

# COVID-19: hemoglobin, iron, and hypoxia beyond inflammation. A narrative review

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## Abstract

Coronavirus disease-19 (COVID-19) has been regarded as an infective-inflammatory disease, which affects mainly lungs. More recently, a multi-organ involvement has been highlighted, with different pathways of injury. A hemoglobinopathy, hypoxia and cell iron overload might have a possible additional role. Scientific literature has pointed out two potential pathophysiological mechanisms: i) severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) interaction with hemoglobin molecule, through CD147, CD26 and other receptors located on erythrocyte and/or blood cell precursors; ii) hepcidin-mimetic action of a viral spike protein, inducing ferroportin blockage. In this translational medicine-based narrative review, the following pathologic metabolic pathways, deriving from hemoglobin denaturation and iron metabolism dysregulation, are highlighted: i) decrease of functioning hemoglobin quote; ii) iron overload in cell/tissue (hyperferritinemia); iii) release of free toxic circulating heme; iv) hypoxemia and systemic hypoxia; v) reduction of nitric oxide; vi) coagulation activation; vii) ferroptosis with oxidative stress and lipoperoxidation; viii) mitochondrial degeneration and apoptosis. A few clinical syndromes may follow, such as pulmonary edema based on arterial vasoconstriction and altered alveolo-capillary barrier, sideroblastic-like anemia, endotheliitis, vasospastic acrosyndrome, and arterio-venous thromboembolism. We speculated that in COVID-19, beyond the classical pulmonary immune-inflammation view, the occurrence of an oxygen-deprived blood disease, with iron metabolism dysregulation, should be taken in consideration. A more comprehensive diagnostic/therapeutic approach to COVID-19 is proposed, includ-

ing potential adjuvant interventions aimed at improving hemoglobin dysfunction, iron over-deposit and generalized hypoxic state.

## Introduction

COVID-19 is the new out-breaking disease, characterized by pneumonia and acute respiratory distress syndrome (ARDS), with sepsis and eventually multi-organ failure.

Most scientific research has focused on the infective-inflammatory component of COVID-19, highlighting several immunological derangements. The infective-immune elements would affect mainly the lungs, which consequently leads to respiratory insufficiency. Moreover, a series of cardiovascular thrombotic events have been described, together with pulmonary embolism, disseminated intravascular coagulation (DIC), liver/myocardial/renal failure.<sup>1,2</sup> An endothelial cell direct viral injury has been suggested as well, potentially contributing to blood and vascular complications.<sup>3</sup> More recently, two innovative pathophysiology hypotheses have been proposed, concerning hemoglobin dysfunction and tissue iron overload, based on preliminary computational and genetic sequencing researches.

A preliminary paper about the viral inhibition of heme metabolism, by binding to beta-chains of hemoglobin through surface glycoproteins,<sup>4</sup> has been followed by another publication about virus-induced hemoglobin denaturation.<sup>5</sup> This hemoglobin alteration would contribute to the oxygen deprived multi-faceted syndrome, which is actually generated by SARS-CoV-2.

Concerning iron dysmetabolism in COVID-19, Ehsani has highlighted a remarkable similarity between the distant amino acid sequence of SARS-CoV-2 spike glycoprotein cytoplasmic tail and the hepcidin protein.<sup>6</sup> Coronaviruses recognize host receptors using their spike proteins, facilitating conformation transition, so to bind cell membrane and enter host cytoplasm; by using host furins and proteases, coronaviruses may cleave their spike polypeptides, thus favoring the cell entry.<sup>7</sup> The found hepcidin mimicry by the virus would take place through this complex mechanism.<sup>6</sup>

Hepcidin is the master regulator of iron metabolism, interacting with ferroportin to favor iron entrance inside the cells;<sup>8</sup> in case of hepcidin-like activity of SARS-CoV-2 a significant iron dysmetabolism may occur, with hyperferritinemia and ultimately ferroptosis.<sup>9</sup>

The altogether of hemoglobinopathy and iron dysmetabolism may seriously compromise the capacity of erythrocytes to

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perform O<sub>2</sub> transport, with hypoxia, while inducing hyperferritinemia-related tissue alterations.

World Health Organization has recently taken into consideration the possible contribution of blood changes within the COVID-19 course.<sup>10</sup>

Whether the original pathologic viral process begins in the lungs, with consequent general anemic hypoxia and iron dysmetabolism, or vice versa hemoglobin/iron dysmetabolism is the leading process that results in multi-organ disease and further hypoxia, is a matter of future research.

SARS-CoV-2 infection strictly depends upon the virus interaction with host cell receptors and proteases. Together with the acknowledged ACE2, cyclophyllins, furins and TMPRSS2, CD147 was identified as an additional receptor on erythrocytes and other cells.<sup>11,12</sup>

Actually, CD147 may also represent the virus entry-point in bone marrow immature cells;<sup>13</sup> interestingly, myeloma colonization of bone marrow is mediated by this receptor.<sup>14</sup> Hypoxia seems to upregulate CD147 expression, which might be detrimental in case of viral linkage to these receptors in erythrocytes/erythroblasts.

Lastly, another receptor, namely DPP4 (CD26), was found to possibly interact with SARS-CoV-2 spikes<sup>12,15</sup> and intriguingly this molecule has a proven role in hematopoiesis as well.<sup>16</sup>

The aim of this narrative review was to provide a complementary overview of the currently available scientific literature on COVID-19, beyond the conventional pneumonia and immune-inflammation conventional view. Based on a few ascertain dysregulated pathomechanisms, which pertain to iron and hemoglobin metabolism and hypoxemia, and on the possible related therapies, we performed a literature search reviewing pertinent articles and documents. PubMed, Google Scholar, Chemrxiv, MedRxiv, BioRxiv, Preprints and ResearchGate were investigated, using the following headings and keywords, linked to the words *COVID-19* or *Sars-CoV-2*: hemoglobin, heme, porphyrin, hematology, erythrocyte, hematopoiesis, erythroblast, thalassemia, hemolysis, RDW, LDH, hypoxia, hypoxemia, nitric oxide, HAPE, ARDS, O<sub>2</sub>, CO<sub>2</sub>, iron, hepcidin, ferroportin, ferritin, ferroptosis, hemochromatosis, chelation, translational medicine, oxidative stress, hormesis, resilience, drugs, nutrition, nutraceuticals, food supplements, polyphenols, mitochondria, lactate, CD147, CD26, GRP78, coagulation, anticoagulants, heparin, vascular, endothelial, thromboembolism, ozone.

Accordingly, literature data provide some evidence on the possible virus interaction with hemoglobin in red blood cells/precursors and with iron metabolism. Based on a translational medicine view, in this narrative review we provide an elaboration concerning the possible role of hemoglobin denaturation and tissue iron overload in COVID-19; potential adjuvant therapeutic interventions, targeting the aforementioned pathomechanisms, are also elucidated.

## Pathophysiological hypotheses

An altogether of several deranged biochemical pathways occur in COVID-19, due to the multiple interplaying levels of viral attack. Notwithstanding the acknowledged role of immune-inflammation processes in the pathophysiology of this disease, hemoglobin alteration, hypoxemia and iron dysmetabolism represent additional key-factors to be investigated within the diagnostic-therapeutic approach.

A viral interaction with hemoglobin molecule, through ACE2, CD147, CD26 and other receptors located on erythrocytes and/or blood cell precursors, has been highlighted. Summarizing the preliminary available data,<sup>4,5</sup> it has been argued that hemoglobinopathy would derive from viral endocytosis, through a linkage between spike proteins and cell receptors. Viral ORF8 protein and surface glycoprotein would bind to

porphyrin, attacking the heme on the 1-beta chain of hemoglobin; SARS-CoV-2 would consequently induce hemolysis and/or form a complex with the released heme, generating a quote of dysfunctional hemoglobin, with reduced oxygen and CO<sub>2</sub> transport.

A similar mechanism was demonstrated for *Plasmodium malariae*, which enters erythrocytes through CD147 core-receptor.<sup>17</sup> A significant role for basigin (CD147) in several metabolic pathways and diseases has been highlighted;<sup>12,14</sup> it has been reported there are about 3000 molecules of CD147 per erythrocyte, determining also the OK blood group;<sup>17</sup> interestingly, pathogenicity of several viral infections is influenced by CD147/OK blood group.<sup>17</sup> Regardless of the limited evidence of the proposed molecular modeling, clinical-instrumental data account for a relevant loss of functioning hemoglobin, especially in later stages of COVID-19.<sup>18</sup> Other hemoglobin-associated markers, such as bilirubin and ferritin, progressively increase concomitantly to the worsening of the disease.<sup>19,20</sup>

This activity of SARS-CoV-2 on hemoglobin has been considered a possible basic pathogenic mechanism,<sup>21</sup> as well as a hypothesis about considering COVID-19 an acquired acute porphyria has been preliminarily published.<sup>22</sup>

SARS-CoV was previously shown to interfere with hemoglobin at erythrocyte and bone marrow level; in fact, through CD147 and/or CD26, the new SARS-CoV-2 attack to bone marrow erythroblasts is plausible, as the larger dimension and cytoplasmic/nuclear material of the precursors would facilitate virus replication and interaction with hemoglobin.

LDH is a reliable marker of hemolysis. The clear-cut evidence from a few large-scale retrospective studies indicates that LDH is exceptionally accurate to screen severe from mild cases of COVID-19. More interestingly, LDH figures increase of a two-three fold in worst scenarios, paralleling hemoglobin decrease.<sup>20,23</sup> Similarly, a relevant hypoferraemia has been reported in ICU patients, with lower serum iron levels associated to severe hypoxemia-related respiratory insufficiency.<sup>24</sup>

The progressively decreased hemoglobin level may lead to a sideroblastic-like anemia pattern, with myelodysplastic features, as per the acute need to replace dysfunctional erythrocytes. Red cell distribution width (RDW) represents a reliable marker of myelodysplasias, being higher when immature cells are produced. COVID-19 literature repeatedly highlights a generally increased RDW, which is significantly higher (>14.5%) in deceased or critical patients.<sup>25,26</sup>

A few case-reports described specific diagnostic/therapeutic features linked to myelodysplastic patterns in COVID-19, with atypical and relevant blood alterations improvement after erythropoietin administration.<sup>27</sup> Other pertinent hematologic findings concern infected thalassemic patients, who seem to exhibit a good prognosis,<sup>28</sup> due to the reduction of hemoglobin beta-chains (potential virus' target); furthermore, thalassemia-associated low hepcidin secretion<sup>8</sup> may represent another "protective" mechanism.

Together with the previously reported molecules, the GRP78 receptor has been considered another SARS-CoV-2 entry-facilitator.<sup>29</sup> This endoplasmic reticulum heat shock protein is also located in bone marrow stem cells. Putatively, this additional receptor would facilitate anti-hemoglobin viral action on hematopoietic stem cells; moreover, ascertained GRP78 downregulation in beta-thalassemia could result beneficial.<sup>30</sup> Overall, the literature data reported above strengthen the hypothesis of erythrocytes/erythroblasts involvement in COVID-19. Of interest, free circulating heme typically injures endothelial cells, as well as ferritin overdeposit may contribute to vascular wall remodeling, hence a form of diffused endotheliitis<sup>3</sup> would be potentially justified by these two pathomechanisms.

Physiologically, anemic hypoxia induces general vasodilation, but also pulmonary vasoconstriction, with an increase of fibrin formation in lung microvasculature.<sup>31</sup> Notably, also hepcidin/ferroportin dysregulated interaction may create pulmonary artery hypertension, via smooth muscle cell proliferation.<sup>32</sup>

Nitric oxide (NO) secretion usually compensates hypoxia inducing vasodilation, but circulating cell-free hemoglobin would impair NO bioavailability. The resulting orthosympathetic activation and systemic vasospastic acrosyndrome could especially affect terminal circulations; in fact, Raynaud phenomenon, chilblains (mostly in children), and other organ histotoxic phenomena linked to hypoxia and possible hypercoagulation have been described.<sup>33,34</sup> During profound hypoxia, tissue injury is caused by mitochondria degeneration; notoriously, in the absence of sufficient oxygen supply, glycolysis anaerobically replaces Krebs cycle and oxidative phosphorylation, with consequent repercussions in mitochondrial metabolism (see the high lactate figure in advanced cases<sup>25</sup>). Cell necrosis and altered apoptosis follow, due to abnormal mitophagy; this kind of histotoxic hypoxia somehow mimics the pathophysiology of chronic cyanide/lead-poisoned hemoglobin, with tissue injuries.<sup>33</sup>

Concerning lung consequences, a systemic hypoxia state, with normal pulmonary tissue compliance, has been highlighted in up to 80% of intensive care unit (ICU) patients exhibiting respiratory distress, which led a few authors to question ARDS diagnosis.<sup>35</sup> Furthermore, a certain similarity between the computed tomography (CT), laboratory, and clinical features of high altitude pulmonary edema (HAPE) and of COVID-19 pneumonia has been highlighted.<sup>36</sup> HAPE is a non-infective, non-inflammatory interstitial pulmonary edema, caused by the low inhaled oxygen and Starling's law disequilibrium, with pulmonary vasoconstriction and hypertension.<sup>36</sup> Interestingly, in COVID-19 patients  $\text{paCO}_2$  does not relevantly rise, if not at the latest, most critical stages.<sup>20</sup> Furthermore, the  $\text{PaO}_2/\text{FiO}_2$  ratio, which is usually high in ARDS, is conversely low in COVID-19 (HAPE-like) lung involvement, until critical respiratory insufficiency occurs.<sup>36</sup> Basically, clinical/instrumental features of lung disease in COVID-19 seem to shift to typical ARDS features only when alveolo-capillary membrane relevantly deteriorates and/or pulmonary circulation is impaired furthermore, possibly due to thromboembolic phenomena and ferroptosis.<sup>9,19,20,25,35,36</sup> Likely, the combination of hypoxemic hypoxia with relatively normal lung compliance and normocapnia, could orientate the conventional *pneumonia* diagnostic/therapeutic approach differently.<sup>35-37</sup>

A kind of *silent* hypoxia is described in these patients, who show progressively worse hypoxemia associated with normal  $\text{CO}_2$ . Normocapnia reflects normal pulmonary gas exchange; being  $\text{CO}_2$  elevation the primary sensor for respiratory distress, patients show relevant respiratory symptoms at later stages only, when  $\text{CO}_2$  increases.<sup>35-38</sup> Lastly, hyperferritinemia progressively affects alveolar-capillary/cell membrane integrity/permeability: inflammation, edema and lung cell necrosis may complicate pulmonary condition ultimately.<sup>39</sup>

Concerning the role of iron toxicity in COVID-19 pathophysiology, the putative hepcidin-mimetic action of SARS-CoV-2<sup>6</sup> may induce ferroportin internalization/blockage, which could explain progressive anemia and hyperferritinemia.

Hepcidin favors iron entrance in cells, downregulating ferroportin, which is the key transporter of iron outside the cells;<sup>8</sup> basically, hepcidin is to iron as insulin is to glucose<sup>6</sup> and hepcidin excess may cause ferroptosis.<sup>9</sup>

Physiologically, hepcidin is respectively up- or down-regulated by high or low serum iron.<sup>8</sup> Other hepcidin-agonists are

inflammation (IL-6, namely), hyperoxemia, obesity and diabetes. Oppositely, hepcidin is antagonized, and ferroportin is upregulated, by hypoxemia, with hypoxia-induced factors (HIF) release, and by anemia.<sup>8</sup>

Interestingly, diabetics' increased hepcidin level pairs the higher level of glycosylated, dysfunctional, hemoglobin; concurrently, obese people and diabetics overexpress CD147 blood receptor,<sup>12</sup> and this altogether of biochemical rearrangements raises their complication risk.

Mimicking hepcidin action, SARS-CoV-2 might remarkably increase circulating and tissue ferritin (affecting liver, spleen, bone marrow and muscles mainly), while inducing serum iron deficiency and lack of hemoglobin, by consequence. Hyperferritinemia gives rise to ferroptosis, with high oxidative stress and lipoperoxidation, ultimately increasing mitophagy with accelerated cell apoptosis/necrosis.<sup>9</sup>

In fact, cell iron overload is tolerated up to a threshold, as with silent hypoxia (COVID-19 first phase). The increasing ferroptosis-linked multi-organ oxidative stress can precipitate the inflammatory/immune over-response (the so-called *interleukine storm*) in later, most critical stages. Laboratory data show a relevantly lower hemoglobin level and higher ferritin levels in non-surviving patients, over the survivors.<sup>19,20,25</sup>

Tissue iron sequestration results in a unique increase of ferritin in the epithelium and immune cells of the lungs; this finding is probably linked to the physiological need to protect lung cells from air oxygen-driven oxidative stress and pathogens.<sup>39</sup>

Hyperferritinemia may induce a series of direct and indirect (*via* autoimmunity) injuries to most organs during COVID-19, such as coagulopathies, macrophage activation syndrome, hemochromatosis-like liver injury, and other ferroptosis-driven syndromes.<sup>40</sup>

SARS-CoV-2 interaction with iron metabolism and oxygen supply could be linked to phylogenetic mechanisms, which were developed in ancestral oxygen-free and iron-rich environments. In fact, viral RNA replication favors this *hostile*-to-humans ground, where Fenton oxidative reaction is highly expressed.<sup>41</sup>

Viruses generally increase iron deposit, to favor their diffusion in host cells;<sup>42</sup> conversely, our immune system tends to control iron metabolism in case of infection, also through transferrin. This key-factor of iron metabolism has ubiquitous (lungs *in primis*) receptors, which are used by many viruses to enter host cells.<sup>43</sup> Possibly, future research could elicit the transferrin receptor

as another target of SARS-CoV-2, which would explain iron dysmetabolism furthermore. Overall, laboratory findings of COVID-19, such as hyperferritinemia, low hemoglobin, low serum iron, thrombocytopenia and anisocytosis, with high figures of RDW, increased lactate and LDH, are reasonably compatible with the hypothesized erythrocyte/bone marrow dysmetabolism and iron dysregulation.<sup>18-20,23-26</sup>

Several organs are directly, or indirectly targeted by SARS-CoV-2 and multiple pathomechanisms have been described, both of immune/inflammatory type and linked to hypoxia and ferroptosis; thromboembolism seems to play a relevant role in later stages as well. Overall, pathophysiological pathways seem to overlap in most cases; however, the found hemoglobinopathy and iron dysmetabolism may induce a series of biological events, which objectively relate to the clinical syndromes highlighted during COVID-19: i) decrease of functioning hemoglobin quote; ii) iron increase in cell/tissues; iii) release of free toxic circulating heme; iv) hypoxemia and systemic hypoxia; v) reduction of NO; vi) coagulation activation; vii) ferroptosis with oxidative stress and lipoperoxidation; viii) mitochondria degeneration.

## Translational medicine-based adjuvant therapeutic options

A few potential therapies in hemoglobin dysfunction and in erythrocyte/erythroblast disease could be represented by selective employment of erythropoietin administration,<sup>27</sup> hyperbaric oxygen, extra-corporeal membrane oxygenation, blood transfusion and glucose/heme-arginine infusion, CD147/CD26-targeting re-purposed drugs or natural compounds<sup>13,44,45</sup> and stem cells.<sup>13,46</sup>

Concerning hepcidin-antagonists (and ferroportin agonists), literature data indicate a few viable pharmaceutical options, such as:<sup>47</sup> spironolactone, metformin, sodium heparin (preferably) or dalteparin/enoxaparin/fondaparinux, erythropoietin, anti-IL6/hepcidin monoclonal antibodies, ferroportin-mimetic antibodies, HIF-stabilizers, cholecalciferol.

Below we report a series of additional adjuvant therapies, targeting hemoglobin denaturation, hypoxia and iron overload, based on a translational medicine-based literature research. Most of these optional treatments are currently under scrutiny in multiple ongoing clinical trials in COVID-19 patients.

## Ascorbic acid

Ascorbic acid (AA) has an immune, anti-infective, and anti-NLRP3 (namely cytokine storm) activity.<sup>48</sup> AA was found to prevent mitochondrial membrane depolarization and early mitophagy, by combating mito-DNA oxidative stress, thus regulating fission and fusion.<sup>49</sup> The virus-related oxidative stress leads to an under-functioning NO; furthermore, hemoglobin denaturation and cell-free heme are potent NO-neutralizers and ARDS-inducers, whereas AA is able to increase NO production by improving heme iron/cell redox balance.

If hemolysis and/or iron dysmetabolism occur, as we speculate, the resulting reactive ferric ion induces oxidative reactions and especially impairs the oxygen-binding capacity of hemoglobin.<sup>50</sup> AA is the main compound erythrocytes use to maintain hemoglobin iron in the ferrous state, which is the only form to bind oxygen.<sup>51</sup> This beneficial activity of AA on hemoglobin is best achieved by combining AA oral supplementation, which has a higher blood early peak and longer half-life, with intravenous sodium ascorbate.<sup>52</sup> Oral AA generates plasma L-ascorbate, which indirectly decreases furin cleavage and reduces endothelial permeability to noxious free heme.<sup>53</sup> Overall, literature data highlight a significant role for AA in ICU patients, with sepsis, pneumonia, multi-organ failure and ARDS.<sup>48</sup>

## Glutathione

Glutathione (GSH) has a protective effect against oxidative stress and viral infections.<sup>54</sup> Ferroptosis-induced oxidative stress is improved by GSH;<sup>9</sup> moreover, GSH may reduce both hemoglobin glycation<sup>55</sup> and, in combination with AA, heme oxidation. Two cases of quick relief from COVID-19 pneumonia-related dyspnea have been described after multiple doses of GSH.<sup>56</sup>

## Zinc

Zinc plays a central role in the immune system, and zinc-deficient individuals experience increased susceptibility to pathogens. This mineral exhibits a series of anti-RNA viruses' activities on polymerases, entry, and virulence,<sup>57</sup> resulting of help in respiratory infections, thanks to its beneficial activity on hemoglobin O<sub>2</sub> affinity as well.<sup>58</sup>

## Vitamin D

Cholecalciferol (vitamin D3) supplementation has been proposed in COVID-19 management, mostly to regulate the pro-inflammatory milieu, modulating innate and adaptive immunity.<sup>59</sup> Vitamin D may also activate a few hepcidin-antagonist pathways, regulating the hepcidin-ferroportin axis. Intubated patients receiving 100.000 IU/day of cholecalciferol for 5 days showed higher and lower levels of hemoglobin and hepcidin respectively.<sup>59</sup> If cholecalciferol insufficiency may be pro-thrombotic, conversely, active form calcitriol may have a net anticoagulant effect.<sup>60</sup> Interestingly, cholecalciferol showed an upregulatory epigenetic action on a few antioxidant systems (GSH complex and AA included).<sup>59</sup>

## Melatonin

Beyond its role in circadian rhythms, psychoneuroendocrinology, and aging,<sup>61</sup> this hormone interacts with a series of immunity and inflammasome pathways typical of COVID-19.<sup>62</sup> Moreover, this molecule mitigates systemic oxidative stress, and specifically in lung mitochondria, which may help modulate pulmonary hypertension. Lastly, melatonin exhibits a proven protective role against hypoxia, ferroptosis, and hemoglobin denaturation.<sup>62,63</sup>

## Polyphenols

Polyphenols are natural compounds, which own several epigenetic activities, mainly by virtue of their hormetic potential and through NrF2-ARE pathway;<sup>61</sup> additionally, their direct antiviral action has been documented in a few studies.<sup>64</sup> Moreover, also heme-oxygenase-1 activation by many polyphenols, curcumin above all, has been documented,<sup>65</sup> which may beneficially impact hemoglobin denaturation. Curcuminoids, anthocyanins (delphinidins mainly) and catechins proved to benefit the following biochemical processes which take place in COVID-19:<sup>61,64-67</sup> i) hemoglobin oxidation, ferroptosis, and lipoperoxidation; ii) derangement of mitochondria function/biogenesis, with altered mitophagy; iii) inflammasome activation and *interleukine storm*; iv) SARS-CoV-2 protease activity; v) SARS-CoV-2 attack to bone marrow; vi) hepcidin upregulation and ferroportin blockage.

## Heparin

The interaction among SARS-CoV-2, endothelial cells, iron metabolism, and erythrocytes, together with the resulting hypoxia, may lead to a series of coagulopathies. The concomitant inflammasome hyperactivation represents another pro-thrombotic factor, even more in the presence of hypoxia. Lastly, viral infection may typically induce an autoimmune-mediated pro-coagulant state.<sup>68</sup> In view of this coagulation activation, heparins are being increasingly employed in these patients. Beyond the classical anticoagulant, anti-inflammatory, angiogenetic, endothelial protective and possible anti-viral effect,<sup>68</sup> heparins also proved to antagonize hepcidin.<sup>47</sup> It is a reminder here that iron dysfunctional metabolism, ferroptosis and Fenton reaction (typical of oxidative stress) may induce fibrinolysis-resistant parafibrin formation.<sup>69</sup> The over-represented ferritin deposit in the lungs under viral infection,<sup>39</sup> together with the consequent fibrin/coagulation derangement, fully justify anticoagulation with multi-functional heparin. Heparin may have a prophylactic and therapeutic role in COVID-19 patients, possibly improving their prognosis;<sup>68</sup> hence anticoagulation has definitely become a cornerstone pharmaceutical treatment with multiple potentials, showing a multi-organ beneficial effect.<sup>70</sup>

## Ozone

Ozone is regarded as a possible adjuvant treatment during SARS-CoV-2 infection.<sup>71,72</sup> Together with its acknowledged direct antiviral action, ozone has other (partly hormesis-mediated), useful biochemical effects:<sup>71,72</sup> i) NO increase; ii) improved erythrocyte rheology; iii) 2,3-diphosphoglycerate increase with better hemoglobin functionality; iv) improved mitochondrial oxidative phosphorylation; v) transient increase of ROS and consequent NrF2/ARE pathway activation; vi) increase of IL10 and decrease of pro-inflammatory cytokines and of TNF $\alpha$ ; vii) upregulation of GSH activity and heme-oxygenase pathway.

This extremely quickly dissipated gas may be administered intravenously or, more rarely, trans-mucosae. Currently, ongoing clinical trials in COVID-19 include intravenous ozone (autohemotransfusion); as to the registered trials, an average of 200 ml of ozonized blood (40mcg/ml) are infused twice a day.

## Mitochondria-targeted interventions

COVID-19 features mitochondria degeneration; these organelles play a fundamental role in innate and adaptive immunity in viral infections. Viruses determine mitochondria membrane depolarization, so to influence mitochondria fusion, fission and apoptosis of the infected cell.<sup>73</sup> Hospitalized COVID-19 patients showed an increased lactate figure (typical of mitochondria dysfunction), which was significantly higher in the deceased subjects.<sup>25</sup> Anthocyanins, AA, curcuminoids, hydroxytyrosol, polydatin, PQQ, ubiquinol, nicotinamide-mononucleotide and specific mitochondria-targeted antioxidants (based on triphenylphosphonium cation) may improve mitochondrial biogenesis/function.<sup>61</sup>

## Iron chelation

Virus-connected iron dysmetabolism, ferroptosis, and oxidative stress influence host immune response.<sup>42</sup> COVID-19 results in remarkably high ferritin levels, which represents a negative prognostic factor, due to a series of detrimental interferences on endothelium, coagulation, and multiple organ cells, lungs *in primis*. Chelation proved to reduce viral replication and related pro-inflammatory pathways.<sup>74</sup> Deferoxamine was shown to work also as HIF-mimetic, downregulating hepcidin. Iron chelation, with deferiprone or deferoxamine, has been proposed in COVID-19, also in view of its demonstrated efficacy in other viral diseases.<sup>74</sup>

## Pulmonary hypoxia/circulation-targeting interventions

COVID-19 combines the so-called anemic hypoxia (low hemoglobin concentration), with the hypoxic hypoxia (low hemoglobin saturation).<sup>33,75</sup> Oxygen deprivation and iron accumulation in lung tissues generate pulmonary vasoconstriction and neoshunting formation, regardless of the incurring pneumonia.<sup>36-39</sup> A debate on the HAPE-like pulmonary involvement has corroborated the role of lung vascular morpho-functional alterations in respiratory insufficiency, which may lead to ARDS over time.<sup>76</sup>

A few literature-backed potentially useful interventions in hypoxia-based pulmonary hypertension and vasoconstriction may be highlighted: i) high concentration/high flow oxygen at low pressure; ii) prostanoids; iii) acetazolamide; iv) calcium

channel blockers; v) sildenafil; vi) NO; vii) adenosine; viii) nitroglycerine-based drugs.<sup>36</sup> Most recently, a critical prognostic role for pulmonary hypertension was detected through echocardiography, highlighting right ventricle dysfunction.<sup>77</sup> Therefore, it follows that targeting pulmonary vasodilation, in COVID-19-related respiratory insufficiency, seems to have a growing scientific background.

## Conclusions

A panoply of symptoms and findings characterize COVID-19, which stimulates scientific community research to improve the investigational/therapeutic approach.

Regardless of the established infective-immune pathways in COVID-19, this narrative review has highlighted a series of possible additional pathophysiological mechanisms, clinical syndromes and specific adjuvant treatments, based on some literature data which elucidate the role of hemoglobin denaturation, iron dysmetabolism/tissue overload and hypoxia.

The limited knowledge about COVID-19 may allow speculative elaborations, which are sometimes based on theoretical modeling, correlations, or on limited evidence. A major scientific research advancement and a more comprehensive disease management are expected in the next future.

The translational medicine-based speculative reasoning provided in this review may represent a contribution to stimulate future studies, so to corroborate or disprove our original elaboration.

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