Received: 25 April 2022 | Revised: 15 July 2022 | Accepted: 2 August 2022

DOI: 10.1002/ajh.26679

RESEARCH ARTICLE

The role of hypoxia and inflammation in the regulation of iron metabolism and erythropoiesis in COVID-19: The IRONCOVID study

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Abstract

Coronavirus Disease (COVID-19) can be considered as a human pathological model of inflammation combined with hypoxia. In this setting, both erythropoiesis and iron metabolism appear to be profoundly affected by inflammatory and hypoxic stimuli, which act in the opposite direction on hepcidin regulation. The impact of low blood oxygen levels on erythropoiesis and iron metabolism in the context of human hypoxic disease (e.g., pneumonia) has not been fully elucidated. This multicentric observational study was aimed at investigating the prevalence of anemia, the alterations of iron homeostasis, and the relationship between inflammation, hypoxia, and erythropoietic parameters in a cohort of 481 COVID-19 patients admitted both to medical wards and intensive care units (ICU). Data were collected on admission and after 7 days of hospitalization. On admission, nearly half of the patients were anemic, displaying mild-to-moderate anemia. We found that hepcidin levels were increased during the whole period of observation. The patients with a higher burden of disease (i.e., those who needed intensive care treatment or had a more severe degree of hypoxia) showed lower hepcidin levels, despite having a more marked inflammatory pattern. Erythropoietin (EPO) levels were also lower in the ICU group on admission. After 7 days, EPO levels rose in the ICU group while they remained stable in the non-ICU group, reflecting that the initial hypoxic stimulus was stronger in the first group. These findings strengthen the hypothesis that, at least in the early phases, hypoxia-driven stimuli prevail over inflammation in the regulation of hepcidin and, finally, of erythropoiesis.

1 | INTRODUCTION

Since the end of 2019, a novel member of the coronavirus family, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), rapidly spread worldwide, becoming a serious threat to public health.[1,2] As of April 2022, more than 490 000 000 confirmed cases have been reported all over the globe, including nearly 6 200 000 deaths.[3] The clinical spectrum of Coronavirus Disease 2019 (COVID-19) is wide, ranging from asymptomatic infection to severe interstitial pneumonia leading to respiratory failure.[4] Severe COVID-19 is characterized by an inflammatory state with multiorgan involvement and significantly increased concentrations of inflammatory markers, like interleukin (IL)-1, IL-2, IL-6, IL-7, IL-10, Tumor necrosis Factor α (TNFα), and interferon γ (IFNγ).[5,6] The prevalence of anemia in SARS-CoV-2 infection varies according to the population under analysis.[7–10] In COVID-19 patients, two main factors that regulate erythropoiesis in the opposite direction, through iron and heme metabolism, are involved: systemic inflammation and hypoxia. On the one hand, inflammation causes the retention of iron in reticuloendothelial cells through the induction of the regulatory hormone hepcidin, which binds ferroportin (FPN), the only known iron exporter, restricting the supply of iron for erythropoiesis, and causing hypoferremia and the so-called anemia of inflammation.[11,12] IL-6 plays an important role in hepcidin induction during several infections, including influenza A.[13] On the other hand, hypoxia induces iron mobilization to sustain erythropoiesis through the suppression of hepcidin, as observed in healthy subjects exposed to high altitude hypoxia, both after acute (i.e., hours) and chronic (i.e., weeks) exposure.[14,15] The main mechanism of adaptation involves the inducible transcription factors hypoxia-inducible factor-1 (HIF-1) and HIF-2, which modulate the expression of genes implicated in response to low oxygen levels.[16] These genes include erythropoietin (EPO), which leads to the production of erythroferrone (ERFE) by the erythroblasts. ERFE suppresses hepcidin and thereby increases iron supply, and finally supports erythropoiesis. HIF-1 and HIF-2 also target genes involved in iron homeostasis (FPN, divalent metal transporter 1, DMT-1) and heme metabolism.[17,18] Previous studies involving healthy volunteers exposed to high altitude[15] and a mouse model mimicking intensive care anemia[19] suggest that erythroid stimulus prevails over inflammation for hepcidin regulation. However, to the best of our knowledge, this has not yet been studied in pathological human conditions. COVID-19 represents a model of combined hypoxia and inflammation.

With this background, we evaluated the prevalence of anemia and the impact of hypoxia and inflammation on hepcidin regulation in a multicentric observational study including patients with SARS-CoV-2 pneumonia admitted to either medical wards or intensive care units during the first and second waves of the COVID-19 pandemic.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

This is a multicentric observational cohort study. We enrolled 481 adult patients diagnosed with SARS-CoV-2 pneumonia admitted to the low and medium intensity medical wards of Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico (Milan) and Azienda Ospedaliera Universitaria Integrata (Verona), to the intensive care unit (ICU) of Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico and Humanitas Clinical and Research Center, IRCCS (Milan) from February 25th, 2020 to November 21st, 2020. Patients younger than 18 years old and those who did not sign written informed consent to enter the COVID-Network registry were excluded from the study.

2.2 | Data collection

We recorded demographic and clinical characteristics of the patients, as well as their medical history. Laboratory parameters were recorded at two time points: on admission to the ward or to the ICU (T0), and 7 days thereafter (T1). We considered different variables, including hematological data, iron status parameters, coagulation, markers of inflammation, and biochemical data. In addition, in a subset of patients, we measured hepcidin, erythropoietin (EPO), and soluble transferrin receptor (sTfR).

2.3 | Measurement technology

All laboratory tests were performed automatically on blood samples collected by venipuncture by the Central Laboratory of the Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico, Humanitas Clinical and Research Center IRCCS, and Azienda Ospedaliera Universitaria Integrata of Verona, which undergo regular quality control.

SARS-CoV-2 detection was performed in nasopharyngeal swabs by nucleic acid amplification test with real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

Hepcidin serum concentrations were determined using an enzyme-linked immunoassay (ELISA) kit from International Intrinsic LifeSciences, Calbiotech Inc. EPO levels were evaluated by means of an ELISA kit from IBL International, Intrinsic LifeSciences. sTfR was analyzed using an ELISA kit from Biomatik Corporation. All the kits were employed according to the manufacturer's protocol.

2.4 | Diagnosis of anemia

Anemia was defined as hemoglobin (Hb) concentrations lower than 13 g/dL in men aged younger than 65 years, and lower than 12 g/dL in women of all ages and in men aged 65 years and older.[20,21] We further classified the degree of anemia as follows: severe, defined as Hb <8 g/dL; moderate, Hb 8–10.9 g/dL; mild, Hb 11–12.9 g/dL in males and Hb 11–11.9 g/dL in females of all ages and in men aged 65 years and older.

2.5 | Aims of the study

The primary endpoint of the study was to evaluate the prevalence of anemia and to perform a descriptive analysis of iron status in COVID-19 adult patients.

As secondary endpoint, we explored the relationship between circulating markers of systemic inflammation, severity of gas exchange impairment, and iron and erythropoietic parameters.

2.6 | Statistical analysis

Continuous variables were presented as mean and standard deviation (SD) or as median and quartiles (IQR). Quantitative and categorical variables at baseline were compared using Wilcoxon rank-sum (Mann–Whitney) test and chi-squared tests, respectively. Spearman's rank correlation coefficient was performed to measure association between variables.

To consider within-subject correlations, the associations between hepcidin, IL-6, serum iron, and P/F ratio were analyzed using random-intercept regression models.

Statistical analysis was performed using Stata 16 software (StataCorp 2019).

3 | RESULTS

3.1 | Demographic, clinical, and laboratory parameters of COVID-19 patients

We collected data of 481 hospitalized patients with laboratory-confirmed SARS-CoV-2 infection, including 286 subjects admitted to the low and medium intensity medical wards (i.e., non-ICU cases), and 195 subjects admitted to the ICU (i.e., ICU cases).

The cohort's baseline demographic and clinical characteristics are described in Table S1. The median age of the patients was 65 years, and male sex was prevalent (70.3% men vs. 29.7% women). During a median hospitalization time of 15 days, 102 patients did not survive (21.2%). The mortality rate was significantly higher in the ICU than in the medical wards (29.7% vs. 15.4%, $p < 0.001$). The overall prevalence of comorbidities was 74.8%, of which hypertension was by far the most common, followed by diabetes, obesity, coronary artery disease, and chronic obstructive pulmonary disease.

Compared to non-ICU patients, ICU subjects were younger and less likely to suffer from comorbidities, such as hypertension, and chronic kidney disease. On the contrary, the prevalence of obese patients was greater in the ICU group (Table S1).

Hematological and biochemical parameters on admission are shown in Table S2. ICU patients displayed a more pronounced inflammatory

TABLE 1 Hematological, inflammatory and iron parameters, hepcidin, EPO and sTfR at T0 and T1

T0	All patients ($n = 481$)	Non-ICU ($n = 286$)	ICU ($n = 195$)	*p* value
Hb, g/dL	12.3 ± 1.9	12.8 ± 1.8	11.6 ± 1.8	**<0.001**
Anemia, *n* (%)	227 (47.2%)	102 (35.7%)	125 (63.9%)	**<0.001**
Mild anemia, n (%)	118 (24.6%)	58 (20.3%)	60 (30.9%)	**<0.001**
Moderate anemia, n (%)	100 (20.9%)	42 (14.7%)	58 (29.9%)	**<0.001**
Severe anemia, n (%)	7 (1.5%)	1 (0.3%)	6 (3.1%)	**<0.001**
Ferritin, µg/L	983 (503–1604)	839 (425–1397)	1284 (775–1944)	**<0.001**
Serum iron, µg/dL	34 (23–53)	34 (23–51)	38 (10–56)	0.73
Transferrin, mg/dL	150 (126–181)	158 (130–192)	136 (120–153)	**<0.001**
TSAT, %	17 (10.6–27)	16 (10–27)	17.7 (11.4–31)	0.27
CRP, mg/dL	9.1 (3.9–15.1)	6.7 (3.1–12.8)	12.4 (6.2–18.6)	**<0.001**
IL-6 ng/L	51 (27–133)	41.9 (23.3–63.6)	68 (27.6–174)	**<0.001**
Hepcidin, ng/mL	203 (124–268), $n = 185$	206 (112–265), $n = 153$	187 (129–274), $n = 32$	0.54
EPO, mUI/mL	15.6 (9.6–28.2), $n = 64$	20.7 (13–31), $n = 32$	11.3 (6.9–25.7), $n = 32$	**0.015**
sTfR, µg/mL	2.67 ± 0.9, $n = 93$	2.59 ± 1, $n = 64$	2.84 ± 0.45, $n = 29$	0.27
T1	**All patients ($n = 439$)**	**Non-ICU ($n = 274$)**	**ICU ($n = 165$)**	***p* value**
Hb, g/dL	11.6 ± 2.1	12.4 ± 2	10.3 ± 1.6	**<0.001**
Anemia, *n* (%)	267 (61.3%)	125 (46.2%)	142 (86%)	**<0.001**
Mild anemia, n (%)	91 (20.9%)	60 (22.2%)	31 (18.8%)	**<0.001**
Moderate anemia, n (%)	165 (37.9%)	63 (23.3%)	102 (61.8%)	**<0.001**
Severe anemia, n (%)	11 (2.5%)	2 (0.7%)	9 (5.4%)	**<0.001**
Ferritin, µg/L	860 (437–1334)	865 (442–1405)	831 (436–1302)	0.51
Serum iron, µg/dL	56 (33–95)	75 (48–111)	29 (23–47)	**<0.001**
Transferrin, mg/dL	151 (129–181)	153 (133–182)	147 (125–172)	0.16
TSAT, %	26 (16–45)	38 (28–50)	14.7 (11–22)	**<0.001**
CRP, mg/dL	4.5 (1.2–11.2)	2.2 (0.9–5.5)	11.2 (5–17.9)	**<0.001**
IL-6 ng/L	34.9 (11–82.5)	9.6 (3.4–20.4)	55.9 (23.8–142)	**<0.001**
Hepcidin, ng/mL	143 (76–231), $n = 211$	146 (75–231), $n = 160$	138 (82–190), $n = 51$	0.83
EPO, mUI/mL	22 (14.9–34.3), $n = 80$	21.5 (12.7–24.9), $n = 27$	23.9 (17.8–37.3), $n = 53$	0.08
sTfR, µg/mL	2.45 ± 1, $n = 95$	2.39 ± 1, $n = 73$	2.64 ± 0.56, $n = 22$	0.50

Note: Data are expressed as mean and standard deviation (SD) or as median and quartiles (IQR). Statistically significant p values are represented in bold. Abbreviations: CRP, C-reactive protein; EPO, erythropoietin; Hb, hemoglobin; IL-6, interleukin-6; PCT, procalcictonin; sTfR, soluble transferrin receptor; TSAT, transferrin saturation.

TABLE 2 Hepcidin, EPO and IL-6 by PaO_2/FiO_2 on admission

T0	PaO_2/FiO_2 < 150 mmHg ($n = 144$)	PaO_2/FiO_2 ≥ 150 mmHg ($n = 284$)	*p* value
IL-6 ng/L	91.9 (37–225), $n = 81$	42.8 (25.9–77.4), $n = 93$	**<0.001**
Hepcidin, ng/mL	183 (101–318), $n = 26$	221 (127–274), $n = 137$	0.72
EPO, mUI/mL	11.9 (7.8–28.3), $n = 16$	16.1 (9.8–26), $n = 45$	0.77
T1			
IL-6 ng/L	57.6 (22.9–56), $n = 69$	17.7 (7.5–56), $n = 78$	**<0.001**
Hepcidin, ng/mL	142 (89–240), $n = 42$	144 (71–200), $n = 145$	0.25
EPO, mUI/mL	24.8 (16.9–35.7), $n = 32$	21.4 (12.9–35), $n = 45$	0.16

Note: Data are expressed as median and quartiles (IQR). IL-6: interleukin-6; EPO: erythropoietin.

pattern with significantly higher leukocyte count (8.8 vs. 6.2 × 10^9/L, $p < 0.001$), C-reactive protein (CRP) (12.4 vs. 6.7 mg/dL, $p < 0.001$), procalcitonin (PCT) (0.3 vs. 0.1 ng/mL, $p < 0.001$), and IL-6 levels (68 vs. 41.9 ng/L, $p < 0.001$). They also showed a reduction in lymphocyte count (0.6 vs. 0.9 × 10^9/L, $p < 0.001$), an elevation in lactate dehydrogenase (LDH) (355 vs. 280 U/L, $p < 0.001$) and d-dimer (1339 vs. 1069 µg/L, p 0.01), known markers of COVID-19 severity.[22] The ratio of the arterial tension to inspiratory fraction of oxygen

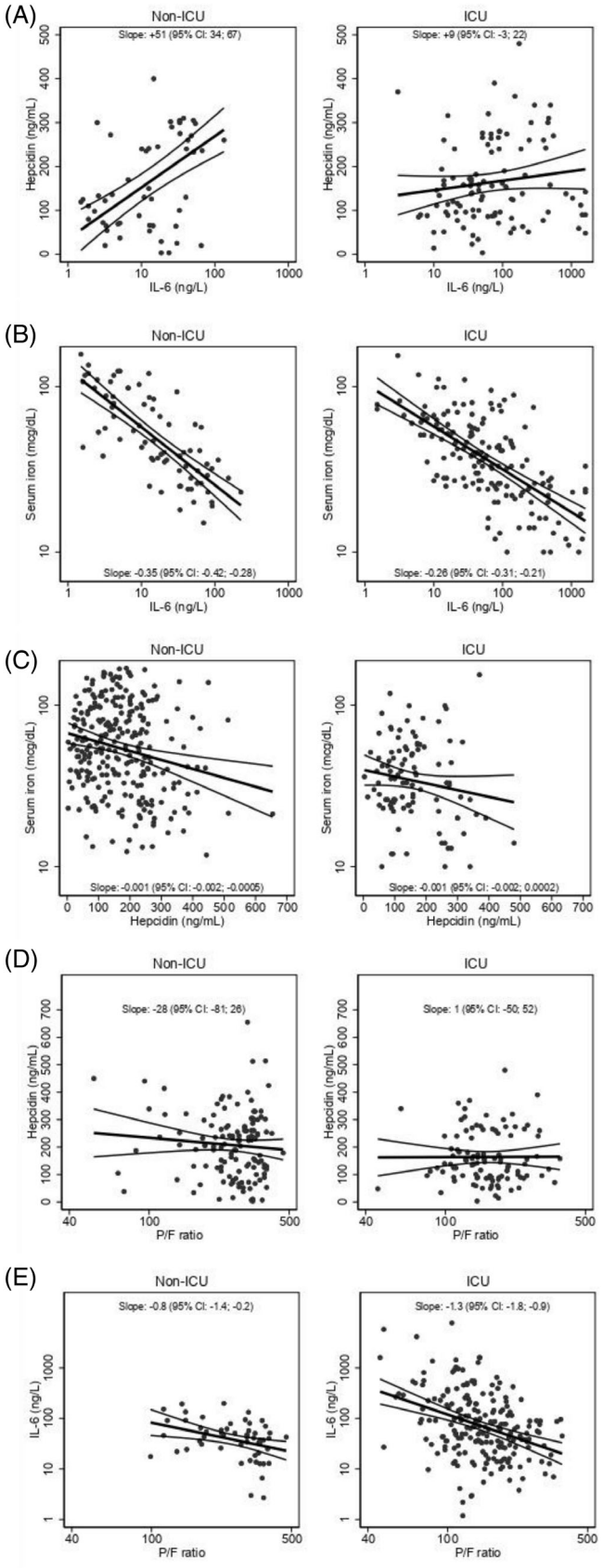

FIGURE 1 (A) Relationship between hepcidin concentration and interleukin-6 (IL-6) levels at T0 among non-ICU and ICU patients: positive correlation in the non-ICU group (rs = 0.33, $p = 0.01$) and no correlation in the ICU group (rs = 0.1, $p = 0.11$). (B) Relationship between serum iron and IL-6 at T0 among non-ICU and ICU patients: negative correlation in both groups (both rs = −0.6, $p = 0.002$). (C) Relationship between serum iron and hepcidin at T0 among non-ICU and ICU patients: negative correlation in both groups (ICU group: rs = −0.3, $p = 0.01$; non-ICU group: rs = −0.02, $p = 0.83$). (D) Relationship between hepcidin and PaO2/FiO2 (P/F) ratio at T0 among non-ICU and ICU patients: no correlation in both groups (ICU group: rs = −0.04, $p = 0.68$; non-ICU group: rs = −0.04, $p = 0.63$). (E) Relationship between IL-6 and P/F at T0 among non-ICU and ICU patients: negative correlation in both groups (ICU group: rs = −0.3, $p < 0.001$; non-ICU group: rs = −0.38, $p = 0.003$). Thick solid line: predicted regression line from a random-intercept linear regression model. Medium thick solid lines: 95% confidence bands. CI: confidence interval.

(PaO_2/FiO_2), expressing the severity of hypoxemia on admission, was lower in those who required intensive care treatment (median PaO_2/FiO_2 126 in the ICU group vs. 281 mmHg in the non-ICU group, $p < 0.001$).

3.2 | Anemia in patients with SARS-CoV-2 infection

Among the 481 enrolled patients, 227 (47.2%) had anemia upon admission (T0), with an overall mean Hb of 12.3 ± 1.9 g/dL (Table 1). 118 patients (24.6%) had mild anemia, whereas 100 patients (20.9%) and 7 patients (1.5%) had moderate and severe anemia, respectively. Overall, Hb levels decreased after 7 days of hospitalization (T1), with the whole cohort displaying a mean Hb of 11.6 ± 2.1 g/dL. The proportion of anemic patients increased over time, reaching 61.3% at T1. Although the number of subjects with mild and severe anemia remained relatively stable, the percentage of patients who developed moderate anemia went up to 37.9% at T1.

At baseline, non-ICU patients showed significantly higher Hb levels than subjects who needed intensive care treatment. Though diminishing over time, the mean Hb levels of non-ICU patients remained normal or in the lower range of normal values. Precisely, we observed a mean Hb of 12.8 ± 1.8 g/dL at T0, and of 12.4 ± 2 g/dL at T1. Patients admitted to the ICU showed a more pronounced reduction in mean Hb levels, which in any case did not fall on average under 10 g/dL: they displayed a mean Hb of 11.6 ± 1.8 g/dL at T0, and 10.3 ± 1.6 g/dL at T1. Compared to the non-ICU group, the ICU group included a significantly higher proportion of anemic subjects during the period of observation: 63.9% versus 35.7% at T0, and 86% versus 46.2% at T1. ICU patients were more likely to develop moderate anemia than non-ICU patients. Severe anemia was quite rare in both cohorts. As for

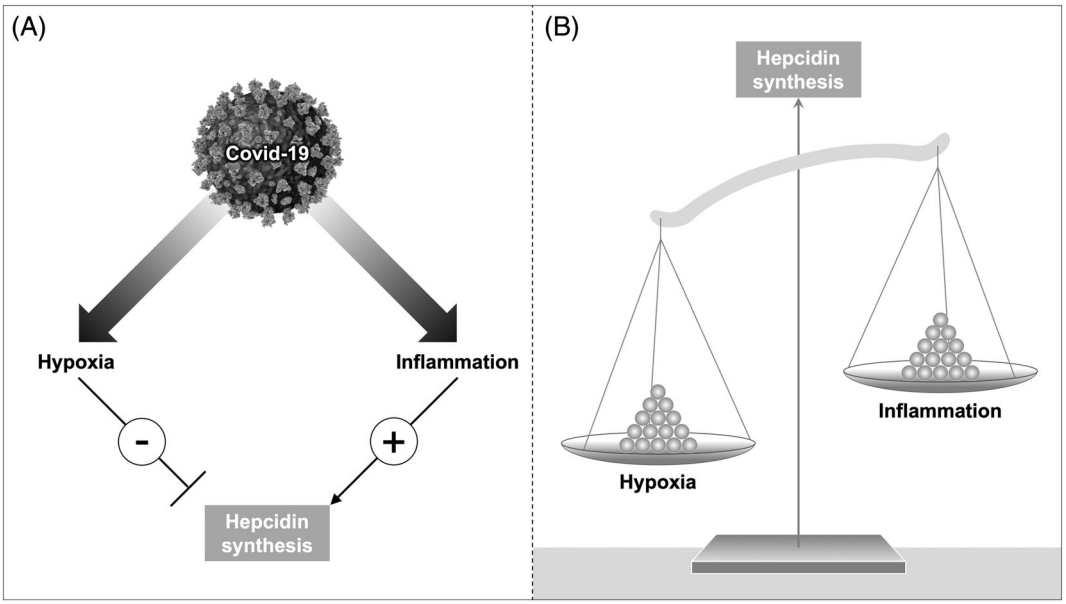

FIGURE 2 (A) Factors implicated in the regulation of hepcidin synthesis in COVID-19: systemic inflammation and profound hypoxia. (B) Hypoxia prevails over inflammation in the regulation of hepcidin synthesis

other hematological parameters, mean corpuscular volume (MCV) and red cell distribution width (RDW) did not differ between the two cohorts.

3.3 | Iron homeostasis in patients with SARS-CoV-2 infection

When analyzing iron parameters on admission, we found that the whole population displayed high ferritin levels associated with low serum iron and low transferrin saturation. These findings were consistent with functional iron deficiency in the setting of acute inflammation. Median serum iron was equally reduced in both ICU and non-ICU patients (38 and 34 μg/dL respectively, $p = 0.73$), as well as median transferrin saturation (17.7% and 16% respectively, $p = 0.27$).

At T1, serum iron and transferrin saturation increased and reached the normal range in non-ICU patients. Specifically, median serum iron and median transferrin saturation were 75 μg/dL (IQR: 48–111 μg/dL) and 38% (IQR, 28% – 50%) at T1. On the contrary, these parameters remained constantly low in the ICU group (median serum iron of 29 μg/dL [IQR: 23–47 μg/dL] at T1, and median transferrin saturation of 14.7% [IQR: 11% – 22%] at T1).

Overall ferritin levels were markedly increased, though showing a decreasing trend over time: the median ferritin concentration was 983 μg/L (IQR: 503–1604 μg/L) at T0, and 860 μg/L (IQR: 437–1334 μg/L) at T1 (Table 1). Compared to patients hospitalized in medical wards, subjects who required ICU admission displayed a more prominent hyperferritinemia at T0 (1284 vs. 839 μg/L, $p < 0.001$). Remarkably, ferritin levels became similar in the two groups at T1 (831 vs. 865 μg/L, $p = 0.51$).

3.4 | Hepcidin, EPO, and sTfR levels in patients with SARS-CoV-2 infection

Hepcidin, EPO, and sTfR measurements were available for a subset of patients (Table 1).

Overall, hepcidin levels were increased during the whole period of hospitalization, though showing a diminishing trend over time (203 ng/mL [IQR: 124–268 ng/mL] at T0, and 143 ng/mL [IQR: 76–231 ng/mL] at T1). As expected, hepcidin showed the same tendency as other acute phase reactants. However, the ICU group (more inflamed) showed lower or similar median hepcidin levels compared to the non-ICU group (less inflamed), both on admission and after 7 days of hospitalization (187 vs. 206 ng/mL at T0, $p = 0.54$, and 138 vs. 146 ng/mL at T1, $p = 0.83$).

On the contrary, EPO displayed a considerably different trend over time in the two groups. While EPO remained relatively stable in the non-ICU group, it doubled in the ICU group at T1. On admission, EPO was significantly lower in the ICU group (11.3 vs. 20.7 mIU/mL, $p = 0.015$), and it reached the non-ICU group's level at T1 (23.9 vs. 21.5 mIU/mL, $p = 0.08$).

When splitting the population according to the severity of hypoxemia on admission ($PaO_2/FiO_2 < 150$ mmHg vs. $PaO_2/FiO_2 \geq 150$ mmHg), hepcidin and EPO showed the same trend (Table 2). Actually, the patients with a more severe gas exchange impairment at presentation (i.e., $PaO_2/FiO_2 < 150$ mmHg) had lower or comparable hepcidin (183 vs. 221 ng/mL, $p = 0.72$) and EPO concentrations at T0 (11.9 vs. 16.1 mIU/mL, $p = 0.77$) in spite of more severe inflammation. At T1, both hepcidin and EPO became similar in the two groups (142 vs. 144 ng/mL, and 24.8 vs. 21.4 mIU/mL, respectively).

sTfR was slightly increased in both groups at T0 and T1, showing a slight decrease over time (2.84 ± 0.45 at T0 and 2.64 ± 0.56 μg/mL

at T1 in the ICU group, and 2.59 ± 1 at T0 and 2.39 ± 1 μg/mL at T1 in the non-ICU group).

3.5 | Correlations between hepcidin, inflammation and iron parameters, and PaO_2/FiO_2 on admission

When analyzing the association between hepcidin at presentation and markers of inflammation, we found that on admission there was a strong positive correlation between hepcidin concentration and IL-6 levels in the non-ICU group ($r_s = 0.33$, $p = 0.01$). On the contrary, this correlation was not observed in the ICU group, which displayed a more severe gas exchange impairment ($r_s = 0.1$, $p = 0.11$) (Figure 1A).

Regarding the association between serum iron and inflammatory markers at T0, we found that serum iron and IL-6 were negatively correlated in both groups ($r_s = -0.6$, $p = 0.002$) (Figure 1B). We also found a negative relationship between hepcidin and serum iron in the ICU group ($r_s = -0.3$, $p = 0.01$) and non-ICU group ($r_s = -0.02$, $p = 0.83$) (Figure 1C).

Unexpectedly, no correlation was observed between hepcidin and PaO_2/FiO_2, neither in the ICU group ($r_s = -0.04$, $p = 0.68$) nor in the non-ICU group ($r_s = -0.04$, $p = 0.63$) (Figure 1D). Finally, IL-6 at T0 tended to be negatively correlated with PaO_2/FiO_2 in both groups (ICU group: $r_s = -0.3$, $p < 0.001$; non-ICU group: $r_s = -0.38$, $p = 0.003$) (Figure 1E).

4 | DISCUSSION

In this multicentric observational study, we found that nearly half of COVID-19 patients admitted either to medical wards or to the ICU were anemic at presentation, displaying mild-to-moderate anemia. The prevalence of anemia increased during the hospital stay, reaching 46.2% in the non-ICU group and 86% in the ICU group. Apart from the raw prevalence of anemia, absolute mean Hb levels at presentation were not as low as expected in a disease characterized by a systemic inflammatory pattern. Actually, non-ICU patients had a mean Hb of 12.8 g/dL on admission, which is considered normal in women of all ages and in men aged 65 years or older, and just slightly decreased in men younger than 65 years.[20,21] Even subjects admitted to the ICU displayed a mean Hb level of 11.6 g/dL, which, in accordance with the WHO guidelines, is diagnostic for mild anemia. In clinical practice, anemia is commonly observed in patients hospitalized with sepsis, and in those who are critically ill and require intensive care treatment.[23–25] Several mechanisms contribute to the lowering of Hb levels during sepsis, and possibly during COVID-19, including hepcidin-induced iron-restricted erythropoiesis, impaired production and response to EPO, as well as increased destruction of red blood cells due to hemolysis and erythrophagocytosis, hemodilution and iatrogenic factors (e.g., repeated phlebotomy).[11,26] Nonetheless, what seems peculiar in COVID-19 is that Hb levels, though decreased in a certain proportion of patients, appear on average unexpectedly high considering the inflammatory milieu (as shown by markedly elevated IL-6 levels and hyperferritinemic response).

In our analysis, iron parameters on admission were in line with those described in previous reports.[7–10,27,28] Hyperferritinemia was a prominent feature of all patients, especially those with a higher burden of disease (i.e., ICU group). Ferritin was still markedly increased after 7 days of hospitalization (T1), though presenting a decreasing tendency. Its trend was consistent with the lowering of acute phase reactants and IL-6 levels, at least in the ICU group. Serum iron and transferrin saturation did not differ between the two groups on admission, being equally low. Noteworthy, iron homeostasis could have also been affected by the therapeutic use of anti-inflammatory drugs (i.e., corticosteroids and/or IL-1 receptor antagonist).[27] As a matter of fact, the patients treated with anakinra in association with dexamethasone (i.e., those who had the a higher burden of disease) displayed the best response in terms of increase in serum iron and transferrin saturation, and decrease in ferritin levels.

We then focused our attention on hepcidin and EPO levels, the key regulators of iron metabolism and erythropoiesis. Hepcidin has been evaluated in other COVID-19 cohorts[27–30]; however, most studies focused on its prognostic role. Similarly to what has been previously reported, our data show that hepcidin was increased during the whole period of hospitalization and displayed the same tendency as other acute phase reactants. However, we observed that the more inflamed and also more hypoxic ICU group showed comparable or even lower median hepcidin levels both on admission and after 7 days of hospitalization compared to the non-ICU group.

Similar results from a smaller cohort including 51 COVID-19 patients stratified according to intubation status (i.e., intubated vs. non-intubated) and disease severity (i.e., mild, severe, and critical illness),[28] showed that hepcidin levels were lower in the intubated and critical groups compared to non-intubated or mild patients.

As for EPO levels, they remained relatively stable in the non-ICU group, whereas they doubled in the ICU group at T1. Likewise, although available for a limited proportion of patients, reticulocyte count doubled in the ICU group at T1. These results were confirmed after dividing the population according to the severity of hypoxemia: the patients who displayed a more severe lung disease at presentation (i.e., $PaO_2/FiO_2 < 150$ mmHg) had lower hepcidin and EPO concentrations, and higher IL-6 levels.

If we consider pathophysiological mechanisms, in patients with SARS-CoV-2 infection, two main factors influence erythropoiesis and iron homeostasis: systemic inflammation and profound hypoxia (Figure 2). On the one hand, systemic inflammation plays a key role in the genesis of anemia and disturbances of iron metabolism. Iron requirements, which are crucial to support Hb synthesis, are mostly satisfied by iron recycling of senescent erythrocytes by macrophages.[31] SARS-CoV-2 induces macrophages to produce IL-6,[32] which stimulates hepcidin synthesis, causing hyperferritinemia and iron-restricted erythropoiesis. On the other hand, hypoxia is a key early feature of patients hospitalized for severe SARS-CoV-2 infection. Previous studies[18,33] indicate that hypoxia affects iron metabolism through the induction of

HIF-1 and HIF-2, increasing iron mobilization by means of DMT1 and FPN upregulation and reducing hepcidin synthesis by means of ERFE via EPO. However, up to now, the impact of low blood oxygen levels on erythropoiesis and iron metabolism in the setting of human hypoxic disease (e.g., pneumonia) has not been fully investigated.

Our data support the hypothesis that, in COVID-19, hypoxia plays a role in the regulation of erythropoiesis by counterbalancing the effect of inflammation on hepcidin regulation (Figure 2). This was postulated in a study by Hippchen et al. including 81 hospitalized COVID-19 patients.[27] The authors found that serum iron levels were negatively correlated with inflammation markers, while, unexpectedly, they did not correlate with hepcidin. Similar results were also shown by Nai et al.[29] Hippchen et al. suggested that the hypoxic state in SARS-CoV-2 patients could elicit EPO-dependent upregulation of ERFE in erythroid precursor cells, thus inhibiting hepcidin synthesis in hepatocytes. Moreover, other ERFE-independent mechanisms could be involved in the response to hypoxia,[34,35] the description of which goes beyond the purpose of our study.

Our data suggest that, in severely ill COVID-19 patients, the hypoxic drive might prevail over inflammation in the regulation of erythropoiesis, at least in the early phase of the disease. Patients in the ICU had circulating hepcidin levels unexpectedly low for the severity of their inflammation. These same patients suffered from more severe respiratory failure (i.e., lower PaO2/FiO2 on admission) and presumably experienced severe hypoxemia (i.e., low PaO2) before or during their ICU stay. Therefore, the positive signal of inflammation on hepcidin regulation could have been mitigated by the negative effect of hypoxemia. Notably, EPO levels were also lower in the ICU group on admission. This could be due to the so-called blunted EPO effect, a consequence of the inhibitory effect of several inflammatory cytokines on EPO production by the kidney, on the expression of its erythroid receptor (EpoR), and on EpoR-mediated signaling.[11] After 7 days of hospitalization, EPO levels rose and nearly doubled in the ICU group while they remained stable in the non-ICU group, probably reflecting that the initial hypoxic stimulus was stronger in the first group. Moreover, according to previous reports,[15,36] EPO response to acute hypoxia peaks only after a few days.

One of the strengths of our study is that, when analyzing our data, we mainly focused on the early phases of hospitalization. Hepcidin, EPO, inflammatory parameters, and Hb levels on admission mirror the direct effect of SARS-CoV-2 infection without other confounding factors (e.g., oxygen therapy, corticosteroids, immunomodulatory medications, blood transfusions, and further infectious complications that might occur during hospitalization).

Our study has also some limitations. Iron parameters, hepcidin, and EPO measurement were not available for all the patients included in the study. Moreover, ERFE levels were not measured. Finally, we were not able to enroll all consecutive patients due to the exceptional clinical workload during the pandemic.

In conclusion, COVID-19 can be considered as a human model of inflammation combined with hypoxia. In this setting, both erythropoiesis and iron metabolism appear to be profoundly affected by inflammatory and hypoxic stimuli, which act in the opposite direction. In patients with SARS-CoV-2 infection, Hb levels tend to be relatively high even in the context of severe disease and inflammation. Moreover, we found that hepcidin levels were lower in patients with a higher burden of disease (i.e., those who needed intensive care treatment or those with a PaO_2/FiO_2 lower than 150 mmHg), strengthening the hypothesis that the relevance of the inflammatory background is mitigated by an opposite force. Thus, in COVID-19, at least in the early phases, hypoxia-driven stimuli might prevail over inflammation in the regulation of hepcidin and, finally, of erythropoiesis. Differently from previous reports, our study did not focus on anemia, hepcidin concentrations or alterations of iron parameters as prognostic factors. Instead, we explored the pathophysiological mechanisms at the base of iron metabolism disturbances and the relationship between inflammation, hypoxia, and erythropoiesis using COVID-19 as a model. Further in-depth analyses are needed to define the role of hypoxia on erythropoiesis and iron metabolism in human pathology.

AKNOWLEDGEMENTS

The authors thank Luigi Ghilardini for his help in designing Figure 2 in the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Maira D, Duca L, Busti F, et al. The role of hypoxia and inflammation in the regulation of iron metabolism and erythropoiesis in COVID-19: The IRONCOVID study. *Am J Hematol*. 2022;1-9. doi:10.1002/ajh.26679