortality.[25]

Theories of COVID-19 attack on Hemoglobin

The first theory was done by Liu and Li using a computational model, sequencing analysis, and molecular docking. It depends upon what's protein can form a conserved domain. Previously reported studies have proved that heme is a lipophilic protein that can't uptake by the liver unless binding with a protein transporter forming a conserved domain.[6] Virus proteins of COVID-19 bind with heme or porphyrin binding or with heme oxygenase activity if they form a similar conserved domain.[6]

According to the study of Liu and Li COVID-19 Hemoglobin (Hb) metabolism will interfere with the

formed conserved domain and separate harmful irons in the blood, which leads to a significant increase in serum ferritin, albumin, ESR, LDH, and CRP, and decrease neutrophils.[6]

Types of proteins that form a conserved domain with Hemoglobin

The structural proteins of COVID-19 that form a conserved domain with porphyrin are E2 glycoprotein, Envelope protein, and Nucleocapsid phosphoprotein. Nonstructural proteins of COVID-19, which include a conserved domain, are ORF1ab, ORF3a, ORF7a, ORF8, and ORF10, which do not provide with the SWISS model[6] are illustrated in **Table 1** and **Table 2**.

There is a difference in the ability of COVID-19 proteins to form a conserved domain with porphyrin according to the number of amino acids and binding energy.

Table 1 illustrates conserved domains between structural proteins and nonstructural proteins of COVID-19 with porphyrin according to the number of amino acids bound to porphyrin and binding energy. E2 glycoprotein forming the most stable conserved domain in the structural proteins, ORF8 is the most stable conserved domain in the nonstructural proteins depending on the highest binding energy.

The possible mechanism of nonstructural proteins of COVID-19 that attack the heme ring and release porphyrin are ORF10, ORF3a, and orf1ab. Porphyrin is directed and binds with ORF8 by ORF6 and ORF10. Finally, ORF8 forms a stable complex with porphyrin.

There are two types of Hemoglobin, oxyhemoglobin, and deoxyhemoglobin; the role, conformation, and the sites of nsps are different in each one.[6, 33]

Table 2 shows the mechanism of heme attack and release of porphyrin by nonstructural proteins of COVID-19. The first step, ORF10, ORF3a, and orf1ab, attack the heme ring and release the porphyrin free. Then porphyrin is directed and binds with ORF8 by ORF6 and ORF10. ORF8 forms a stable complex with porphyrin.[6]

The second theory found that there is no evidence between COVID-19 and Hemoglobin defect[7] experimentally. When comparing the laboratory results of 21 patients with positive COVID-19 in ICU with 21 patients non-COVID-19 selected in the Acute Lung Harm Registry (ALIR) from various etiologies at UPMC. At the beginning of clinical studies, they record Hb, total bilirubin, venous blood gas values of the partial pressure of carbon dioxide (PvCO2), the partial pressure of oxygen (PvO2), pH, and venous oxygen saturation of Hemoglobin (SvO2) levels.

They found no Hb affinity variation to carry oxygen in positive COVID-19 patients, so no Hb value variation means no association between Hb and COVID-19 [7,27]. Patients with COVID-19 have a higher incidence rate of Thromboembolism [28] and no relation between it and Hb toxicity. No absorbance occurs to free heme when measured at the wavelength range.[29]

Hopp, Marie-Thérèse et al., were proposing a study of common pathways performed by superimposing two knowledge graphs, namely the "Heme KG"and the "COVID-19 KG", which contained information about theme-driven and COVID-19 pathophysiology on a molecular level.[29]

Several biomarkers from proinflammatory pathways (cytokines, such as TNF), the complement system (e.g., C3), the blood and coagulation system (e.g., ferritin, platelets), and organ-specific indicators (i.e., LDH and bilirubin) developed to support the evidently shared clinical[22].

Four proteins involved in SARS-CoV-2 entry into host cells (the two viral proteins S protein and protein 7a, as well as the two human host cell proteins ACE2 and TMPRSS2) were suggested as potential heme-binding proteins in a second approach (out of six analysed COVID-19-related proteins). With the use of the web tool HeMoQuest and manual refining, the heme-binding potential of these proteins was determined, revealing two possible HBMs on the surface of protein 7a, three for S protein, five for ACE2, and ten motifs for TMPRSS2.[29]

Finally, a link between COVID-19 infection symptoms and the consequences of excess heme does not have to be relevant for every patient; but, in certain circumstances, it may correlate or even create a more severe illness development due to pre-existing hemolytic diseases or hemolysis-provoking events.[29]

Role of Fetal Hemoglobin in COVID-19:

Hemoglobin F (HBF) may be one of the main reasons for decreasing the prevalence of COVID-19 in pediatrics.[30] The hypothesis based on the molecular docking study approved that SAR-COV2 attacks the Beta chain, causing a release of iron from porphyrin and iron toxicity.[6] The pilot study results showed that patients with hemoglobinopathies like beta-thalassemia and sickle cell anemia had low mortality and fatality rates toward COVID-19.[30]

From the previous results of studies, some clinical trials started to use HBF inducers agent s in protocols for treatment COVID-19 like Hydroxyurea and Panobinostat [31]. However, some literature and case studies approved that beta-thalassemia patients had a higher risk of COVID-19 complications due to iron overload and splenectomy[32,34].

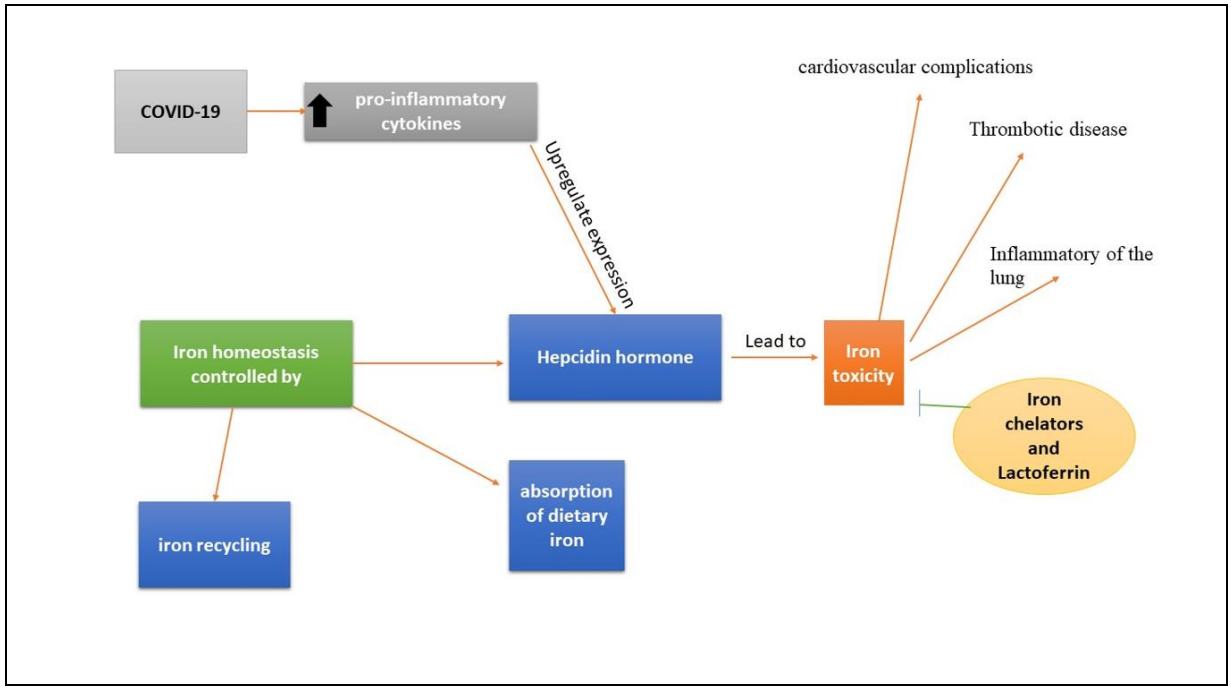

Figure 3. The relationship between COVID-19 and iron homeostasis. In addition to the complications of the increase iron overload and treatment of iron toxicity.

Table 1. Conserved domains between structural proteins and nonstructural proteins of COVID-19 with porphyrin according to the number of amino acids bound to porphyrin and binding energy

Types of COVID-19 proteins		Number of amino acids bound to porphyrin	Binding energy
Structural proteins	E2 glycoprotein	18 amino acids	219,317.76 kcal/mol
	Nucleocapsid	22 amino acids	15,532,506.53 kcal/mol
	Envelope protein	18 amino acids	219,317.76 kcal/mol
Non-structural proteins	ORF8	18 amino acids	12,804,859.25 kcal/mol
	orf1ab	18 amino acids	561,571.10 kcal/mol
	ORF7a	15 amino acids	37,123.79 kcal/mol

Blood group type and COVID-19

Here is no clear evidence between blood grouping and COVID-19. However, some studies have shown that blood type O individuals had a low risk of developing COVID-19, while blood group A was associated with a higher risk [(35)]. Blood iron state studies reported that some serum iron indicators are lower in patients with blood group O than those with other blood groups[8]. Increasing amounts of serum iron may contribute to complications associated with COVID-19, like inflammation and hypercoagulation.[35]

Prognostic role of Hemoglobin in COVID-19

COVID-19 individuals with severe illness had considerably lower hemoglobin levels than those with milder forms, according to a meta-analysis of four relevant studies.[36] In another study, it was discovered that a drop in Hb level following a pneumonia diagnosis might be a predictor of deteriorating pneumonia in 23 COVID-19 patients with pneumonia, admitted to the Kumamoto City Hospital, 23 COVID-19 patients with pneumonia, admitted to the Kumamoto City Hospital.[37]

Table 2. The mechanism of heme attack and release of porphyrin by nonstructural proteins of COVID-19

Type of nsps	Oxyhemoglobin	Deoxyhemoglobin
ORF1ab	Configuration change in alpha and beta chain when attack both of them. Form a stable complex with porphyrin.	Configuration changes in globin protein when attacking both alpha chain. Form a stable complex with porphyrin.
ORF3a	Attack beta chain and reveal heme	Drive 2 alpha chain to attack beta chain and reveal heme
ORF10	Attach with a beta chain to release its iron atom to form porphyrin.	Attach with 1- beta chain to release its iron atom to form porphyrin.

The study reported done by Anai M., et al. found that Hemoglobin concentration is not related to overall survival., Hemoglobin is affected by factors like red blood cell population, age, lactate dehydrogenase. Also adding the ratio of arterial partial oxygen pressure to inspired oxygen percentage. Anemia is a condition that affects the old and feeble, and it can have a detrimental impact on their health.[38]

World Health Organization has recently taken into consideration the possible contribution of blood changes within the COVID- 19 course.[39]

Medicine -Based Drug Options

In a previous study concerning hemoglobinemia and the erythrocyte disease, few therapies are found based on medical works of literature.[40] Recommendations for drugs and additional adjuvant therapies targeting hemoglobin dysfunction are used currently under scrutiny in multiple ongoing clinical trials in COVID-19 patients.

Iron chelators and Lactoferrin options for treatment COVID-19

Lactoferrin (Lf) is a glycoprotein produced from different mucosal epithelial cells and is one of the transferrin family members and has an affinity to bind to iron more than transferrin [21,41]. Lactoferrin had a preventive, therapeutic such as antifungal, antibacterial, antiviral, and anticancer, and biological activities like neutralizing bioactive substances like lipopolysaccharide (LPS)[42,43].

Lactoferrin has anti-inflammatory through its immunomodulation action on the cellular functions of both T and B lymphocytes in addition to Lf have receptors expressed on all T-cell subsets. [44] As well as Lf modulate cytokine/chemokine production to regulate the production of ROS and immunity cells and act as an iron scavenger [42,43].

Lf has antiviral activity through inhibiting both naked and enveloped, DNA and RNA viruses' activities like Cytomegalic Virus, Human Immunodeficiency Virus (HIV), Poliovirus, Hepatitis B and C (HCV) viruses, Echovirus 6, Influenza A virus [45].

Lf also owns the ability to obstruct viral entry by binding to a host's viral or cell surface receptors, or both. Like, bind to Angiotensin-converting enzyme 2 (ACE II) receptor by adhesion molecules called heparan sulfate proteoglycans (HSPGs) [46,47], so it is possible to use LF for preventing entry of COVID-19 to a cell of humans [45].

Iron chelators own multiple pharmacological actions, such as chelating iron, inhibiting redox properties exerted by free iron, reduction of hepcidin. It acts as anti-ferritin by removing iron from its proteins and preventing iron from participating in Fenton reactions, leading to inhibition of hydroxyl radical production, ROS formation, and production ferroptosis [48–51]. Furthermore, it induces, lowering of glutathione and change of glutathione peroxidase 4 (GPX4), which act as a ROS regulator [52,53].

Accordingly, iron toxicity patients get treated with iron chelators like deferasirox, deferoxamine, and deferiprone. [54] And it's a potential therapeutic option in COVID-19. Iron chelation, with deferiprone or deferoxamine, has been proposed in COVID-19, also in view of its demonstrated efficacy in other viral infections.[55]

Cures work potentially useful interventions in hypoxia-based pulmonary hypertension and vasoconstriction may be highlighted recommendation for treatment in literature: Nitroglycerine-based drugs; Prostanoids; Calcium channel blockers; high concentration/ high flow oxygen at low pressure; Vadenosine; Acetazolamide; Sildenafil. [56]

Mitochondrial organelles play a role in innate and adaptive immunity in viral infections. Hospitalized COVID-19 patients showed an increased lactate level, a marker for mitochondrial degeneration.[57] Specific mitochondria-targeted antioxidants (based on triphenylphosphonium cation) as Curcuminoids, Polydatin, Ubiquinol, Nicotinamide-mononucleotide may improve mitochondrial biogenesis/ function.[58]

SARS-CoV2 attacks endothelial cells, iron metabolism, and erythrocytes which may lead to a series

of coagulopathies. In COVID-19 patients are treated with anticoagulants as heparins. Anticoagulants are showing a multi-organ beneficial effect and become essential in pharmaceutical treatment.[59]

Ozone may be the function in the treatment of COVID-19 patients. ***Ozone*** has many biochemical effects.[60,61] These effects including (increase GSH activity and heme oxygenase pathway; Elevation of IL10 and decrease of proinflammatory cytokines and TNFα; Transient increase of ROS and consequent NrF2/ARE pathway activation; Improved mitochondrial oxidative phosphorylation; 2,3- diphosphoglycerate increase with better hemoglobin functionality; Improved erythrocyte rheology and NO elevation).

Polyphenols own several epigenetic activities through the NrF2-ARE pathway.[62] Polyphenols have direct antiviral action documented in a few studies. Curcuminoids, anthocyanins, and catechins proved to benefit processes in COVID-19. Polyphenols are effective in inflammasome activation *interleukin storm and* SARS-CoV-2 attack to bone marrow [63].

Melatonin exhibits a proven protective role against hypoxia, ferroptosis, and hemoglobin denaturation.[64]

Vitamin D showed an epigenetic action on a few antioxidant systems and modulate immune system. [55]

Rifkind and Heim refer to the central role of zinc in the immune system. It helps in respiratory infections and activity on hemoglobin O_2 affinity as well. [61]

Horowitz et al. register quick relief from COVID-19 pneumonia-related dyspnea in two cases after multiple doses of ***GSH***. [62]

Ascorbic acid constitutes a significant role in Intensive Care Unites due to its immune, anti-infective, and anti-cytokine storm activity. Ascorbic acid can increase NO production by improving heme iron/cell redox balance.[63]

Clinically, ***dexamethasone and other corticosteroids*** are among the very few widely used treatments and diagnoses for COVID-19 [64], which significantly correlates with the course of the disease. The severity of the disease is associated with the serum levels of ***hepcidin*** and ***ferritin***. Serum Ferritin is an indicator of inflammation and infection.[65]

CONCLUSION

This review highlights the genomic organization of the COVID-19 virus, and the Pathophysiology of SARS-CoV-2 discusses the mechanism of the COVID-19 virus attack on the cell and hemoglobin. Moreover, Scientists have not found the proper mechanism of COVID-19 that creates them to interconnect between theoretical modeling and limitation of clinical studies. The virus can attack cells via interaction with the highly expressed ACE2 receptor in many organs and tissues. The study also includes details on medicine-based medications and other adjuvant treatments for haemoglobin malfunction that are currently being tested in numerous COVID-19 patients.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Wang, C.; Horby, P. W.; Hayden, F. G.; Gao, G. F. A Novel Coronavirus Outbreak of Global Health Concern. *The Lancet* **2020**, 470–473. https://doi.org/10.1016/S0140-6736(20)30185-9.
2. Goud, P. T.; Bai, D.; Abu-Soud, H. M. A Multiple-Hit Hypothesis Involving Reactive Oxygen Species and Myeloperoxidase Explains Clinical Deterioration and Fatality in Covid-19. *Int. J. Biol. Sci.* **2020**, *17* (1), 62–72. https://doi.org/10.7150/ijbs.51811.
3. (Li, T.; Lu, H.; Zhang, W. Clinical Observation and Management of COVID-19 Patients. *Emerg. Microbes Infect.* **2020**, 687–690. https://doi.org/10.1080/ 22221751.2020.1741327.
4. Zhang, J.; Wang, X.; Jia, X.; Li, J.; Hu, K.; Chen, G.; Wei, J.; Gong, Z.; Zhou, C.; Yu, H.; Yu, M.; Lei, H.; Cheng, F.; Zhang, B.; Xu, Y.; Wang, G.; Dong, W. Risk Factors for Disease Severity, Unimprovement, and Mortality in COVID-19 Patients in Wuhan, China. *Clin. Microbiol. Infect.* **2020**, *26* (6), 767–772. https://doi.org/10.1016/j.cmi.2020.04.012.
5. (Liu, Q.; Zhou, Y. H.; Yang, Z. Q. The Cytokine Storm of Severe Influenza and Development of Immunomodulatory Therapy. *Cellular Mol. Immunol*. **2016**, 3–10. https://doi.org/10.1038/cmi.2015.74.
6. Wenzhong, Liu; Hualan, L. COVID-19: Attacks the 1-Beta Chain of Hemoglobin and Captures the Porphyrin to Inhibit Human Heme Metabolism. **2020**. https://doi.org/10.26434/ chemrxiv. 11938173.v4.
7. DeMartino, A. W.; Rose, J. J.; Amdahl, M. B.; Dent, M. R.; Shah, F. A.; Bain, W.; McVerry, B. J.; Kitsios, G. D.; Tejero, J.; Gladwin, M. T. No SARS-CoV-2 Evidence Infection of Hemoglobin Damage By. *Haematologica* **2020**, *105* (12), 2769–2773. https://doi.org/10.3324/ haematol.2020.264267.

8. Martin Ositadinma, I.; Grace Ifeoma, A.; Amauche Martina, N.; Okorie, O. G.; Ejike, O. A. Ferritin and Serum Iron Levels among the ABO Blood Groups in Enugu, South Eastern Nigeria. *J. Blood Disord. Transf.* **2014**, *5* (4), 204. https://doi.org/ 10.4172/2155-9864.1000204.
9. Rohaim, M. A.; El Naggar, R. F.; Clayton, E.; Munir, M. Structural and Functional Insights into Non-Structural Proteins of Coronaviruses. *Microbe. Pathogen.* **2021**, 104641. https://doi.org/ 10.1016/j.micpath.2020.104641.
10. Li, Y.; Zhou, W.; Yang, L.; You, R. Physiological and Pathological Regulation of ACE2, the SARS-CoV-2 Receptor. *Pharmacol. Res.* **2020**. https://doi.org/ 10.1016/ j.phrs.2020.104833.
11. Tang, D.; Comish, P.; Kang, R. The Hallmarks of COVID-19 Disease. *PLoS Pathogens* **2020**. https://doi.org/10.1371/journal.ppat.1008536.
12. Galijasevic, S.; Abdulhamid, I.; Abu-Soud, H. M. Potential Role of Tryptophan and Chloride in the Inhibition of Human Myeloperoxidase. *Free Radic. Biol. Med.* **2008**, *44* (8), 1570–1577. https://doi.org/ 10.1016/j.freeradbiomed.2008.01.003.
13. Abu-Soud, H. M.; Maitra, D.; Shaeib, F.; Khan, S. N.; Byun, J.; Abdulhamid, I.; Yang, Z.; Saed, G. M.; Diamond, M. P.; Andreana, P. R.; Pennathur, S. Disruption of Heme-Peptide Covalent Cross-Linking in Mammalian Peroxidases by Hypochlorous Acid. *J. Inorg. Biochem.* **2014**, *140*, 245–254. https://doi.org/10.1016/j.jinorgbio.2014.06.018.
14. Galijasevic, S.; Saed, G. M.; Diamond, M. P.; Abu-Soud, H. M. Myeloperoxidase Up-Regulates the Catalytic Activity of Inducible Nitric Oxide Synthase by Preventing Nitric Oxide Feedback Inhibition. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (25), 14766–14771. https://doi.org/10.1073/ pnas.2435008100.
15. Vatansever, F.; de Melo, W. C. M. A.; Avci, P.; Vecchio, D.; Sadasivam, M.; Gupta, A.; Chandran, R.; Karimi, M.; Parizotto, N. A.; Yin, R.; Tegos, G. P.; Hamblin, M. R. Antimicrobial Strategies Centered around Reactive Oxygen Species - Bactericidal Antibiotics, Photodynamic Therapy, and Beyond. *FEMS Microbiology Reviews*. **2013**, 955–989. https://doi.org/ 10.1111/1574-6976.12026.
16. Ivanova, D.; Zhelev, Z.; Aoki, I.; Bakalova, R.; Higashi, T. Overproduction of Reactive Oxygen Species – Obligatory or Not for Induction of Apoptosis by Anticancer Drugs. *Chinese J. Cancer Res.* **2016**, 383–396. https://doi.org/ 10.21147/j.issn.1000-9604.2016.04.01.
17. Podrez, E. A.; Abu-Soud, H. M.; Hazen, S. L. Myeloperoxidase-Generated Oxidants and Atherosclerosis. *Free Radic. Biol. Med.* **2000**, *28* (12), 1717–1725. https://doi.org/10.1016/S0891-5849(00)00229-X.
18. Ottestad, W.; Seim, M.; Mæhlen, J. O. Covid-19 Med Stille Hypoksemi. *Tidsskr. den Nor. Laegeforening* **2020**, *140* (7). https://doi.org/10.4045/ TIDSSKR.20.0299.
19. (Gell, D. A. Structure and Function of Haemoglobins. *Blood Cells, Molecules, and Diseases*. **2018**, 13–42. https://doi.org/10.1016/ j.bcmd.2017.10.006.
20. Nagababu, E.; Rifkind, J. M. Formation of Fluorescent Heme Degradation Products during the Oxidation of Hemoglobin by Hydrogen Peroxide. *Biochem. Biophys. Res. Commun.* **1998**, *247* (3), 592–596. https://doi.org/10.1006/bbrc.1998.8846.
21. Lepanto, M. S.; Rosa, L.; Paesano, R.; Valenti, P.; Cutone, A. Lactoferrin in Aseptic and Septic Inflammation. *Molecules* **2019**, *24* (7), 1323. https://doi.org/10.3390/molecules24071323.
22. Shah, A.; Frost, J. N.; Aaron, L.; Donovan, K.; Drakesmith, H. Systemic Hypoferremia and Severity of Hypoxemic Respiratory Failure in COVID-19. *Crit. Care* **2020**, *24* (1), 1–4. https://doi.org/10.1186/S13054-020-03051-W.
23. Zhao, K., Huang, J., Dai, D., Feng, Y., Liu, L., Nie, S. Serum Iron Level as a Potential Predictor of Coronavirus Disease 2019 Severity and Mortality: A Retrospective Study. *Open Forum Infect. Dis.* **2020**, *7* (7). https://doi.org/10.1093/OFID/OFAA250.
24. Pan, L.; Mu, M.; Yang, P.; Yu Sun, Y.; Wang, R.; Yan, J.; Pibao Li, P.; Hu, B, Wang, J., Chao, H., Yuan Jin, Y.; Niu, X., Ping, R.; Du, Y., Li, T., Xu, G.; Hu, Q.; Tu, L. Clinical Characteristics of COVID-19 Patients with Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am. J. Gastroenterol.* **2020**, *115* (5), 766–773. https://doi.org/10.14309/AJG. 0000000000000620.
25. BM, H.; G, A.; J, W.; S, B.; J, V.; M, P.; G, L. Lactate Dehydrogenase Levels Predict Coronavirus Disease 2019 (COVID-19) Severity and Mortality: A Pooled Analysis. *Am. J. Emerg. Med.* **2020**, *38* (9), 1722–1726. https://doi.org/10.1016/ J.AJEM.2020.05.073.
26. JW, S. Simple, Accurate Equations for Human Blood O2 Dissociation Computations. *J. Appl. Physiol.* **1979**, *46* (3), 599–602. https://doi.org/ 10.1152/ JAPPL.1979.46.3.599.
27. Smeeth, L.; Cook, C.; Thomas, S.; Hall, A. J.; Hubbard, R.; Vallance, P. Risk of Deep Vein Thrombosis and Pulmonary Embolism after Acute Infection in a Community Setting. *Lancet* **2006**, *367* (9516), 1075–1079. https://doi.org/10.1016/S0140-6736(06)68474-2.
28. Pires, I. S.; Belcher, D. A.; Palmer, A. F. Quantification of Active Apohemoglobin Heme-

Binding Sites via Dicyanohemin Incorporation. *Biochemistry* **2017**, *56* (40), 5245–5259. https://doi.org/10.1021/ acs.biochem.7b00683.
29. Hopp, M.-T.; Domingo-Fernández, D.; Gadiya, Y.; Detzel, M. S.; Graf, R.; Schmalohr, B. F.; Kodamullil, A. T.; Imhof, D.; Hofmann-Apitius, M. Linking COVID-19 and Heme-Driven Pathophysiologies: A Combined Computational–Experimental Approach. *Biomolecules* **2021**, *11* (5). https://doi.org/10.3390/BIOM11050644.
30. E, S.; H, S. A Hypothesis about the Role of Fetal Hemoglobin in COVID-19. *Med. Hypotheses* **2020**, *144*. https://doi.org/10.1016/J.MEHY.2020.109994.
31. AH, H.; EM, S.; MH, S. Fetal Hemoglobin in Sickle Cell Anemia: The Arab-Indian Haplotype and New Therapeutic Agents. *Am. J. Hematol.* **2017**, *92* (11), 1233–1242. https://doi.org/10.1002/AJH.24872.
32. Okar, L.; Ali, M.; Parengal, J.; Yassin, M. A. COVID-19 and Thalassemia Beta Major in Splenectomized Patient: Clinical Case Progression and Literature Review. *Clin. Case Reports* **2020**, *8* (12), 2918–2922. https://doi.org/10.1002/ccr3.3345.
33. Karimi, M.; Haghpanah, S.; Azarkeivan, A.; Zahedi, Z.; Zarei, T.; Akhavan Tavakoli, M.; Bazrafshan, A.; Shirkavand, A.; De Sanctis, V. Prevalence and Mortality in B-thalassaemias Due to Outbreak of Novel Coronavirus Disease (COVID-19): The Nationwide Iranian Experience. *Br. J. Haematol.* **2020**, *190* (3), e137–e140. https://doi.org/ 10.1111/bjh.16911.
34. Wu, Y.; Feng, Z.; Li, P.; Yu, Q. Relationship between ABO Blood Group Distribution and Clinical Characteristics in Patients with COVID-19. *Clin. Chim. Acta* **2020**, *509*, 220–223. https://doi.org/10.1016/j.cca.2020.06.026.
35. Lippi, G.; Mattiuzzi, C. Hemoglobin Value May Be Decreased in Patients with Severe Coronavirus Disease 2019. *Hematol. Transfus. Cell Ther.* **2020**, *42* (2), 116. https://doi.org/10.1016/ J.HTCT.2020.03.001.
36. Anai, M., Akaike, K., Iwagoe, H., Akasaka, T., Higuchi, T., Miyazaki, A., Naito, D., Tajima, Y., Takahashi, H., Komatsu, T., Masunaga, A., Kishi, H., Fujii, K., Fukuda, K., Tomita, Y., Saeki, S., Ichiyasu, H., Sakagami, T. Decrease in Hemoglobin Level Predicts Increased Risk for Severe Respiratory Failure in COVID-19 Patients with Pneumonia. *Respir. Investig.* **2021**, *59* (2), 187–193. https://doi.org/10.1016/J.RESINV. 2020.10.009.
37. Cavezzi, A.; Troiani, E.; Corrao, S. COVID-19: Hemoglobin, Iron, and Hypoxia beyond Inflammation. A Narrative Review. *Clin. Pract.* **2020**, *10* (2). https://doi.org/10.4081/cp.2020.1271.
38. Habib, H. M.; Ibrahim, W. H.; Schneider-Stock, R.; Hassan, H. M. Camel Milk Lactoferrin Reduces the Proliferation of Colorectal Cancer Cells and Exerts Antioxidant and DNA Damage Inhibitory Activities. *Food Chem.* **2013**, *141* (1), 148–152. https://doi.org/10.1016/j.foodchem.2013.03.039.
39. Abu-Serie, M. M.; El-Fakharany, E. M. Efficiency of Novel Nanocombinations of Bovine Milk Proteins (Lactoperoxidase and Lactoferrin) for Combating Different Human Cancer Cell Lines. *Sci. Rep.* **2017**, *7* (1), 1–12. https://doi.org/10.1038/ s41598-017-16962-6.
40. Habib, H. M.; Ibrahim, S.; Zaim, A.; Ibrahim, W. H. The Role of Iron in the Pathogenesis of COVID-19 and Possible Treatment with Lactoferrin and Other Iron Chelators. *Biomed. Pharmacother.* **2021**, *136*, 111228. https://doi.org/10.1016/j. biopha. 2021.111228.
41. Mancinelli, R.; Rosa, L.; Cutone, A.; Lepanto, M. S.; Franchitto, A.; Onori, P.; Gaudio, E.; Valenti, P. Viral Hepatitis and Iron Dysregulation: Molecular Pathways and the Role of Lactoferrin. *Molecules* **2020**, *25* (8), 1997. https://doi.org/10.3390/ molecules25081997.
42. Chang, R.; Sun, W.; Ng, T. B. Title: Lactoferrin as Potential Preventative and Treatment for COVID-19. *Authorea Prepr.* **2020**. https://doi.org/10.22541/AU.58630054.41052564.
43. (Ammendolia, M. G.; Agamennone, M.; Pietrantoni, A.; Lannutti, F.; Siciliano, R. A.; de Giulio, B.; Amici, C.; Superti, F. Bovine Lactoferrin-Derived Peptides as Novel Broad-Spectrum Inhibitors of Influenza Virus. *Pathog. Glob. Health* **2012**, *106* (1), 12–19. https://doi.org/10.1179/2047773212Y.0000000004.
44. Kell, D. B.; Heyden, E. L.; Pretorius, E. The Biology of Lactoferrin, an Iron-Binding Protein That Can Help Defend Against Viruses and Bacteria. *Front. Immunol.* **2020**, *11*, 1221. https://doi.org/ 10.3389/fimmu. 2020.01221.
45. Wang, B.; Timilsena, Y. P.; Blanch, E.; Adhikari, B. Lactoferrin: Structure, Function, Denaturation and Digestion. *Critical Reviews in Food Science and Nutrition*. **2019**, 580–596. https://doi.org/10.1080/ 10408398.2017.1381583.
46. Stockwell, B. R.; Friedmann Angeli, J. P.; Bayir, H.; Bush, A. I.; Conrad, M.; Dixon, S. J.; Fulda, S.; Gascón, S.; Hatzios, S. K.; Kagan, V. E.; Noel, K.; Jiang, X.; Linkermann, A.; Murphy, M. E.; Overholtzer, M.; Oyagi, A.; Pagnussat, G. C.; Park, J.; Ran, Q.; Rosenfeld, C. S.; Salnikow, K.; Tang, D.; Torti, F. M.; Torti, S. V.; Toyokuni, S.; Woerpel, K. A.; Zhang, D. D. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell*. **2017**, 273–285. https://doi.org/10.1016/j.cell.2017.09.021.
47. Liu, W.; Zhang, S.; Nekhai, S.; Liu, S. Depriving Iron Supply to the Virus Represents a Promising Adjuvant Therapeutic Against Viral Survival.

Current Clinical Microbiol. Reports. **2020**, 13–19. https://doi.org/ 10.1007/s40588-020-00140-w.

48. Alvarez, S. W.; Sviderskiy, V. O.; Terzi, E. M.; Papagiannakopoulos, T.; Moreira, A. L.; Adams, S.; Sabatini, D. M.; Birsoy, K.; Possemato, R. NFS1 Undergoes Positive Selection in Lung Tumours and Protects Cells from Ferroptosis. *Nature* **2017**, *551* (7682), 639–643. https://doi.org/ 10.1038/ nature24637.
49. Kolnagou, A.; Kontoghiorghe, C. N.; Kontoghiorghes, G. J. New Targeted Therapies and Diagnostic Methods for Iron Overload Diseases. *Front. Biosci. - Sch.* **2018**, *10* (1), 1–20. https://doi.org/10.2741/s498.
50. Kim, S.; Thiessen, P. A.; Bolton, E. E.; Chen, J.; Fu, G.; Gindulyte, A.; Han, L.; He, J.; He, S.; Shoemaker, B. A.; Wang, J.; Yu, B.; Zhang, J.; Bryant, S. H. PubChem Substance and Compound Databases. *Nucleic Acids Res.* **2016**, *44* (Database issue), D1202. https://doi.org/10.1093 /NAR/GKV951.
51. WB, G.; H, L.; SL, M.; CA, B.; CB, F.; JL, A.; HP, B. Evidence That Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. *Nutrients* **2020**, *12* (4). https://doi.org/10.3390/NU12040988.
52. Levy, T. J.; Richardson, S.; Coppa, K.; Barnaby, D. P.; McGinn, T.; Becker, L. B.; Davidson, K. W.; Cohen, S. L.; Hirsch, J. S.; Zanos, T. P.; Bodenstein, H.; Debnath, S.; Dominello, A. J.; Falzon, L.; Gitman, M.; Goldstein, J. M.; Herron, C.; Kim, E. J.; Lau, L.; Lockerman, Z. S.; Makhnevich, A.; Mogavero, J. N.; Molmenti, E. P.; d. Paradis, M.; Tóth, V. Development and Validation of a Survival Calculator for Hospitalized Patients with COVID-19. *Med Rxiv Prepr. Serv. Heal. Sci.* **2020**. https://doi.org/10.1101/2020.04.22.20075416.
53. MM, M.; P, S.; P, S. Oxidative Stress, Antioxidants, Hormesis and Calorie Restriction: The Current Perspective in the Biology of Aging. *Arch. Gerontol. Geriatr.* **2021**, *95*, 104413–104413. https://doi.org/10.1016/J.ARCHGER.2021.104413.
54. Paranjpe, I., Fuster, V., Lala, A., Russak, A. J., Glicksberg, B. S., Levin, M. A., Charney, A. W., Narula, J., Fayad, Z. A., Bagiella, E., Zhao, S., & Nadkarni, G. N. Association of Treatment Dose Anticoagulation with In-Hospital Survival Among Hospitalized Patients With COVID-19. *J. Am. Coll. Cardiol.* **2020**, *76* (1), 122–124. https://doi.org/10.1016/J.JACC.2020.05.001.
55. Rowen, R. J.; Robins, H. A Plausible Penny Costing Effective Treatment for Corona Virus - *Ozone Therapy*. **2020**.
56. Valdenassi, L., Franzini, M., Ricevuti, G., Rinaldi, L., Galoforo, A. C., Tirelli, U. Potential Mechanisms by Which the Oxygen-Ozone (O2-O3) Therapy Could Contribute to the Treatment against the Coronavirus COVID-19. *Eur. Rev. Med. Pharmacol. Sci.* **2020**, *24* (8), 4059–4061. https://doi.org/10.26355/EURREV_202004_20976.
57. Cavezzi, A., Ambrosini, L., Colucci, R., Ionna, G. D., & Urso, S. U. Aging in the Perspective of Integrative Medicine, Psychoneuro-endocrineimmunology and Hormesis. *Curr. Aging Sci.* **2020**, *13* (2), 82–91. https://doi.org/10.2174/ 1874609812666191129095417.
58. M, D. Polyphenols as Antimicrobial Agents. *Curr. Opin. Biotechnol.* **2012**, *23* (2), 174–181. https://doi.org/10.1016/J.COPBIO.2011.08.007.
59. Zhang, R., Wang, X., Ni, L., Di, X., Ma, B., Niu, S., Liu, C., Reiter, R. J. Melatonin as a Potential Adjuvant Treatment. *Life Sci.* **2020**, *250*. https://doi.org/10.1016/J.LFS.2020.117583.
60. Rifkind, J. M.; Heim, J. M. Interaction of Zinc with Hemoglobin: Binding of Zinc and the Oxygen Affinity. *Biochemistry* **2002**, *16* (20), 4438–4443. https://doi.org/10.1021/BI00639A017.
61. RI, H.; PR, F.; J, B. Efficacy of Glutathione Therapy in Relieving Dyspnea Associated with COVID-19 Pneumonia: A Report of 2 Cases. *Respir. Med. case reports* **2020**, *30*. https://doi.org/10.1016/J. RMCR.2020.101063.
62. (Cheng, R. Z. Can Early and High Intravenous Dose of Vitamin C Prevent and Treat Coronavirus Disease 2019 (COVID-19)? *Med. Drug Discov.* **2020**, *5*, 100028. https://doi.org/10.1016/J.MEDIDD. 2020.100028.
63. Group, T. W. R. E. A. for C.-19 T. (REACT) W.; Sterne, J. A. C.; Murthy, S.; Diaz, J. V.; Slutsky, A. S.; Villar, J.; Angus, D. C.; Annane, D.; Azevedo, L. C. P.; Berwanger, O.; Cavalcanti, A. B.; Dequin, P.-F.; Du, B.; Emberson, J.; Fisher, D.; Giraudeau, B.; Gordon, A. C.; Granholm, A.; Green, C.; Haynes, R.; Heming, N.; Higgins, J. P. T.; Horby, P.; Jüni, P.; Landray, M. J.; Gouge, A. Le; Leclerc, M.; Lim, W. S.; Machado, F. R.; McArthur, C.; Meziani, F.; Møller, M. H.; Perner, A.; Petersen, M. W.; Savović, J.; Tomazini, B.; Veiga, V. C.; Webb, S.; Marshall, J. C. Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-Analysis. *JAMA* **2020**, *324* (13), 1330–1341. https://doi.org/10.1001/JAMA.2020.17023.
64. DM, B.; M, F.; SM, G.; M, J.; AA, K.; S, L.; GM, L.; M, M.; A, S.; DR, S.; K, Z.; P, M. Patient Blood Management during the COVID-19 Pandemic: A Narrative Review. *Anaesthesia* **2020**, *75* (8), 1105–1113. https://doi.org/10.1111/ANAE.15095.