C O R R E S P O N D E N C E  /  C A S E  R E P O R T S

# Iron overload and Hepcidin overexpression could play a key role in COVID infection, and may explain vulnerability in elderly, diabetics, and obese patients

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**Summary.** *Background:* The COVID epidemic hit like a tsunami worldwide. At the time of its arrival in Italy, available literary data were meager, and most of them concerned its epidemiology. World Health Organization proposed guidelines in march 2020, a strategy of treatment has been developed, and a significant number of subsequent articles have been published to understand, prevent, and cure COVID patients. *Methods:* From the observation of two patients, we performed a careful analysis of scientific literature to unearth the relation between COVID infection, clinical manifestations as pneumonia and thrombosis, and to find out why it frequently affects obese, diabetics, and elderly patients. *Results:* The analysis shows that hepcidin could represent one of such correlating factors. Hepcidin is most elevated in older age, in non-insulin diabetics patients and in obese people. It is the final target therapy of many medicaments frequently used. Viral disease, and in particular SARS-CoV19, could induce activation of the hepcidin pathway, which in turn is responsible for an increase in the iron load. Excess of iron can lead to cell death by ferroptosis and release into the bloodstream, such as free iron, which in turn has toxic and pro-coagulative effects. *Conclusions:* Overexpression of hepcidin and iron overload might play a crucial role in COVID infection, becoming potential targets for treatment. Hepcidin could also be considered as a biomarker to measure the effectiveness of our treatments and the restoration of iron homeostasis the final intent. (www.actabiomedica.it)

**Key words:** COVID, sars-cov, diabetes, elderly, iron, hepcidin, ferritin, heparin, erythropoietin, chloroquine

## Introduction

COVID-19 hit the hospital of Piacenza heavily, overwhelming it since the beginning of the epidemic and making it one of the most affected hospitals in Italy. When it hit us, we started using the therapy proposed by the World Health Organization (1), including antivirals such as ritonavir/lopinavir, darunavir, and chloroquine, heparin, azithromycin, high oxygen doses, and intubation. Meanwhile, other hospitals have started to perform and test other treatments, such as monoclonal antibodies, but the results are still ongoing. The number of deaths has been remarkably high,

and we found ourselves observing such symptoms as respiratory failure, frequent thrombosis, and high fever. Unfortunately, we were helpless for many of the patients. At the beginning of the pandemic, a wing of our hospital started to use high dose heparin to improve recovery, but data have still to be published. As reported by the literature (2), also in our experience, high mortality was associated with diabetes, high BMI, hypertension, and age. The lack of extensive expertise does not allow us to explain why a selected population of patients affected by these comorbidities appears to be most hit by COVID infection. Starting from the discussion of two different cases, apparently without

any relationship linking them, we analyzed the medical literature, and we found that iron and hepcidin might play a significant role in this contest, suggesting their potential value as targets for treatment.

## Case Reports

### Case 1

An 82-year old patient with previous history of mild Hypertension in treatment with bisoprolol and diabetes treated with insulin, was diagnosed with pancreatic cancer and responded to neoadjuvant therapy. He underwent spleen preserving total pancreatectomy at the beginning of February 2020. The postoperative course was remarked by mild fever treated with piperacillin-tazobactam and gastric outlet syndrome, which required total parenteral nutrition with intravenous insulin infusion and subcutaneous enoxaparin. One week before the first lockdown in Italy, the patient underwent a CT scan with evidence of interstitial pneumonia with ground-glass opacity in the right and medium right lung and opacity in the inferior lobe. Tigecycline was started, and bronchoalveolar lavage was performed. The lockdown started, and antibiotic therapy was changed with vancomycin, meropenem, and fluconazole. The patient continued to deteriorate with a worsening of physical conditions, while all around him, other patients started to die from acute respiratory failure. The patient then stabilized, and a follow-up CT scan, performed 17 days after the first one, showed the disappearance of the ground glass lesions in the superior and medium lobe. While the gastric outlet syndrome persisted, the occurrence of hypoglycemia required insulin withdrawal for seven days. Outlet syndrome eventually improved, and oral intake was started. Intravenous insulin was substituted with subcutaneous administration three times a day. The patient progressively improved and was subsequently transferred to a post-hospital structure. Before discharge, blood samples revealed low serum iron with 22µgr/dl (NV 53-167) without anemia and a low increase of c-reactive protein. The patient is still alive at the moment.

### Case 2

A 63-year old patient with mild Hypertension, treated with enalapril and lercanidipine, developed intense asthenia, anosmia, ageusia with interstitial pneumonia, and tested positive for COVID-19. The patient was treated at home with lopinavir/ritonavir, azithromycin, and chloroquine, plus enoxaparin 4000ui. Twenty days later, symptoms disappeared, and the swab became negative. At the end of the quarantine, a CT Scan of the thorax showed ground-glass congestion of 15% of the left lung and blood samples with augmented ferritin 769mg/dl (NV 12-300) and reduced transferrin 146 mg/dl (NV200-360) and serum iron 45µgr/dl (NV 53-167) with a normal hematocrit of 46 (NV 39-49). The patient is in good condition at the moment.

## Discussion

### 1st: The questions.

We were wondering how patient 1 could have healed after acute respiratory failure, despite being so fragile, with diabetes, hypertension, and old age. Between the two CT scans, antiviral drugs were not administered to the patient, who received only antibiotics, high-dose intravenous insulin, bisoprolol, and standard enoxaparin to prevent thromboembolism. Insulin infusion was applied to the patient; however, due to persistent hypoglycemia, insulin infusion was discontinued for several days. As thromboembolism is suspected to be one of the main causes of death in COVID patients, we investigate the relationship between insulin, glycemia, and coagulation. For the same reason, in consideration of high ferritin and reduced transferrin and iron levels in patient 2, we investigate the relationship between iron and coagulation.

### 2nd: The hypothesis.

In 2012, in a study with diabetic patients, Lipinsky and Pretorius (3) reported a particular iron-related coagulation pathway. This mechanism is not linked to the classical coagulation pathway and causes proteol-

ysis-resistant protein clotting. The authors refer to the latter as the cause of diabetic microangiopathy and indicate the use of hydroxyl radical scavengers, such as salicylates, and iron polyphenols chelating agents for prevention. In 2015 Ciciliano (4) reported a second mechanism activating the coagulation caused by iron, in its configuration FeCl3, and mediated by charge-based binding proteins. The effect of FeCl3 on blood cells and proteins in the absence of endothelial cells led Ciciliano to postulate a charge-based mechanism for aggregation and formation of clots. Also, insulin and glucose concentration seem to affect coagulation. In 2006 Stregenga (5) reported a direct correlation between hyperglycemia activating coagulation, and on the contrary, by hyperinsulinemia impairing fibrinolysis. It could explain why high dosage insulin in patient 1 could have prevented pulmonary thrombosis. The complex relation between iron and coagulation, as well as between insulin and coagulation, prompted us to investigate the relationship between insulin and iron metabolism. In 2014 Wang (6) described the effect of insulin on iron metabolism by directly effecting hepcidin production. Hepcidin itself is one of the most important regulators of iron metabolism, modifying intracellular and extracellular iron's concentration. In a recent publication, Haddadi (7) described a remarkable therapeutic effect of erythropoietin in a COVID patient. He relates the effect of erythropoietin in modulating iron distribution away from the intracellular virus. This finding suggests us to verify how erythropoietin induces modification in iron metabolism. Searching the literature, we can find that its effect occurs in reducing hepcidin production and in addressing iron to the bone marrow, stimulating erythropoiesis. Interestingly, also heparin, with its anticoagulant effect, has a direct role in hepcidin inhibition (8). The modification of iron distribution, as observed in patient 2, and its relation with insulin, coagulation, and erythropoietin, led us to investigate in literature whether iron plays a role in viral replication. In general, the importance of intracellular iron for viral replication is well known, and as reported by Armitage in 2014 (9), there is a close relation between hepcidin and iron regulation in HIV-1, HBV, and HCV patients. This relationship has been described for hepatitis virus C already in the 2000s by treating patients with the use of phlebotomy

to promote iron reduction to improve interferon treatment response (10). In a recent publication, Smidth (11) underlines the importance of intracellular iron for viral replication, and this replication is influenced via Human Hemochromatosis Protein (HFE) and hepcidin. The basic consequence of this mechanism is the concentration of intracellular iron and the reduction of the extracellular one. As we have seen before, hepcidin is one of the most important regulators in iron balance. It has two main functions: the first one is to store iron into the cell to allow cellular duplication and DNA and RNA synthesis; the second one is its antibacterial effect by depriving the microorganism of iron for replication outside the host. This mechanism prevents bacterial infection but could facilitate viral replication. Its effect acts on duodenal enterocytes and the reticuloendothelial system by promoting ferroportin degradation which reduces enteric absorption of iron but retaining iron in the cell (12-13) . The gene responsible for its production is the HAMP (hepcidin antimicrobial protein, also known as HEPC; PLTR; HFE2B; LEAP1) that could be expressed mainly in the liver, but also the brain, lung, body fat and others organs (14). The excess of this intracellular iron is excreted as ferritin. As reported by Clark and Pedzernik (15), intracellular iron surplus results in the detachment of Iron-Regulatory protein (IRP) from the Ferritin mRNA, with consequent ferritin formation. Similar to what we observed in patient 2, also Battaille (16) described high ferritin levels related to the infection, even in asymptomatic patients with negative c-reactive protein, and proposed this factor as an helpful and simple screening to use in COVID disease. Interestingly Connelly, in 1997 (17), related high serum ferritin levels as a predictor of the acute respiratory distress syndrome (ARDS), which is one of the main problems in the actual COVID infection. How ARDS or acute lung injury (ALI) occurs was first described by Dixon in 2012 (18) as "Ferroptosis," and recently, Liu (19) investigated this in COVID infection, proposing iron chelation as a beneficial adjuvant in treating these patients. This is a different way from apoptosis, necrosis or autophagic cell death, and is considered as a novel type of cell death, which mainly results from iron-dependent lipid peroxidation, and is characterized by mitochondrial shrinkage in consequence of intracel-

lular iron overload, and this intracellular overload is related to hepcidin expression. Studies utilizing bronchoalveolar lavage fluid (BALF) have demonstrated increased non-heme iron in patients with acute lung injuriy compared to normal individuals (20). Moreover, Huang (21) has reported an excessive redox-active iron mobilization in lung injury induced by ischemia, from intracellular iron accumulation to the vascular space, and iron stress in the vascular space could enhance the generation of highly damaging reactive oxygen species extracellularly. In 2018 Cao (22) reported the inhibition of the ferroptosis process through the regulation of TRL4, one of the main factors involved in hepcidin production, using Ulinastatin and Liu (23) in a recent publication reported the same effect by using Ferrostatin-1. In addition, Sauler (24) advocates that ferroptosis is dependent on the presence of iron. In lung transplantation, the role of iron in pulmonary injury is well known, and the use of iron-depleting therapy has prospected as possible prevention in the lung allograft injury (25). Lagan (26) in 2008 suggested a distinction in the genetic background in ferritin light-chain gene genotype conferring susceptibility to ARDS, while the heme oxygenase 2 haplotype as a protective one. Considering these observations, we could even go so far as to hazard the hypothesis that free iron binds to oxygen, in oxido-reductive reaction, causing a reduction in the arterial oxygen pressure. Finally, recent publications start to quote deferoxamine as a potential treatment in Covid infection (27), since the evidence of its action on Enterovirus 71 infection (28), by improving B cell levels and mortality in infected mice (29).

These data suggest that COVID infection has a major effect on iron by the hepcidin pathway.

### 3rd: COVID symptoms, iron, and hepcidin relations.

As seen before, COVID infection could be related to iron overload and hepcidin, by increasing intracellular iron, promoting pulmonary ferroptosis, mobilizing the iron in vascular space, and activating coagulation via an independent pathway.

Some other symptoms could be interpreted by linking the infection with iron and, in particular, with hepcidin. For example, profound asthenia, fatigue, and heart failure could be linked to the accumulation of

iron in the mitochondria, generating reactive oxygen species (ROS) and exacerbating the damage. Halon-Golabek (30) reported iron accumulation as a cause of skeletal muscle damage and atrophy correlating sarcopenia to the high level of ferritin. Bayeva (31) reported that reducing mitochondrial iron can potentially protect failing hearts. The presence of high serum D-Dimer, without modification of coagulative parameters, was reported by Martinson after iron sucrose infusion (32). In a report from Vuppalanchi (33), high serum hepcidin levels were seen in patients with higher BMI and independent of liver disease. This observation suggests that the synthesis of hepcidin by adipose tissue may be higher in obesity.

The serum hepcidin levels seem to increase progressively with aging and it is higher in males until their 60s; subsequently, it presents the same increase in both men and women (34). Also in hypertension, an increasing level of hepcidin mRNA was observed in response to angiotensin infusion (35). This mechanism could explain high morbidity in these patients. As previously seen, insulin is directly related to hepcidin downregulation, whereas metformin is related to hepcidin upregulation (36).

It is well showed that COVID virus overexpresses several key biomarkers of the inflammatory cascade, and hepcidin is highly involved in this process. Consequently, several symptoms of COVID disease can be related to hepcidin action and iron imbalance.

### 4th: Hepcidin stimulators and inhibitors

The production of hepcidin appears to be determined by several factors with a complex mechanism of up and down-regulation (Figure 1). In this pathway, we can distinguish upregulation process as (13):

-Hereditary Hemochromatosis Gene (HFE) that is bound to Transferrin Receptors TfR1 and TfR2; -HFE seems to stimulate also Toll-Like receptor 4 (TLR4); - TLR4 in macrophages seems to upregulate hepcidin directly; - Interleukin 6 (IL6) directly stimulates hepcidin and promotes the synthesis of STAT. STAT linked to Bone Morphogenic Protein (BMP) activates hepcidin; - Bone Morphogenic Protein (BMP) on one side binds to STAT as seen before, and on the other hand by linking Hemojuvelin
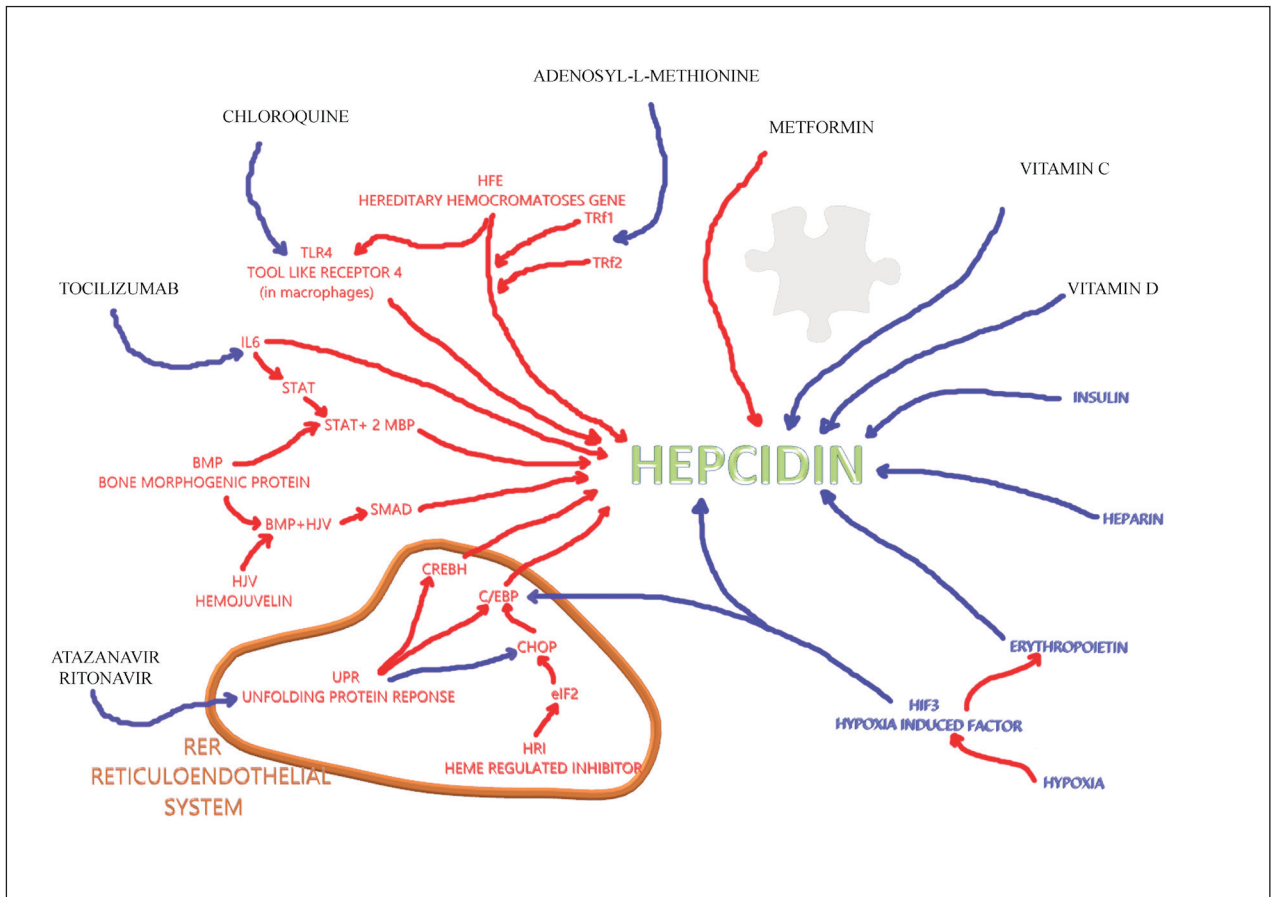
**Figure 1.** Hepcidin interaction. Molecules that have interactions with hepcidin. Red words: endogenous molecules stimulating hepcidin; Blue words: endogenous molecules inhibiting hepcidin; Black words: exogenous molecules effecting on Hepcidin; Red arrow: way of stimulation; Blue arrows: way of inhibition

(HJV) in synthesizing SMAD that stimulates hepcidin; -Hemojuvelin by linking with BMP; - Unfolding Protein Response by stimulating CREBH, which directly stimulates hepcidin, and by stimulating C/EBP that link CHOP, while these subsequently stimulate hepcidin; - Heme Regulator Inhibitor (HRI) that stimulates eIF2 with subsequent CHOP production. Between exogenous molecules that induce hepcidin, we found Metformin (35), and for virus also the Cov/SARS (37).

Between the endogenous molecules that reduce hepcidin we have: -Insulin (5), -heparin (8), -erythropoietin (38); among the exogenous one : Vitamin D (39), Vitamin C by interaction with oxidation (40), Adenosyl-L-Methionine by inhibiting TRf (41), chloroquine by inhibiting TLR4 (42), tocilizumab by inhibiting IL6 (43), ritonavir, in particular in association with atanazavir, by inhibiting UPR (44) , carvedilol by inhibiting TLR4 (45). In the literature, we can find a various number of substances that interact with hepcidin, inducing, or inhibiting it (46-47- 48-49- 50-51) in different ways and with different intensity. Among inhibitors we found: enoxaparin, fondaparinux, momelotinib, imatinib, spironolactone, siltuximab, tocilizumab, curcumin, dorsomorphin (small molecule), aspirin, Angelica Sinensis polysaccharide, and many others.

This suggests that several substances act directly or indirectly on hepcidin levels and could influence the response to current treatments.

## Conclusion

The analysis of literature reveals that, as most of viral infection, also COVID disease acts variably in the hepcidin-iron pathway. The complex interaction between iron, hepcidin, and other molecules has to be investigated to understand which relation the COVID virus has in this process. Iron overload, related to Hepcidin overexpression, seems to play a major role in COVID infection, and this overexpression could be more harmful than protective and could be related to most of the symptoms. Measurement of serum hepcidin and ferritin levels could be proposed as an indicator of infection severity, and the restoration of iron overload, by Hepcidin down-regulation and iron chelation, a possible way to improve symptoms in COVID patients. In order to confirm our research ourselves, we will soon start measuring hepcidin levels in our patients. Further investigation is warranted.

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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