DERLEME REVIEW

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COVID-19 and Iron Metabolism: Traditional Review

COVID-19 ve Demir Metabolizması: Geleneksel Derleme

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ABSTRACT Viruses invade cells to reproduce, and they require an iron-filled cell for efficient reproduction. Together with other viruses, the coronavirus disease-2019 (COVID-19) virus can alter the expression of proteins involved in iron homeostasis. For example, in COVID-19 patients, an increase in pro-inflammatory cytokines such as interleukin-6 may stimulate the synthesis of hepcidin, the regulatory hormone of iron metabolism, thereby suppressing ferroportin-mediated cellular iron export. Increased serum levels of ferritin in COVID-19 virus infection is associated with a poor prognosis and may be partly due to the virus itself. Some viruses selectively infect iron acceptor cells (e.g. macrophages) by binding to transferrin receptor 1during cell entry. Moreover, human airway secretions in the major route of entry of COVID-19 include transferrin and lactoferrin, and this glycoproteins can bind iron and maintain a chemically inert form. Understanding how iron metabolism and viral infection interact in the COVID-19 outbreak may suggest new ways to control the disease.

Keywords: SARS-CoV-2; COVID-19; iron; ferritin; hepcidin

ÖZET Virüsler, çoğalmak için hücreleri istila eder ve verimli üreme için demir dolu bir hücreye ihtiyaç duyarlar. Diğer virüslerle birlikte koronavirüs hastalığı-2019 [coronavirus disease-2019 (COVID-19)] virüsü, demir homeostazına dâhil olan proteinlerin ekspresyonunu değiştirebilir. Örneğin COVID-19 hastalarında, interlökin-6 gibi proinflamatuar sitokinlerdeki bir artış, demir metabolizmasının düzenleyici hormonu olan hepsidin sentezini uyarabilir ve böylece ferroportin aracılı hücresel demir dışa aktarımını baskılayabilir. COVID-19 virüs enfeksiyonunda artan serum ferritin seviyeleri kötü prognozla ilişkilidir ve kısmen virüsün kendisinden kaynaklanıyor olabilir. Bazı virüsler, hücre girişi sırasında transferin reseptörü 1'e bağlanarak seçici olarak demir alıcı hücreleri (örneğin makrofajlar) enfekte eder. Dahası, COVID-19'un ana giriş yolundaki insan hava yolu salgıları arasında transferrin ve laktoferrin bulunur ve bu glikoproteinler demiri bağlayabilir ve kimyasal olarak inert bir formu koruyabilir. COVID-19 salgınında demir metabolizmasının ve viral enfeksiyonun nasıl etkileşime girdiğini anlamak, hastalığı kontrol etmenin yeni yollarını önerebilir.

Anahtar Kelimeler: SARS-CoV-2; COVID-19; demir; ferritin; hepsidin

Worldwide, coronavirus disease-2019 (COVID-19) [severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2)] is a new epidemic disease characterized by pneumonia.[1] There are many studies in the literature reporting the epidemiological and clinical characteristics of patients with COVID-19, but the pathophysiological characteristics of infected individuals are very limited in these articles.[2] In recent studies, hyper-inflammation, which may be encountered in COVID-19, may be associated with oxidative imbalance, impaired iron metabolism, and hyper-activated immune response.[1,3]

Currently, the state of iron metabolism in the genesis and prognosis of COVID-19 is still unclear. For the first time in the literature, we have suggested in our previous articles that increased serum ferritin levels as a result of hyper-inflammation associated with COVID-19 may indicate a vicious iron cycle

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and lead to increased tissue damage due to increased ferroptosis.[4,5] Recently, innovative pathophysiology hypotheses regarding iron overload in tissue have been supported by many scientists.[3] Indeed, hyperferritinemia has been identified as a key feature predicting a high risk of death in several studies of severe COVID-19 patients.[6,7] Because of this strong association with serum ferritin levels and mortality in COVID-19 patients, the presence of hyper-ferritinemia may be a systemic marker of pathogenesis in COVID-19 patients.[6,7] It may also be thought that hyper-ferritinemia is associated with a state of iron toxicity that may result from increased ferritin leakage from damaged tissue.[8,9] For example, the cognitive impairment, ageusia and anosmia (loss of taste and smell) that occur in COVID-19 disease may be the result of ferroptosis.[10] Of course, excessive cellular iron deposition and intracellular mitochondrial dysfunction can be associated with many organ failures.[11]

Regardless, systemic changes in serum iron levels can be detected in some patients but this finding may not be general.[6,7] Also, iron metabolism tests can show serious changes in the presence of iron deficiency in the patients.[9] Therefore, investigating current iron parameters such as transferrin saturation (TfSat), plasma iron levels, non-transferrin-binding iron and hepcidin in COVID-19 patients may be more important in the future.[3,10]

The free form of cellular iron (Fe^{+2}) can interact with hydrogen peroxide to form reactive oxygen species (ROS). The excess ROS produced by iron by the Fenton, Haber-Weiss and other reactions can contribute greatly to the oxidative damage of cellular components of many organs. In addition, the complex interaction between iron metabolism and reactive nitrogen species (RNS) and reactive sulfur species (RSS) are iron metabolism and newly described interactions of reactive species.[3,10] It is known that free oxidized iron in the cell accelerates coagulation by interacting with proteins of coagulation factors.[12] For example, dysfunction of platelet mitochondria by iron overload in the blood can trigger thrombosis in COVID-19. Also, the coagulopathy common in COVID-19 may be the result of intracellular iron overload.[6-8,13]

SARS-CoV-2 PROTEINS

SARS-CoV-2 has a positive single-stranded RNA, protein spike (S), membrane (M), and envelope (E) proteins, as well as the nucleocapsid (N) protein containing structural amino acids.[14,15] The angiotensin converting enzyme 2 (ACE2) receptor, found in many organs in humans, is the main receptor for SARS-CoV-2.[1,14,15]

ANCIENT ORIGIN OF CORONAVIRUSES

According to molecular clock studies, the common ancestors of corona viruses existed millions of years ago.[13] First of all, coronaviruses are enveloped, non-segmented, positive sense RNA viruses; therefore their stability is lower, but the mutation potential is higher compared to DNA analogs. Due to the lack of reread activity of RNA virus polymerases, RNA viruses have a relatively high mutation tendency and adaptive capacity. An oxygen-deprived iron rich habitat, reminiscent of the atmosphere at the beginning of life on Earth, is a situation that RNA viruses so desire because simple RNA viruses such as SARS-CoV-2 and many other infectious agents can use free iron for catalysis.[13] May be the decrease in the oxygen saturation in severe COVID-19, that is, the hypoxemic state, provides an advantage to the viruses.[13-16] Iron is also required for processes such as viral replication, mitochondrial function, ATP production, and nucleic acid synthesis. SARS-CoV-1 viral replication in the previous coronavirus outbreak requires large amounts of ATP hydrolysis for the activity of RNA strands, and this process requires plenty of iron in the environment likely to happen.[5,16,17]

COVID-19 AND IRON METABOLISM

The first way; food (Fe^{+2}) or ferric (Fe^{+3}) iron is absorbed by intestinal enterocytes by the divalent metal transporter (DMT1). The iron absorbed into the intestinal cells is then released from the intestinal enterocytes into the bloodstream via ferroportin-mediated transferrin-bound iron (TBI). However, unlike the various pathways of cellular iron uptake, only two cellular iron release mechanisms are known (Figure 1).[3,4,18] In general, iron release from a cell occurs via ferro-

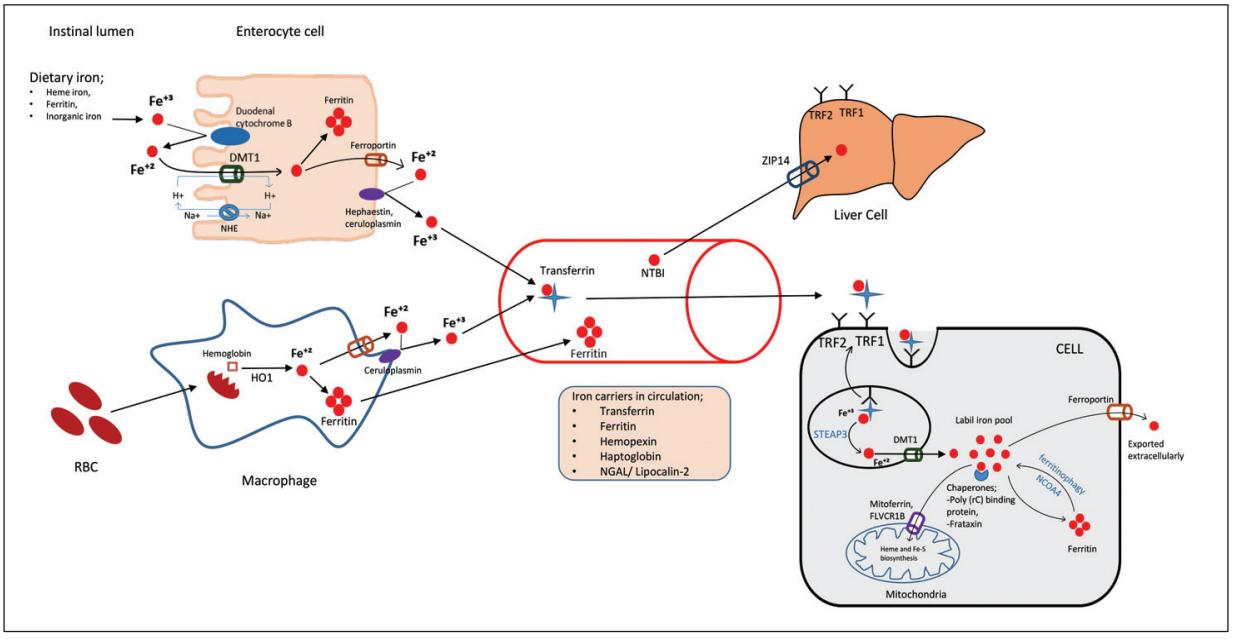

FIGURE 1: Iron transfer metabolism-intracelluler iron transfer.
DMT1: Divalent metal transporter 1; NHE: Na+/H+ exchanger; TRF1: Transferrin receptor 1; TRF2: Transferrin receptor 2; ZIP14: Zinc transporter; HO1: Heme oxygenase-1; NTBI: Non-transferrin bound iron.

portin (Fpn), a membrane bound iron exporter controlled by hepcidin derived from hepatocytes.[18,19]

Fpn-releasing iron, which is exported from the cell when needed or to maintain iron balance, is then transferred directly to TBI in the blood with the help of ferroxidase hephaestin and ceruloplasmin, which contains of 6 copper atoms in its structure.[18-20]

Because, as stated in the "Iron World Hypothesis", viruses have been using iron in their replication for catalytic purposes since primitive times.[16] For example, the increased pro-inflammatory cytokines in COVID-19 such as interleukin-6 (IL-6) protect the cell against harmful reactions such as Fenton because cytokines, particularly IL-6, stimulate hepcidin production.[21] Indeed, in COVID-19, hepcidin and ferritin levels can be reduced with anti-IL6 treatment.[22,23] When the Tf binding capacity is exceeded, it can release the iron in the environment in ferric form (Fe^{+3}) that can damage the organism. In addition, Tf synthesis can be altered by inflammation and hypoxia.[18-20,24] Hyperferritinemia, one of the laboratory findings of COVID-19, may be valuable as it binds free iron and provides antioxidant defense.[20,25] In COVID-19 and other inflammatory events, the increased ferritin in serum can bind to its own ferritin receptors or TfR and thus enter the cell through endocytosis.[18,20,26]

Studies have also demonstrated the in vitro antiviral activity of iron oxide nanoparticles. Studies show that Fe_2O_3 and Fe_3O_4 are effective against SARS-CoV-2 glycoproteins.[27] Similarly, the beneficial effect of recombinant human erythropoietin in the treatment of COVID-19 disease may occur as a result of free iron uptake from the lung tissue required for erythropoiesis.[28]

COVID-19 DISEASE AND ALVEOLAR OXIDATIVE STRESS

Broncho alveolar lavage fluid obtained from ARDS patients has been shown to contain increased molecular iron compared to normal healthy controls.[29,30]

Free iron can often increase virulence and prooxidant reactions and contribute to alveolar oxidative damage. As a result, as with many viral infections, SARS-CoV-2 can prefer to replicate in the lungs, making the patient hypoxaemic/hypoxic. In macrophages, Nrf2 can regulate genes involved in hemoglobin catabolism, iron storage and iron export.

It has been shown that Nrf2 is activated by iron-derived pro-oxidants and triggers bone morphogenetic protein 6 expression in liver sinusoid endothelial cells, which increases hepcidin synthesis in neighboring hepatocytes. What's more, Nrf2 directs the activation of antioxidant defenses that are crucial for protecting against iron toxicity.[17-31]

In addition, iron-related parafibrin formation can occur through membrane lipid peroxides and protein oxidation. Peroxidation and protein oxidation can be triggered by the hydroxyl radicals produced by free iron and trigger cell death through ferroptosis.[24,29,31,32]

Hydroxyl radical formation by Fenton or Heiber-Weiss reactions, which are critical for ferroptosis, can induce lysosomal lipid peroxidation.[33] Decreased oxygen saturation leads to superoxide radical and H_2O_2 formation in the cell by mitochondria. As a result of the susceptibility of erythrocytes to oxidative stress (OS), free hemoglobin can decompose and be reduced by heme-oxygenase (HO-1), which may further contribute to free iron production.[30,34] Therefore, it is hypothesized that free heme contributes to most of the inflammatory events seen in critically ill COVID-19 patients, and that HO-1 induction or heme use may provide protection.[30-34]

Moreover, excess intracellular iron enhances NF-kappa B expression in mitochondria via inducible iNOS and thus the generation of RNS that ultimately cause OS.[35] Additionally, the SARS-CoV-2 virus can reduce the body's enzymatic antioxidant defense system by strategically blocking Nrf2, an important molecule in the defense system, thereby causing further oxidant damage.[11,30] In addition, the increase in alveolar iron can inhibit interferon synthesis by T cells, leading to a significant weakness in combating viral infection.[36,37] It creates a self-sustaining loop between impaired iron metabolism and increased ROS production, and this can lead to multiple organ failure in COVID-19 patients who eventually progress to sepsis and shock.[3,20]

SARS-CoV-2 SPIKE (S) PROTEIN AND HEPCIDIN

Inflammatory conditions that increase in COVID-19 disease stimulate the synthesis of ferritin and hepcidin especially through pro-inflammatory cytokines such as IL-6.[38-40] The primary task of hepcidin is to limit the activity of ferroportin, the only known cellular iron exporter. Circulating hepcidin can bind to ferroprotin, cause internalization, and trap iron in hepatocytes, macrophages, and absorbent enterocytes in all patients (Figure 2).[40,41] In this situation, using plasma iron or ferritin tests alone to diagnose COVID-19 patients may be problematic.[42,43]

Interestingly, a remarkable similarity was found between the last amino acid sequence of the cytoplasmic tail of the SARS-CoV-2 (S) glycoprotein and the amino acid sequence of hepcidin hormone. The SARS-CoV-2 S protein and hepcidin are both rich in cysteine amino acids.[44] The SARS-CoV-2-specific protein S can potentially act on alveolar macrophages, similar to hepcidin, and promote iron sequestration, thereby impairing the immunological response of the host.[25,44]

Glycosaminoglycan heparin, which has recently been given to severe COVID-19 patients, is a form of heparan sulfate, and this anticoagulant agent is also a very potent hepcidin inhibitor.[45] Zhang et al. have shown that, the levels of hepcidin in the blood increase with ARDS developing as a result of iron overload.[46]

COVID-19 DISEASE AND GASTROINTESTINAL IRON METABOLISM

Additionally, the presence of common gastrointestinal symptoms has been identified in COVID-19 patients.[47] There is a group of serine protease enzymes in our cells that help the S protein binding to ACE2 receptors.[47,48] The serine proteases are called Transmembrane serine protease 2 and transmembrane serine protease 4. They mainly act as enzymes that act as degraders and allow fusion of the virus with the host cell membrane by breaking down the S protein. For example, Type-2 pneumocyte cells in the lungs, enterocyte cells in the small intestine are suitable for the entry of the virus.[48] In addition, the "iron gate" which iron uses to enter cells can be an important site for the virus to invade cells through the intestines.[3,49]

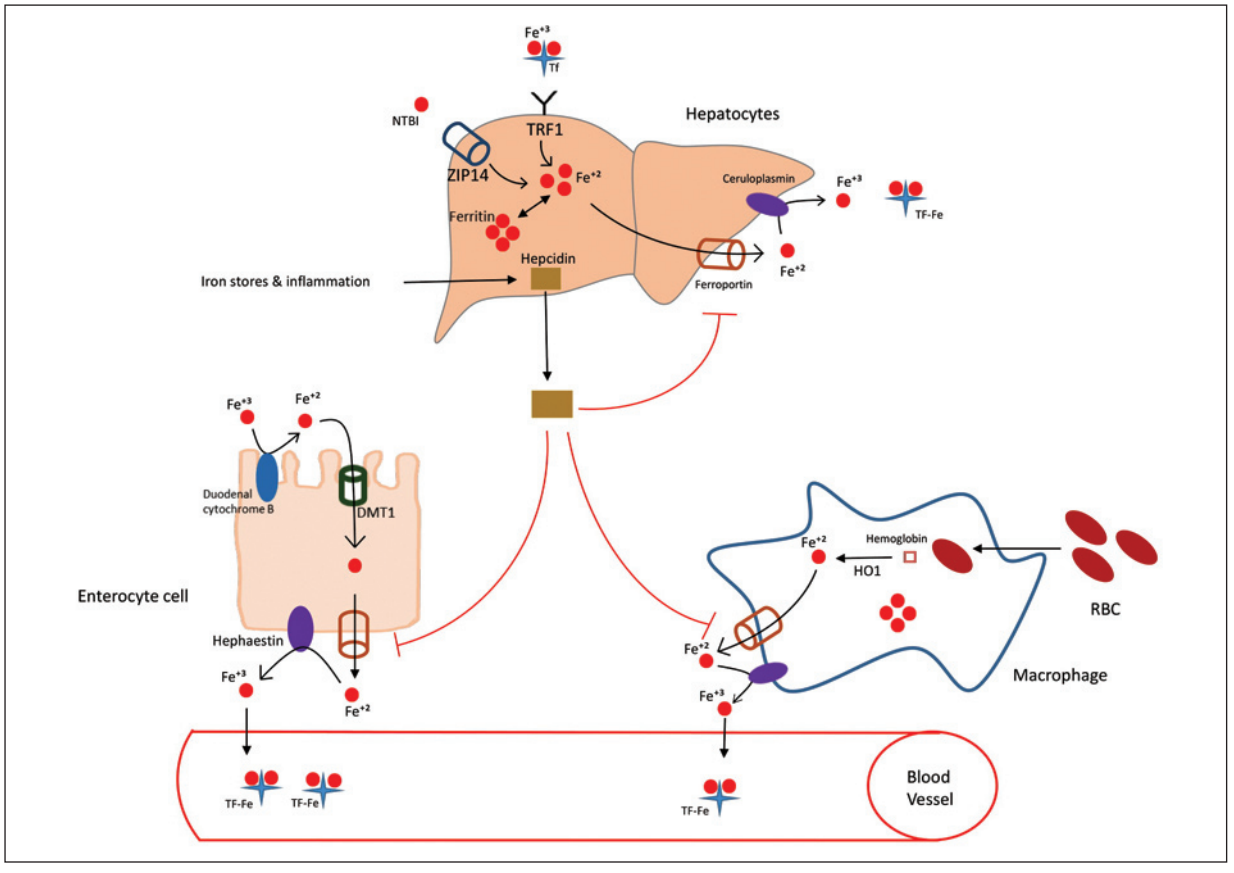

FIGURE 2: The role of hepcidin in iron methabolism.
DMT1: Divalent metal transporter 1; NHE: Na^+/H^+ exchanger; TRF1: Transferrin receptor 1; ZIP14: Zinc transporter; HO1: Heme oxygenase-1; NTBI: Non-transferrin bound iron.

COVID-19 DISEASE AND TRANSFERRIN RECEPTOR

The cell entrance gate of Tf is TfR and is homodimeric in nature. Most importantly, viral attackers can target the same gates as iron in intracellular acidic compartments.[50,51] For example, in the case of OS that occurs in inflammatory diseases such as COVID-19, cells can increase the synthesis of apo-ferritin, where iron is stored in the cell, thereby increasing ferritin and decreasing TfR synthesis. Expression of these iron metabolism proteins is regulated at the post-transcriptional level by IRP-1 ve IRP-2.[52] These mechanisms are under the influence of ROS and inflammatory mediators.[18,53] At this point, it is useful to remember the "cytokine storm" in severe COVID-19 patients. At least theoretically, there is a possibility that COVID-19 disease may activate supportive molecular mechanisms for ferritin production in iron metabolism (Figure 3).

Many cells such as alveoli abundantly express the DMT1, ZRT -IRE-like protein 14 (ZIP14) and ZIP8.[54,55] The abundance of these metal carrier proteins may contribute to high systemic and / or local iron overload in the lungs.[56] It appears to have measurable functional consequences such as iron overload, increased stiffness of the lungs, and hypoxemia.[55-57] High hepcidin decreases the amount of iron bound to Tf, or conversely, decreased hepcidin may be associated with increased Tf saturation as free iron in the environment decreases.[40-43]

Increased iron burden in lung alveoli could have a significant impact on the development of COVID-19 disease. Iron, which locks into alveoli and other cells by this mechanism, can cause anemia and hy-

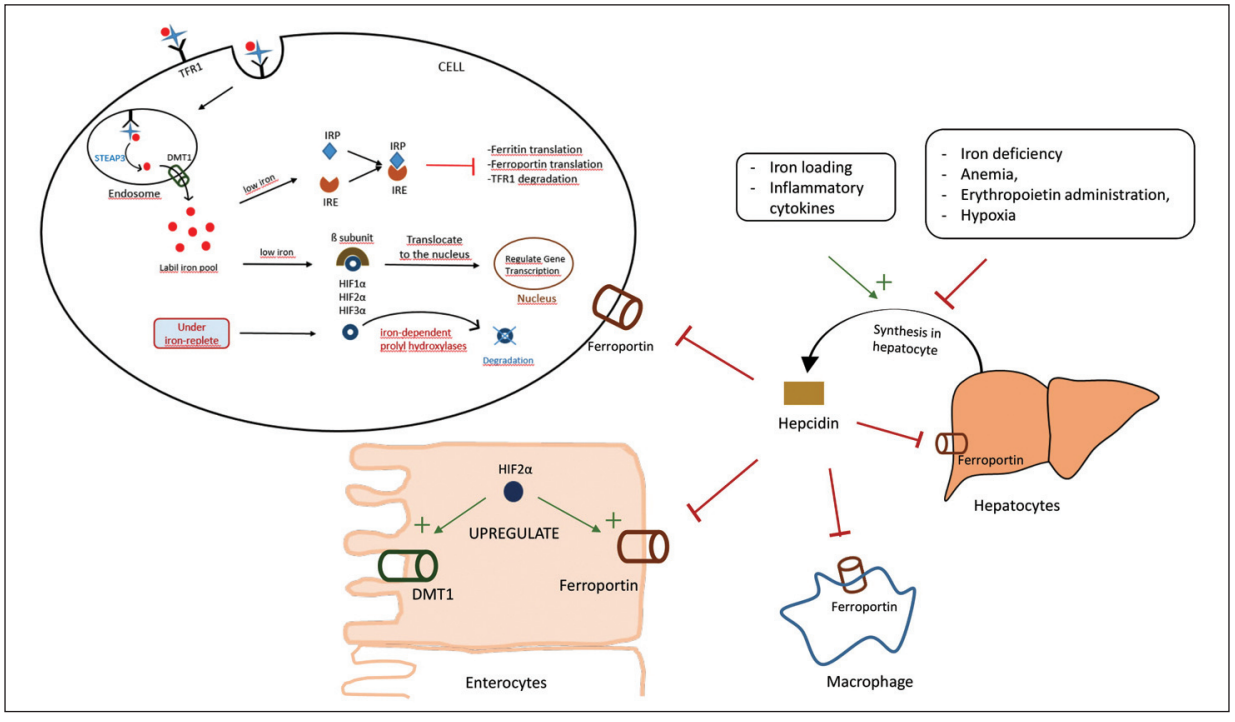

FIGURE 3: Iron metabolism hepcidin and ferropotin.
DMT1: Divalent metal transporter 1; TRF1: Transferrin receptor 1; IRP: Iron regulatory protein; IREs: Iron responsive elements; HIF: Hypoxia inducible factor.

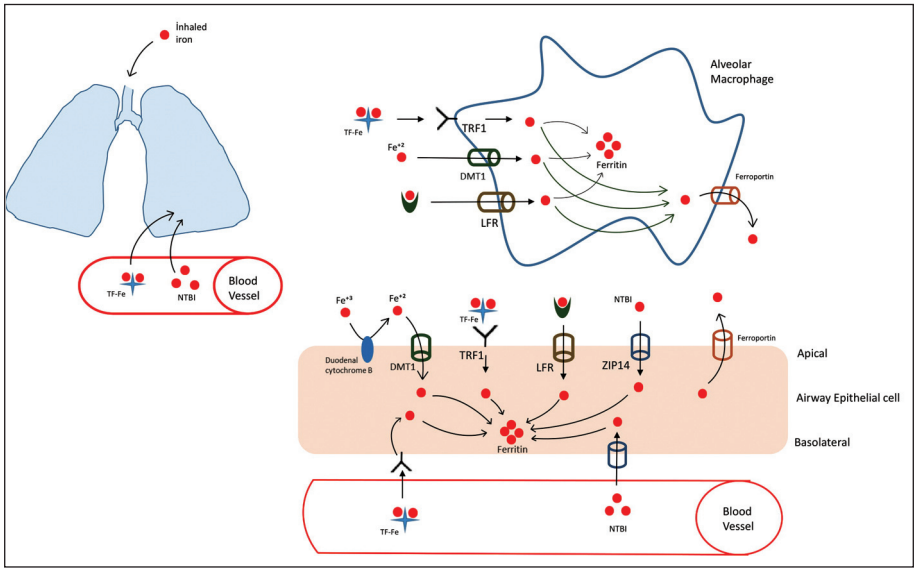

FIGURE 4: COVID-19 and iron metabolism of alveolar macrophages.
DMT1: Divalent metal transporter 1; TF: Transferrin; TRF1: Transferrin receptor 1; ZIP14: Zinc transporter; NTBI: Non-transferrin bound iron; LFR: Lactoferrin receptor.

poxia even in those without iron deficiency.[3,55-57] If iron remains locked in the cell, it can contribute to the increase of hypoxia in vital organs in COVID-19. Macrophages can absorb iron from ion channels with hemoglobin or ingestion of damaged and infected cells or by TfR endocytosis or with CD163 on them (Figure 4).

Iron taken into macrophages is stored in the form of ferritin complex. In diseases such as COVID-19, higher levels of hepcidin due to increased levels of

pro-inflammatory cytokines may occur with lower levels of ferroportin, because usually the ultimate effect of pro-inflammatory cytokines is iron storage in the cell of macrophages and hepatocytes.[18,58,59] Processes related to iron metabolism are always dynamic processes during illnesses and can change rapidly.[42,51] For example, in one study, TfSat was found to be very low initially in severe COVID-19 intensive care patients, but it increased significantly after 3 days in all patients tested. A similar pattern was seen in the serum iron level. Serum iron level increased after the 3rd day in the intensive care unit during the hospital stay. Serum iron reached more than twice the value of ICU admission. However, hyper-ferritinemia that occurred in the same patients remained unchanged during hospitalization.[60]

While macrophages have been reported to secrete ferritin, there is also evidence that serum ferritin is caused by cell damage.[60] Also, the SARS-CoV-2 virus found in macrophages can take advantage of increased iron stores and possibly increase viral replication in macrophages.[61] The proper functioning of the lymphatic system can play an important role in the treatment of COVID-19. However, the lymphatic system will need to release macrophages back into the bloodstream. Consequently, SARS-CoV-2 virus inhibits the recycling of more iron from macrophages and may contribute to inflammatory conditions. Age and aging-related lung diseases, which are among the important risk factors of COVID-19 disease, can be associated with impaired lung iron homeostasis leading to burden.[56-58] In many chronic lung diseases, it has now been shown that alveolar macrophages accumulate iron and the percentage of iron-laden macrophages increases with disease severity.[58-61]

COVID-19 DISEASE AND TRANSFERRIN

Many severe cases of COVID-19 are characterized by increased blood clotting and thrombosis. As is known, some studies on COVID-19 patients have shown that Tf, a procoagulant molecule, increases with age and is higher in men than in women, and Tf in the glycoprotein structure may have an important place in COVID-19 pathophysiology. It has even been suggested that Tf can indicate the risk of COVID-19 disease, that is, it can be a biomarker.[45,50] TfR protein levels in the lungs increase significantly in ARDS due to viral infections.In the treatment of viral infections, blocking TfR and antibody-mediated neutralization against TfR may reduce virus pathogenesis, perhaps the entry of SARS-CoV-2 into the cell.[62] As a result, the exact mechanism by which TfR is involved in viral input in COVID-19 is still not defined.[63]

COVID-19 DISEASE AND FERRITIN

Pathogens, particularly RNA viruses such as primitive coronaviruses, compete with the host for the iron necessary to reproduce due to the limited availability of iron in the human body and basic processes using iron.[13,32,37] Because the nuclear export of the transcribed viral RNA also depends on iron.[17,37] Especially pro-inflammatory cytokines in COVID-19 increase the synthesis of ferritin and hepcidin for protection purposes. Therefore, cytokines such as IL-6 inhibit ferroportin mRNA expression to keep iron export low and keep iron away from pathogens.[21,64] This sequence of events ultimately lead to iron accumulation in monocytes/macrophages and iron restriction in effective erythropoiesis. This response to infection can cause large amounts of iron to remain locked mainly within cells and unavailable for other essential functions such as blood oxygen transport.[36-39] This can lead to anemia and hypoxia even in those who are not previously iron deficient (Figure 5).[36,61]

The consistently high ferritin levels in COVID-19 suggest that ferritin may be suitable for assessing

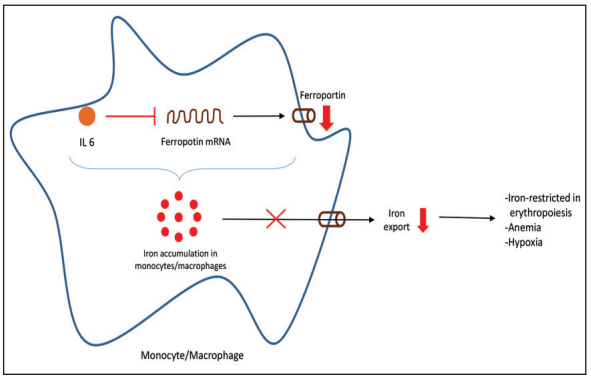

FIGURE 5: The effect of cytokines on iron metabolism.

COVID-19 severity, but not for monitoring the course of the disease.[40-43] Ferritin is a cellular defense molecule that develops during the evolutionary process of host protection from viral infections.[5,13,16] Significantly higher ferritin characterizes the severity of COVID-19 and the worse prognosis suggests that the mortality rate may be due to viral-induced hyper-inflammation.[6] After viral infections, serum iron and TfSat may decrease first, but with this method, the pathogen can be prevented from reaching iron.[17,18] Ferritin is essential for cell integrity, especially under increased operating system conditions, as the production of ferritin, which reduces excess free iron in the cell, almost completely buffers the excess iron.[20,31] In addition, it not only contributes to ferritin synthesis, but also to antioxidant defense in the process of ferritin iron release. Because in the release of iron from ferritin, the reduction of mineralized Fe^{+3} and direct iron release mechanisms are used.[20,30] The release of iron from ferrite contributes to the antioxidant activity. Because, during the release of iron from ferritin, small reducing molecules such as O_2, ascorbate radical and NO are consumed. The antioxidant property of iron release from its ferritin in the cell may change according to the ferritin structure. In particular, H-chain expression of ferritin provides stronger protection against OS in viral inflammatory events. L-chain-rich isoforms of ferritin have no ferroxidase activity.[20,64]

Serum ferritin levels are generally considered as a general indicator of inflammation and infection, and all iron metabolism tests may vary in the presence of iron deficiency. In this case, the use of iron metabolism tests or ferritin to diagnose COVID-19 patients may be problematic in patients with COVID-19. Iron homeostasis in COVID-19 may be a more specific pathophysiological basis for this infection. First, the levels of various serum markers for iron biology in the clinic can be measured and analyzed more systematically and comprehensively. If iron accumulates in the cell as a result of stimulation of hepcidin with excessive synthesis of IL-6 and restriction of iron export of ferroportin by hepcidin, a series of reactions leading to cell ferroptosis may occur. This is because free iron deposited inside the cell can interact with molecular oxygen to produce ROS. Excess ROS that iron will produce with Fenton and other reactions can greatly contribute to the oxidative damage of the cellular components of many organs. In addition, the complex interaction between iron metabolism and RNS and RSS are newly described interactions of iron metabolism and reactive species.

Therefore, ferritin synthesis and degradation act as a protector at the cellular level. Of course, the regulator of ferritin synthesis is the increase of iron in the environment, but ferritin synthesis is also caused by inflammatory cytokines such as IL-1β, TNF-alpha and IL-6, which are increased in COVID-19.[35,42,53]

Cellular ferritin levels are regulated at the translational level through the m-RNA binding proteins IRP-1 and IRP-2. Intracellular excess iron can increase the expression of proteins such as ferritin, using the IRP1 and IRP2 pathways. Furthermore, ferritin synthesis caused by the action of the NF-κB / Nrf2 pathway may increase H chain synthesis of ferritin. There is also increasing evidence that ferritin has both immunosuppressive and proinflammatory effects through its specific receptors.[53,54,58,59] It is still unclear whether the high serum ferritin that can be measured in COVID-19 is in the bloodstream by leaky cells or as a result of ferroptosis, or whether ferritin serum is actively secreted from cells.[65,66] This observation may indicate that serum ferritin may be suitable for assessing the severity of COVID-19, but not for monitoring the course of the disease.[42,43]

COVID-19 DISEASE AND FERROPTOSIS

Ferroptosis is a newly recognized form of cell death, unlike conventional necrosis, apoptosis, or autophagic cell death. A viral agent entering the cell can be a trigger for ferroptosis formation.[17,33] Ferroptosis, which is actually a cellular defense mechanism, can cause unwanted functional damage by releasing excess free iron. Ferritinophagia, which has gained importance in understanding senescent cells, is a form of selective autophagy mediated by nuclear receptor coactivator 4 (NCOA4) responsible for the degradation of intracellular ferritin. After all, ferroptosis, a

unique pathway of cell death different from apoptosis, depends on the presence of iron released by ferritinophagy.[14,33] Thanks to this defense mechanism developed by the organism during evolution, viruses and other pathogens are prevented from reaching iron. As a result of the reduced Fpn activity by hepcidin, cellular iron lock positively regulates expression of ferritin at both transcriptional and translational levels to protect against unstable iron-mediated oxidative stress. Its receptor promotes NCOA4-mediated autophagic degradation, cellular ROS accumulation, and thus ferroptotic cell death.[18,19,33] Increasing ferroptosis can cause a disproportionate increase in human immune response. In fact, while trying to defend itself, the body can harm itself with the disproportionate immune response (cytokine storm) it can generate, whereas the organism tries to clean the infected cells with ferroptosis.[17,33,63,67]

COVID-19 DISEASE AND PARAFIBRIN

It has been suggested that viral damage associated with COVID-19 in the endothelial cell contributes to coagulation disorders and vascular complications that may be seen in severe patients.[45,63] In addition, autopsy results, which describe the irregular coagulation affecting the lungs of COVID-19 patients who died, are quite remarkable.

Oxidized iron interacts with proteins as the coagulation stage accelerates serum coagulation. In fact, excess free Fe^{+3} in the medium can initiate the conversion of fibrinogen to hydroxyl radical catalyzed parafibrin, which is highly resistant to proteolytic dissolution, thereby further increasing intravascular accumulation.[2,3]

Recently, it has been suggested in some studies that Fe^{+3} can transform fibrinogen molecules into harmful insoluble fibrin-like polymer parafibrin by Fenton reaction. As a result, fibrin polymer parafibrin, which can be produced very densely in viral infections such as COVID-19, takes on the properties of a foreign body and can attract macrophages leading to a persistent inflammatory state.[68,69] Unfortunately, there is no known molecule or known biological process in the human body that can re-dissolve parafibrin. Although an article showing the formation of parafibrin in COVID-19 is not included in the literature, a theoretical relationship can be expected.[12,43,45,69]

COVID-19 DISEASE AND LACTOFERRIN

We previously published a presentation showing the possible relationship between COVID-19 and lactoferrin (LF), a highly conserved pleiotropic iron-binding immunomodulatory glycoprotein (80 kDa) of the transferrin family found in most body fluids.[70,71] Human LF is globular double lobed and consists of 691 amino acids. LF, which is found in breast milk as a protector from natural neonatal infections, also functions against infection in the respiratory tract. It has 60% amino acid sequence similar to Tf.[72,73] However, it has a very different function from Tf. LF has mainly antifungal, antiviral, antiparasitic, anti-inflammatory and immunomodulatory activities. However, an important production purpose of LF is to remove the iron needed by viruses for replication and catalysis. Due to its similarity to transferrin, the main iron transport molecule in serum, α-LF has iron-binding abilities and can chelate two ferric iron atoms (Fe^{+3}). The most important feature that distinguishes LF from transferrin is that it does not release iron even at pH 3.5, indicating that LF can be effective against oxidative stressors such as COVID-19.[70,72,73]

This information is important for the COVID-19 outbreak because LF can be a potential therapeutic agent. LF has strong antiviral activity against a broad spectrum of both naked and enveloped DNA and RNA viruses. Generally, LF blocks the entry of viral particles into the host. By attaching viral particles directly to viral particles or by blocking their cellular receptors, LF can block S proteins from binding to host cells, thus creating a host defense mechanism against the virus.[72-74]

Sepsis caused by serious infections can break down erythrocytes. Erythrocytes can be broken down due to viral infection or as a result of hemolysis, hemoglobin or heme is released into the bloodstream by hemolysis, hemoglobin haptoglobins and heme are captured by circulating hemopexin.[34,52] Circulating ceruloplasmin with serum ferroxidase activity is

important for the smooth running of this process. In addition, the direct transfer of ferric iron from ceruloplasmin to LF prevents both the formation of potentially toxic hydroxyl radicals and the use of iron by pathogenic bacteria. Besides these effects, the LF receptor is found in many tissues and organs. LF can step in by binding to the input receptors of viruses and can act as an important element in host defense mechanisms. Another important pathway for LF binding at the cell surface is through heparan sulphate proteoglycans (HSPGs). In fact, HSPGs can play an important role in the corona viruses cell entry process. LF can prevent viral infections by interacting with HSPGs cell receptors that allow binding to the first anchorage site on the cell surface in viruses and particularly coronaviruses. Moreover, LF can help prevent thrombocytopenia and hyper-coagulation, both of which are hallmarks of COVID-19 infection.[72-74]

In fact, LF can bind many metals, among which zinc can stand out, as zinc is recommended as a supplement in the treatment of COVID-19. Fortunately, LF saturated with zinc apparently has a stronger antiviral effect because LF saturated with zinc has been observed to inhibit viral infection when incubated with cells after viral binding, and the inhibition is directly related to the degree of zinc saturation. There is no doubt that the pathophysiology of COVID-19 will be better understood over time, treatment options such as LF administration may be involved in the regulation of iron metabolism in COVID-19 patients. At least, LF, one of the natural defense molecules against microbes and obtained during the evolution process, can be among the treatment options for COVID-19.

CONCLUSION

Infection in the body induces hepcidin, the main iron regulator, to cause inflammatory anemia because there is competition between the host and the pathogen for iron, a nutrient that infectious virus or bacteria need to survive and reproduce. Of course, reducing the iron needed by pathogens in the body is a critical part of innate immunity. Anemia, which can be seen in COVID-19, can occur due to inflammation and a decrease in hemoglobin can be detected. Moreover, the coronavirus is an RNA virus whose replication is based on an RNA duplex intermediate. These types of viruses, unlike DNA or retroviruses, may not need much iron to replicate their genomes.

Until there is evidence that cellular high iron levels exist in COVID-19 and are related to COVID-19 disease, it is doubtful whether iron chelation will be effective. Therefore, different treatment approaches, such as hepcidin antagonists, cytokine antagonists or spike-functionalized ferritin vaccines may be included in supportive treatments.[22,75]

MAIN POINTS

1. We had presentations in the literature suggesting the possible relationship between iron metabolism and COVID-19 disease.[3,71] Today, the number of articles showing the importance of iron metabolism in the COVID-19 epidemic is increasing.

2. COVID-19 disease is characterized by cytokine storm. As a result of the increase in pro-inflammatory cytokines, the production of hepcidin, the main hormone that regulates iron metabolism, may increase.

3. Many RNA viruses such as SARS-CoV-2 require the presence of iron in the environment for replication. Alveoli macrophages may be the primary cells in which iron is stored as ferritin in the cell. Increased intracellular iron may cause oxidative stress and ferroptosis.

4. Parafibrin formation may contribute to the coagulation disorders that can be seen in COVID-19 disease.

5. Lactoferrin, which can bind iron strongly, may be an option in the treatment of COVID-19 disease.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise,

working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Necat Yılmaz;* ***Design:*** *Necat Yılmaz;* ***Control/Supervision:*** *Esin Eren;* ***Data Collection and/or Processing:*** *Necat Yılmaz, Cemile Öz;* ***Analysis and/or Interpretation:*** *Esin Eren Zafer Kalaycı, Ferhat Saribek;* ***Literature Review:*** *Necat Yılmaz, Cemile Öz;* ***Writing the Article:*** *Esin Eren;* ***Critical Review:*** *Esin Eren, Zafer Kalaycı.*

REFERENCES

1. Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, et al. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. J Virol. 2005;79(23):14614-21. [Crossref] [PubMed] [PMC]
2. Sun P, Lu X, Xu C, Sun W, Pan B. Understanding of COVID-19 based on current evidence. J Med Virol. 2020;92(6):548-51. [Crossref] [PubMed] [PMC]
3. Cavezzi A, Troiani E, Corrao S. COVID-19: hemoglobin, iron, and hypoxia beyond inflammation. A narrative review. Clin Pract. 2020;10(2):1271. [Crossref] [PubMed] [PMC]
4. Yılmaz N, Eren E, Öz C. COVID-19 and ozone. Cyprus J Med Sci. 2020;5(4):365-72. [Crossref]
5. Garrick MD, Ghio AJ. Iron chelation may harm patients with COVID-19. Eur J Clin Pharmacol. 2021;77(2):265-6. Erratum in: Eur J Clin Pharmacol. 2021 Feb;77(2):265-266. [Crossref] [PubMed] [PMC]
6. Lin Z, Long F, Yang Y, Chen X, Xu L, Yang M. Serum ferritin as an independent risk factor for severity in COVID-19 patients. J Infect. 2020;81(4):647-79. [Crossref] [PubMed] [PMC]
7. Kappert K, Jahić A, Tauber R. Assessment of serum ferritin as a biomarker in COVID-19: bystander or participant? Insights by comparison with other infectious and non-infectious diseases. Biomarkers. 2020;25(8):616-25. [Crossref] [PubMed]
8. Altschul DJ, Esenwa C, Haranhalli N, Unda SR, de La Garza Ramos R, Dardick J, et al. Predictors of mortality for patients with COVID-19 and large vessel occlusion. Interv Neuroradiol. 2020;26(5):623-8. [Crossref] [PubMed] [PMC]
9. Taneri PE, Gómez-Ochoa SA, Llanaj E, Raguindin PF, Rojas LZ, Roa-Díaz ZM, et al. Anemia and iron metabolism in COVID-19: a systematic review and meta-analysis. Eur J Epidemiol. 2020;35(8):763-73. [Crossref] [PubMed] [PMC]
10. Edeas M, Saleh J, Peyssonnaux C. Iron: Innocent bystander or vicious culprit in COVID-19 pathogenesis? Int J Infect Dis. 2020;97:303-5. [Crossref] [PubMed] [PMC]
11. Shenoy S. Coronavirus (Covid-19) sepsis: revisiting mitochondrial dysfunction in pathogenesis, aging, inflammation, and mortality. Inflamm Res. 2020;69(11):1077-85. [Crossref] [PubMed] [PMC]
12. Giannis D, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. J Clin Virol. 2020;127:104362. [Crossref] [PubMed] [PMC]
13. Wertheim JO, Chu DK, Peiris JS, Kosakovsky Pond SL, Poon LL. A case for the ancient origin of coronaviruses. J Virol. 2013;87(12):7039-45. [Crossref] [PubMed] [PMC]
14. Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, et al. Coronavirus Disease 2019-COVID-19. Clin Microbiol Rev. 2020;33(4):e00028-20. [Crossref] [PubMed] [PMC]
15. Yoshimoto FK. The Proteins of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2 or n-COV19), the Cause of COVID-19. Protein J. 2020;39(3):198-216. [Crossref] [PubMed] [PMC]
16. Wächtershäuser G. Groundworks for an evolutionary biochemistry: the iron-sulphur world. Prog Biophys Mol Biol. 1992;58(2):85-201. [Crossref] [PubMed]
17. Drakesmith H, Prentice A. Viral infection and iron metabolism. Nat Rev Microbiol. 2008;6(7):541-52. [Crossref] [PubMed]
18. Chifman J, Laubenbacher R, Torti SV. A systems biology approach to iron metabolism. Adv Exp Med Biol. 2014;844:201-25. [Crossref] [PubMed] [PMC]
19. Cohen LA, Gutierrez L, Weiss A, Leichtmann-Bardoogo Y, Zhang DL, Crooks DR, et al. Serum ferritin is derived primarily from macrophages through a nonclassical secretory pathway. Blood. 2010;116(9):1574-84. [Crossref] [PubMed]
20. Arosio P, Levi S. Cytosolic and mitochondrial ferritins in the regulation of cellular iron homeostasis and oxidative damage. Biochim Biophys Acta. 2010;1800(8):783-92. [Crossref] [PubMed]
21. Zhang Q, Huang X. Induction of interleukin-6 by coal containing bioavailable iron is through both hydroxyl radical and ferryl species. J Biosci. 2003;28(1):95-100. [Crossref] [PubMed]
22. Innes AJ, Cook LB, Marks S, Bataillard E, Crossette-Thambiah C, Sivasubramaniam G, et al. Ruxolitinib for tocilizumab-refractory severe COVID-19 infection. Br J Haematol. 2020;190(4):e198-e200. [Crossref] [PubMed] [PMC]
23. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: A single center experience. J Med Virol. 2020;92(7):814-8. [Crossref] [PubMed] [PMC]
24. Li Y, Zhou Y, Zhang D, Wu WY, Kang X, Wu Q, et al. Hypobaric hypoxia regulates iron metabolism in rats. J Cell Biochem. 2019;120(8):14076-87. [Crossref] [PubMed]
25. Perricone C, Bartoloni E, Bursi R, Cafaro G, Guidelli GM, Shoenfeld Y, et al. COVID-19 as part of the hyperferritinemic syndromes: the role of iron depletion therapy. Immunol Res. 2020;68(4):213-24. [Crossref] [PubMed] [PMC]
26. Quiros Roldan E, Biasiotto G, Magro P, Zanella I. The possible mechanisms of action of 4-aminoquinolines (chloroquine/hydroxychloroquine) against Sars-Cov-2 infection (COVID-19): A role for iron homeostasis? Pharmacol Res. 2020;158:104904. [Crossref] [PubMed] [PMC]
27. Abo-Zeid Y, Ismail NSM, McLean GR, Hamdy NM. A molecular docking study repurposes FDA approved iron oxide nanoparticles to treat and control COVID-19 infection. Eur J Pharm Sci. 2020;153:105465. [Crossref] [PubMed] [PMC]
28. Hadadi A, Mortezazadeh M, Kolahdouzan K, Alavian G. Does recombinant human erythropoietin administration in critically ill COVID-19 patients have miraculous therapeutic effects? J Med Virol. 2020;92(7):915-8. [Crossref] [PubMed] [PMC]
29. Sukhomlin T. Could an acute respiratory distress syndrome in COVID-19 infected patients be calmed down simply by iron withdrawal from lung tissues? J Med Virol. 2021;93(2):577-8. [Crossref] [PubMed]
30. Cecchini R, Cecchini AL. SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. Med Hypotheses. 2020;143:110102. [Crossref] [PubMed] [PMC]

31. Lim PJ, Duarte TL, Arezes J, Garcia-Santos D, Hamdi A, Pasricha SR, et al. Nrf2 controls iron homeostasis in haemochromatosis and thalassaemia via Bmp6 and hepcidin. Nat Metab. 2019;1(5):519-31. [Crossref] [PubMed] [PMC]
32. 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):ofaa250. [Crossref] [PubMed] [PMC]
33. Sun Y, Chen P, Zhai B, Zhang M, Xiang Y, Fang J, et al. The emerging role of ferroptosis in inflammation. Biomed Pharmacother. 2020;127:110108. [Crossref] [PubMed]
34. Wagener FADTG, Pickkers P, Peterson SJ, Immenschuh S, Abraham NG. Targeting the heme-heme oxygenase system to prevent severe complications following COVID-19 infections. Antioxidants (Basel). 2020;9(6):540. [Crossref] [PubMed] [PMC]
35. Cooper CE. Nitric oxide and iron proteins. Biochim Biophys Acta. 1999;1411(2-3):290-309. [Crossref] [PubMed]
36. Gan ZS, Wang QQ, Li JH, Wang XL, Wang YZ, Du HH. Iron reduces M1 macrophage polarization in RAW264.7 Macrophages Associated with Inhibition of STAT1. Mediators Inflamm. 2017;2017:8570818. [Crossref] [PubMed] [PMC]
37. Arber N, Moshkowitz M, Konikoff F, Halpern Z, Hallak A, Santo M, et al. Elevated serum iron predicts poor response to interferon treatment in patients with chronic HCV infection. Dig Dis Sci. 1995;40(11):2431-3. [Crossref] [PubMed]
38. Kanamori Y, Murakami M, Sugiyama M, Hashimoto O, Matsui T, Funaba M. Hepcidin and IL-1β. Vitam Horm. 2019;110:143-56. [Crossref] [PubMed]
39. Agarwal AK, Yee J. Hepcidin. Adv Chronic Kidney Dis. 2019;26(4):298-305. [Crossref] [PubMed]
40. Girelli D, Nemeth E, Swinkels DW. Hepcidin in the diagnosis of iron disorders. Blood. 2016;127(23):2809-13. [Crossref] [PubMed] [PMC]
41. Rishi G, Wallace DF, Subramaniam VN. Hepcidin: regulation of the master iron regulator. Biosci Rep. 2015;35(3):e00192. [Crossref] [PubMed] [PMC]
42. Vargas-Vargas M, Cortés-Rojo C. Ferritin levels and COVID-19. Rev Panam Salud Publica. 2020;44:e72. [Crossref] [PubMed] [PMC]
43. Velavan TP, Meyer CG. Mild versus severe COVID-19: Laboratory markers. Int J Infect Dis. 2020;95:304-7. [Crossref] [PubMed] [PMC]
44. Di Paola L, Hadi-Alijanvand H, Song X, Hu G, Giuliani A. The discovery of a putative allosteric Site in the SARS-CoV-2 spike protein using an ınтegrated structural/dynamic approach. J Proteome Res. 2020;19(11):4576-86. [Crossref] [PubMed] [PMC]
45. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al; Global COVID-19 Thrombosis Collaborative Group, Endorsed by the ISTH, NATF, ESVM, and the IUA, Supported by the ESC Working Group on Pulmonary Circulation and Right Ventricular Function. COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;75(23):2950-73. [Crossref] [PubMed] [PMC]
46. Zhang V, Ganz T, Nemeth E, Kim A. Iron overload causes a mild and transient increase in acute lung injury. Physiol Rep. 2020;8(12): e14470. [Crossref] [PubMed] [PMC]
47. Patel KP, Patel PA, Vunnam RR, Hewlett AT, Jain R, Jing R, et al. Gastrointestinal, hepatobiliary, and pancreatic manifestations of COVID-19. J Clin Virol. 2020;128:104386. [Crossref] [PubMed] [PMC]
48. Zang R, Gomez Castro MF, McCune BT, Zeng Q, Rothlauf PW, Sonnek NM, et al. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. Sci Immunol. 2020;5(47):eabc3582. [Crossref] [PubMed] [PMC]
49. Auguet T, Aragonès G, Berlanga A, Martínez S, Sabench F, Binetti J, et al. Hepcidin in morbidly obese women with non-alcoholic fatty liver disease. PLoS One. 2017;12(10): e0187065. [Crossref] [PubMed] [PMC]
50. Gammella E, Buratti P, Cairo G, Recalcati S. The transferrin receptor: the cellular iron gate. Metallomics. 2017;9(10):1367-75. [Crossref] [PubMed]
51. Bellmann-Weiler R, Lanser L, Barket R, Rangger L, Schapfl A, Schaber M, et al. Prevalence and predictive value of anemia and dysregulated iron homeostasis in patients with COVID-19 infection. J Clin Med. 2020;9(8):2429. [Crossref] [PubMed] [PMC]
52. Muckenthaler MU, Galy B, Hentze MW. Systemic iron homeostasis and the iron-responsive element/iron-regulatory protein (IRE/IRP) regulatory network. Annu Rev Nutr. 2008;28:197-213. [Crossref] [PubMed]
53. Gao G, Li J, Zhang Y, Chang YZ. Cellular Iron Metabolism and Regulation. Adv Exp Med Biol. 2019;1173:21-32. [Crossref] [PubMed]
54. Toniati P, Piva S, Cattalini M, Garrafa E, Regola F, Castelli F, et al. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in Brescia, Italy. Autoimmun Rev. 2020;19(7):102568. [Crossref] [PubMed] [PMC]
55. Neves J, Haider T, Gassmann M, Muckenthaler MU. Iron Homeostasis in the Lungs-A Balance between Health and Disease. Pharmaceuticals (Basel). 2019;12(1):5. [Crossref] [PubMed] [PMC]
56. Dorward DA, Russell CD, Um IH, Elshani M, Armstrong SD, Penrice-Randal R, et al. Tissue-Specific Immunopathology in Fatal COVID-19. Am J Respir Crit Care Med. 2021;203(2):192-201. [Crossref] [PubMed] [PMC]
57. Ghio AJ, Wang X, Silbajoris R, Garrick MD, Piantadosi CA, Yang F. DMT1 expression is increased in the lungs of hypotransferrinemic mice. Am J Physiol Lung Cell Mol Physiol. 2003;284(6):L938-44. [Crossref] [PubMed]
58. Soares MP, Hamza I. Macrophages and iron metabolism. Immunity. 2016;44(3):492-504. [Crossref] [PubMed] [PMC]
59. Ganz T. Iron and infection. Int J Hematol. 2018;107(1):7-15. Erratum in: Int J Hematol. 2017 Dec 2. [Crossref] [PubMed]
60. Bolondi G, Russo E, Gamberini E, Circelli A, Meca MCC, Brogi E, et al. Iron metabolism and lymphocyte characterisation during Covid-19 infection in ICU patients: an observational cohort study. World J Emerg Surg. 2020;15(1):41. [Crossref] [PubMed] [PMC]
61. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Nat Rev Immunol. 2020;20(6):355-62. Erratum in: Nat Rev Immunol. 2020. [Crossref] [PubMed] [PMC]
62. Woodward JE, Bayer AL, Chavin KD, Boleza KA, Baliga P. Anti-transferrin receptor monoclonal antibody: a novel immunosuppressant. Transplantation. 1998;65(1):6-9. [Crossref] [PubMed]
63. McLaughlin KM, Bechtel M, Bojkova D, Münch C, Ciesek S, Wass MN, Michaelis M, Cinatl J Jr. COVID-19-Related Coagulopathy-Is Transferrin a Missing Link? Diagnostics (Basel). 2020;10(8):539. [Crossref] [PubMed] [PMC]
64. Chen ST, Ni YH, Li CC, Liu SH. Hepcidin correlates with interleukin-1β and interleukin-6 but not iron deficiency in children with Helicobacter pylori infection. Pediatr Neonatol. 2018;59(6):611-7. [Crossref] [PubMed]
65. Sabelli M, Montosi G, Garuti C, Caleffi A, Oliveto S, Biffo S, et al. Human macrophage ferroportin biology and the basis for the ferroportin disease. Hepatology. 2017;65(5):1512-25. [Crossref] [PubMed] [PMC]

66. Hirayama M, Kohgo Y, Kondo H, Shintani N, Fujikawa K, Sasaki K, et al. Regulation of iron metabolism in HepG2 cells: a possible role for cytokines in the hepatic deposition of iron. Hepatology. 1993;18(4):874-80. [Crossref] [PubMed]

67. Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol. 2020;39(7):2085-94. [Crossref] [PubMed] [PMC]

68. Oxley TJ, Mocco J, Majidi S, Kellner CP, Shoirah H, Singh IP, et al. Large-Vessel Stroke as a Presenting Feature of Covid-19 in the Young. N Engl J Med. 2020;382(20):e60. [Crossref] [PubMed] [PMC]

69. Jafarzadeh A, Chauhan P, Saha B, Jafarzadeh S, Nemati M. Contribution of monocytes and macrophages to the local tissue inflammation and cytokine storm in COVID-19: Lessons from SARS and MERS, and potential therapeutic interventions. Life Sci. 2020;257: 118102. [Crossref] [PubMed] [PMC]

70. Root-Bernstein R. Age and Location in Severity of COVID-19 Pathology: Do Lactoferrin and Pneumococcal Vaccination Explain Low Infant Mortality and Regional Differences? Bioessays. 2020;42(11):e2000076. [Crossref] [PubMed]

71. Ehsani S. COVID-19 and iron dysregulation: distant sequence similarity between hepcidin and the novel coronavirus spike glycoprotein. Biol Direct. 2020;15(1):19. [Crossref] [PubMed] [PMC]

72. Berlutti F, Pantanella F, Natalizi T, Frioni A, Paesano R, Polimeni A, et al. Antiviral properties of lactoferrin--a natural immunity molecule. Molecules. 2011;16(8):6992-7018. [Crossref] [PubMed] [PMC]

73. Lang J, Yang N, Deng J, Liu K, Yang P, Zhang G, et al. Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. PLoS One. 2011;6(8):e23710. [Crossref] [PubMed] [PMC]

74. Peroni DG, Fanos V. Lactoferrin is an important factor when breastfeeding and COVID-19 are considered. Acta Paediatr. 2020;109(10): 2139-40. [Crossref] [PubMed] [PMC]

75. Powell AE, Zhang K, Sanyal M, Tang S, Weidenbacher PA, Li S, et al. A Single Immunization with Spike-Functionalized Ferritin Vaccines Elicits Neutralizing Antibody Responses against SARS-CoV-2 in Mice. ACS Cent Sci. 2021;7(1):183-99. [Crossref] [PubMed] [PMC]