SARS-CoV-2

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Alterations of iron homeostasis as a potential druggable driver of long COVID

Oriana Marques & Martina U. Muckenthaler

Drivers of persistent symptoms after acute COVID-19 remain largely unknown. Alterations in immune function, iron homeostasis and dysregulated erythropoiesis are described as treatable correlates of post-acute sequelae of COVID-19.

A link between altered iron homeostasis and COVID-19 was established early on. Consistent with the idea that iron is essential for effective immune function, several studies have shown that iron-related biomarkers such as ferritin and hepcidin are predictive of disease severity[1]. In this issue of *Nature Immunology*, Hanson et al.[2] use an insightful longitudinal analysis of 214 individuals with COVID-19, followed for up to 1 year after the onset of symptoms, to show that dysregulation of iron homeostasis for longer than 2 weeks after acute COVID-19 is associated with dysfunctional erythropoiesis and is predictive for the development of long COVID, irrespective of the initial disease severity.

Iron is essential for vital biological processes such as energy production, oxygen transport and cell proliferation. Upon demand, almost every cell in the human body can take up transferrin-bound iron from plasma. A transferrin saturation of at least 16% is required to prevent iron deficiency, which in turn can cause anemia owing to the lack of iron for the hemoglobinization of red blood cells. By contrast, iron overload may cause toxicity and cellular damage due to oxidative stress catalyzed by unbound iron. Transferrin saturation is maintained within a physiological range by the liver hormone hepcidin, which controls iron absorption in the duodenum and iron release from iron storage sites, such as reticuloendothelial macrophages and hepatocytes, by promoting the degradation of ferroportin (FPN), the only known iron exporter[3].

Infections can activate hepcidin transcription through pathogen-associated molecular patterns (PAMPs) and/or the secretion of inflammatory cytokines from innate immune effector cells. Increased levels of hepcidin reduce FPN expression, resulting in iron retention in macrophages, reduced absorption of dietary iron and consequently, decreased plasma iron levels (hypoferremia). Inhibition of FPN-mediated iron export during an immune response also occurs owing to direct transcriptional repression of the ferroportin gene (*SLC40A1*) by PAMPs and pro-inflammatory cytokines in relevant cell types (such as macrophages). As macrophages recycle vast amounts of iron from aging and effete red blood cells, reduced iron export in infectious and inflammatory conditions causes the accumulation of macrophage iron and hypoferremia (Fig. 1). In vertebrates, this innate immune mechanism, also known as 'nutritional immunity', limits iron availability for the proliferation and pathogenicity of extracellular microorganisms. Increased plasma hepcidin further protects against iron-dependent pathogens by limiting the appearance of non-transferrin-bound iron in the plasma that would be available for rapid pathogen growth[4].

Prolonged restriction of iron supplies for erythropoiesis results in 'anemia of inflammation' — as found in individuals with acute or chronic inflammation, including COVID-19[4,5]. Anemia of inflammation is a multifactorial condition, and, depending on the underlying primary disease, is associated with a reduction in the circulatory half-life of erythrocytes, reduced production and activity of erythropoietin (EPO), and reduced proliferation and differentiation of erythroid progenitor cells, along with hypoferremia. Iron is also required for the proliferation and function of T cells, and, as such, for functional adaptive immune responses[6]. One may therefore envisage that hypoferremia may contribute to delayed disease resolution in individuals with infectious diseases, including COVID-19. In addition to biomarkers indicating decreased plasma iron levels, hypoferremia has been linked to disease severity and poor outcome in individuals with COVID-19[1,7]. However, whether hypoferremia just reflected disease severity or whether there is a causal relationship between iron levels and COVID-19 progression remained unknown.

Persistent inflammation and/or the delay in establishing an effective adaptive immune response have been suggested as the culprit in severe acute COVID-19 disease, and more recently, long-lasting symptomatology beyond the acute phase (also known as long COVID or post-acute sequelae of SARS-CoV-2 infection)[8]. Hanson et al.[2] suggest a model in which hypoferremia caused by persistent inflammation can drive the symptoms of long COVID. The authors[2] evaluated 214 SARS-CoV-2-infected individuals, across a spectrum of disease severity (asymptomatic, mildly symptomatic, moderately symptomatic without supplemental oxygen required, moderately symptomatic with supplemental oxygen given as maximal respiratory support, and severely affected with assisted ventilation), up to 1 year after disease onset, and 73 healthy control individuals for reference. Blood, plasma and serum collected from these individuals were analyzed at defined time points (0–14, 15–30, 31–90, 91–180, 181–270 and 271–360 days after onset).

In addition to the delayed resolution of immune cell abnormalities, such as persistent T cell and B cell lymphopenia beyond 30 days and an increased ratio of activated/naive T cells up to one year, the authors observed dysregulation of iron homeostasis in individuals with moderate-to-severe disease[2]. These individuals had markedly reduced serum levels of iron and transferrin saturation compared with healthy controls at 0–14 days after symptom onset, correlating with increased levels of hepcidin and its activating cytokine interleukin-6 (IL-6). Consistent with the crucial role of iron for erythropoiesis, decreased hemoglobin levels were observed in individuals with moderate disease in the first 30 days, and up to 90 days in individuals with severe disease.

In conditions in which inflammation inhibits steady-state erythropoiesis, extramedullary (stress) erythropoiesis increases the number of red blood cells. Accordingly, early after symptom onset (0–14 days),

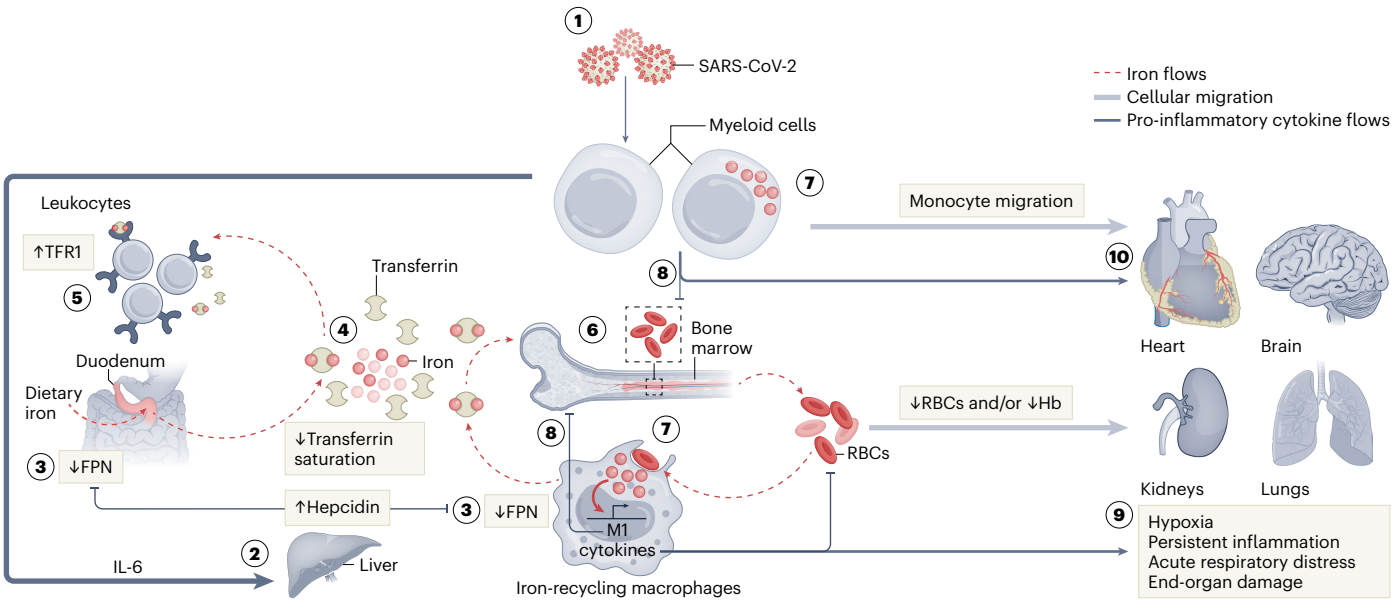

Fig. 1 | Iron dysregulation in COVID-19 infections causes an anemia of inflammation -like phenotype that may contribute to symptoms of long COVID. (1) During SARS-CoV-2 infection, myeloid cells are activated and produce pro-inflammatory cytokines. (2) Hepatocyte stimulation with the pro-inflammatory cytokine IL-6 induces the expression of hepatic hormone hepcidin. (3) Increased plasma levels of hepcidin block dietary iron absorption and iron export from red blood cell (RBC)-recycling macrophages by degrading the iron exporter FPN. (4) Decreased absorption of dietary iron and decreased release of iron from macrophages reduces transferrin saturation (hypoferremia). (5) Transferrin-bound iron is necessary for leukocyte proliferation and function. Increased iron demand during leukocyte activation is reflected by increased expression of the transferrin receptor 1 (TFR1). Hypoferremia affects leukocyte function and delays resolution of inflammation and the establishment of adaptive immune responses. (6) Reduced iron availability affects erythropoiesis by reducing the proliferation and differentiation of erythroid progenitors, resulting in anemia. (7) Reduced iron export through the FPN exporter causes accumulation of iron in myeloid cells, which further deprives leukocytes of iron and promotes the expression of pro-inflammatory cytokines, contributing to persistent inflammation. (8) Pro-inflammatory cytokines directly affect erythropoiesis, damage RBCs and reduce their half-life. (9) Reduced RBC numbers with lower concentrations of hemoglobin (Hb) contribute to hypoxia and systemic and tissue-specific manifestations of acute COVID-19 and post sequelae. (10) Migration of iron-loaded activated monocytes into inflamed tissues is expected to promote further organ damage.

reticulocyte counts, which reflect new erythrocyte production, were low in all groups. The counts had resolved to control levels by 30 days in symptomatic and mildly symptomatic patients. Individuals with moderate to severe disease displayed a steep increase in reticulocyte counts between 31 and 90 days after symptom onset, albeit with reduced reticulocyte hemoglobin and mean corpuscular hemoglobin concentration, which suggests that reticulocytosis progresses despite insufficient iron availability for hemoglobin production (defective stress erythropoiesis).

To identify biological variables that discriminate individuals experiencing persisting symptoms between 3 and 5 months after disease onset, the authors conducted a partial least-squares discriminant analysis correlating with immune cell counts, serum and hematological parameters collected during the time span. After correcting for age and severity, the authors found that individuals with persisting symptoms displayed significantly lower serum iron and transferrin saturation at 15–30 days, higher reticulocyte counts at 31–90 days, and increased levels of C-reactive protein (CRP) and IL-6 at 91–180 days after disease onset. In these individuals, a whole-blood transcriptome analysis revealed increased expression of genes associated with heme metabolism and hypoxia, ROS, IL-6–JAK–STAT3 signaling and iron homeostasis at 15–30 days after disease onset. Thus, the authors identified a multivariate signature encompassing prolonged inflammation with profound dysregulation of iron homeostasis and erythropoiesis beyond 2 weeks after SARS-CoV-2 infection that differentiated individuals reporting long COVID at 3–5 months, independently of age and initial disease severity.

In addition to affecting erythropoiesis and the recovery from anemia, inflammatory hypoferremia is also expected to affect leukocyte function[6]. By analyzing two publicly available whole blood transcriptome datasets, Hanson et al.[2] observed the differential expression of genes associated with the control of iron homeostasis in individuals with moderate-to-severe disease in the first 2 weeks after disease onset, in comparison to healthy controls. Gene responses to both iron overload (such as genes involved in the glutathione peroxidase pathway) and iron deficiency (increased expression of *TFRC* (also known as *TFR1*), which encodes the receptor for transferrin-bound iron, and *NCOA4*, which encodes a protein involved in the release of iron from ferritin in conditions of increased iron demand) were observed, which suggests that populations of blood cells may be affected in different ways. Subsequent, cellular deconvolution of the iron signatures in several single-cell datasets led to the identification of cell population-specific responses. The authors concluded that iron-deprivation signatures were predominant in proliferating $CD4^+$ and $CD8^+$ T cells, especially in

the first 2 weeks after disease onset. By contrast, myeloid cells displayed a signature consistent with iron overload and their known role in iron sequestration during inflammatory hypoferremia.

Of note, iron accumulation in macrophages polarizes macrophages towards a pro-inflammatory phenotype and increases their susceptibility to reactive oxygen species (ROS)-induced cell death[9,10]. Iron-loaded macrophages may thus contribute to persistent inflammation up to a point at which the FPN-mediated iron export from macrophages is normalized (such as when inflammation is resolved). The combination of cellular iron dysregulation, hypoferremia and hypoxia in a setting of a highly inflammatory viral infection is expected to contribute to the systemic and tissue-specific manifestations of acute COVID-19 and correlate with the development of post-acute sequelae, including fatigue, dysregulated energetics and immune function (Fig. 1).

If the results by Hanson et al.[2] are validated in larger patient cohorts, they may provide the basis for clinical studies aimed to prevent iron deficiency in viral infections, including COVID-19, in which post-acute sequelae are common. However, such a therapeutic strategy would have to be taken with caution. Hypoferremia is part of the host's nutritional immunity program and serves the purpose of limiting iron availability for pathogens, at the cost of impairment of erythropoiesis and inadequate adaptive immune responses. Although it is commonly accepted that hypoferremia may be advantageous during bacterial and protozoal infections, the same has not been clearly demonstrated during viral diseases. In fact, opportunity studies in individuals with β-thalassemia that are prone to iron overload due to ineffective erythropoiesis and blood transfusions or in patients with heart failure who receive intravenous iron supplementation suggest that increased iron availability may protect from severe COVID-19 disease[11,12].

These data indicate that the correction of iron maldistribution or hypoferremia early after disease onset during viral infections may be therapeutically beneficial to correct erythropoiesis and immune defects and prevent severe disease and post-acute sequelae. Such treatment may be particularly important for (premenopausal) women, who are frequently affected by iron deficiency owing to menstruation and child birth. The authors speculate that the absolute iron deficiency common in women may get aggravated by the functional iron deficiency (hypoferremia) in response to COVID-19, explaining the finding that women are more frequently affected by long COVID compared to men. Several drugs that directly target hepcidin or the cytokines that induce hepcidin expression are currently in clinical trials or approved by the appropriate institutional agencies. These approaches have shown promising results in normalizing iron distribution and correcting erythropoiesis in the context of other inflammatory disorders[4]. Whether or not such treatments may enhance the chance of secondary bacterial infections requires further investigation. These therapies will have to be evaluated for their capacity to overcome COVID-19-associated iron anomalies as well as long-term recovery of patients to prevent prolonged ill-health after infection, which place an increasing burden on healthcare systems and are associated with high socio-economic costs.

Oriana Marques [1,2] **& Martina U. Muckenthaler** [1,2,3,4] ✉
[1]Department of Pediatric Oncology, Hematology and Immunology and Hopp Children Cancer Center (KiTZ), University Hospital Heidelberg, Heidelberg, Germany. [2]Molecular Medicine Partnership Unit (MMPU), EMBL and University of Heidelberg, Heidelberg, Germany. [3]Translational Lung Research Center Heidelberg (TLRC), German Center for Lung Research (DZL), University of Heidelberg, Heidelberg, Germany. [4]DZHK (German Centre for Cardiovascular Research), Partner Site Heidelberg/Mannheim, Heidelberg, Germany.
✉e-mail: martina.muckenthaler@med.uni-heidelberg.de

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Competing interests

The authors declare no competing interests.