Research Article

Role of hematic iron and anemia in SARS-CoV-2 pathogenesis

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Article Info

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Abstract

Background: The role of anemia and iron deficit in the pathogenesis of SARS-CoV-2 is not well established. Anemia is a common finding in patients infected with SARS-CoV-2, however few studies analyze the impact of iron metabolism changes in disease progression during SARS-CoV-2 infection. Our study analyses the influence of hemoglobin and red blood cell iron deficit at the time of infection in the prognosis of patients with COVID.

Materials and Methods: This observational retrospective study collected and analyzed data from a cohort of unvaccinated patients, collecting data on variables such as erythrocyte indices associated with iron deficiency, hemoglobin and several analytical variables associated with inflammation, and analyzing its correlation with clinical outcome. Patients were classified into three groups: non-anemic, anemic (non-iron deficiency) and iron deficiency anemic (IDA). We looked for the impact of those parameters and classification on disease progression.

Results: We collected data of 435 patients with COVID infection, 322 patients with anemia and 113 without anemia as controls. Among patients with anemia, 159 had IDA and 163 were non-IDA patients. As expected, anemic patients had worse clinical evolution compared to non-anemic patients: ward admission 71.7% vs. 42.4%, $p<0.001$; ICU admission 18% vs. 7%, $p=0.03$. Interestingly, patients presenting with IDA at the onset of infection showed a better outcome when compared to non-iron deficiency anemic patients, with lower rate (56.6% vs. 86.5%, $p<0.001$) and duration (8 vs. 15 days, $p<0.001$) of admission to ward, ICU admission (8.1% vs. 27.6%, $p<0.001$) and length of ICU stay (17 vs. 23 days, $p<0.001$). Furthermore, patients with IDA showed less pronounced signs of an inflammatory process, as reflected by lower CRP (114 vs. 168 mg/L, $p<0.001$) and ferritin levels (301 vs. 1026 g/L, $p<0.001$). Other factors as age, sex, presence of comorbidities, ratio lymphocytes/neutrophils and maximum COHb concentration exhibited a significant influence on patient's outcome. Multivariate regression analysis showed that presence of IDA remains an independent prognostic factor that protect patients from admission to ward and/or ICU.

Conclusion: Our findings highlight the importance of evaluating the iron status, particularly iron deficiency anemia, in patients with COVID-19, as it is associated with a more favorable prognosis. Patients with iron deficiency anemia exhibit a more favorable outcome compared to other anemic patients. This association remains significant even after adjusting for confounding factors such as age, sex, and the presence of other comorbidities.

Introduction

The clinical manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are highly variable, and chronic conditions and comorbidities have a great impact on prognosis. Anemia is a common finding in patients infected with SARS-CoV-2. It may exist as a prior condition, or appear during the infection, possibly due to the hemolytic effect of the coronavirus [1,2]. There is contradictory data about the impact of anemia on the outcome of the disease. Some studies have reported similar hemoglobin (Hb) concentrations in fatal COVID-19 cases or those requiring intensive care admission (ICU) to those with milder clinical presentations [3]. Other studies report a clear association between anemia and prognosis [4]. Anemia may influence mortality through tissue hypoperfusion, or it may indirectly affect elderly, frail patients and impact their quality of life. Iron deficiency is the most common cause of anemia.

Iron is a fundamental element for immune system development and function, specifically for the proliferation and maturation of immune cells. Iron is involved in the inflammatory response. A deficiency of this mineral significantly impairs the body's ability to mount an immune response against infectious agents, causing a lower leukocyte count (particularly lymphocytes), and hinders its capacity to neutralize pathogens. The importance of iron in immunity and infection is evidenced by the fact that mammalian hosts have evolved multiple mechanisms of innate immunity (ex. transferrin and lactoferrin proteins) that limit the availability of the essential nutrient iron to infecting microbes [1].

On the other hand, iron overload may result in uncontrolled inflammation and immune dysfunction. Free unbound iron is very reactive and potentially toxic due to its role in the generation of reactive oxygen species (ROS) [5,6]. Excess iron is toxic to the body's cells as it produces peroxidation of cell membranes and intracellular organelles. Excessive levels of iron can be detrimental to the immune system by promoting oxidative stress and inflammation. Studies have suggested that iron may have different effects on different subsets of T cells, and that its effects on Treg cells may depend on the context and timing of exposure. Indeed, several of the manifestations of COVID-19, such as inflammation, hypercoagulation, hyperferritinemia, and immune dysfunction may arise directly or indirectly from iron overload, which in turn might be derived from virus-dependent dissociation of hemoglobin [7–9]. Many of the more severe clinical manifestations of COVID-19 arise from an exacerbated inflammatory process. In the lungs, uncontrolled inflammation causes alveolar damage, hyaline membrane formation and pulmonary oedema. These results in impaired gas exchange, low blood oxygen levels and facilitates the development of pneumonia [10,11]. Systemic immune dysregulation can lead to an excessive release of pro-inflammatory cytokines in response to an infection, resulting in a cytokine storm [12]. This can lead to damage in various organs and tissues, including the brain, gut, and kidneys.

The aim of the study is to examine the relationship between anemia, iron metabolism, and patient prognosis in COVID-19, and to determine whether these associations vary by age, sex, and the presence of chronic medical conditions.

Material and methods

This is a single-centre, observational, retrospective cohort study carried out in a third-level public health hospital in northern Spain. The study collected data from patients admitted to the emergency department of our hospital with PCR-confirmed SARS-CoV-2 infection and anaemia during the first wave (from 15 March to 31 December 2020). We recorded the laboratory findings and clinical course of the patients from laboratory and clinical information systems. Main outcomes were admission to ward or UCI and death. The study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Cantabria. (CEIm of Cantabria; 2023.416).

Study sample

We selected all the subjects who arrive at the Emergency Unit of the University Hospital "Marqués de Valdecilla" at Santander (Cantabria) between March and June of 2020 who were infected with SARS-CoV[2] virus (PCR confirmed) and with concomitant anemia. Presence and classification of anemia were established by previous diagnosis documented in clinical records and/or by data of the hemogram at admission: Hb less than 120 g/L for woman and 130 g/L for man, mean corpuscular volume (MCV) less than $8x^{10}$-14 L and mean corpuscular haemoglobin concentration (MCHC) less than 30 g/L. We also recorded main risk factors and comorbidities, such as chronic obstructive pulmonary disease (COPD), asthma, cancer, heart disease, diabetes, obesity, smoking, hypertension, and kidney disease. Data of unvaccinated patients with PCR-confirmed SARS-CoV-2 infection and without anemia (Hb greater than 140g/L) were collected as controls

Different biochemical and hematological parameters from routine analysis were collected, including C-reactive protein (CRP), lactate dehydrogenase (LDH), carboxyhaemoglobin, iron profile, D-dimer, hemoglobin and leucocyte count. These values were recorded both at admission and at their peak levels. Hemograms were analyzed using the Beckman Coulter DXH 800 autoanalyzer, coagulation was assessed using the Werfen ACL TOP 700, biochemistry was measured using the Siemens Atellica autoanalyzer, and blood gas analyses were conducted using the Radiometer ABL 800 Flex blood gas analyzer.

The study recorded the duration and nature of hospital admission for all patients included in the analysis. Patients were classified based on whether they were not admitted, admitted to a general ward, admitted to the intensive care unit (ICU), or deceased.

Variables were presented as either n (%) or medians with 25th

to 75th percentiles, as most biomarkers were not normally distributed. Differences between groups were evaluated using the Mann-Whitney-U test and Kruskal-Wallis test. Correlations were assessed using Pearson chi-square tests. The Mann-Whitney-U test was used to compare parameters between non-anemic and anemic patients, as well as between iron-deficient and non-iron-deficient patients within the latter group. Logistic regression was utilized to assess whether comorbidities impacted the disease's progression, with hospital admission, ICU admission, and mortality serving as endpoints. Statistical analysis was performed using SPSS version 26.0

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Results
In this retrospective study, we gathered and analyzed data of 322 unvaccinated patients with PCR-confirmed SARS-CoV-2 infection and anemia (159 with iron deficiency anemia and 163 with non-iron deficiency anemia) who attended our hospital in the period of the study. Data of 113 unvaccinated patients with PCR-confirmed SARS-CoV-2 infection and without anemia attended in the same period were collected as controls. The subjects included were 206 men and 229 women, with a median age of 69 years (range 19-103 years). Out of all the subjects analyzed, 285 (64.3%) required hospitalization, 71 (16%) were admitted to the ICU, and 52 (11.7%) succumbed to the disease.

Patients with SARS-CoV-2 infection and concurrent anemia had higher rates of hospitalization (71.7% vs. 42.4%, p<0.001) and longer hospital stays (11 vs. 9 days, p < 0.025) than infected patients without anemia. Furthermore, they were more frequently transferred to the ICU (18.0% vs. 7%, p=0.003) and experienced higher mortality (13.0% vs. 6.0%, p=0.031) (Table 1).

Furthermore, Table 1 also reveals that individuals with IDA had a significantly lower likelihood of requiring hospitalization (56.6% vs. 86.5%, p<0.001) and ICU admission (8.1% vs. 27.6%, p<0.001) compared to anemic patients without iron deficiency.

Table 1: Frequency of admission and fatal outcome, and length of hospital stays, depending on the presence of anemia and/or iron deficiency.

	Anemic n (%)			**Non anemic n (%)**	**p**
	322 (74.0%)			113 (26.0%)	
	Non-IDA n (%)	**IDA n (%)**	**p**		
	163 (51.0%)	159 (49.0%)			
Age	74 [19 - 103]			65 [30-99]	**0.006**
	77 [22-103]	67 [19-99]	**<0.001**		
Ward Admission	231 (71.7 %)			48 (42.4 %)	**< 0.001**
	141 (86.5 %)	90 (56.6 %)	**<0.001**		
Length of Ward stay (days)	11 [1 - 151]			9 [3-31]	**0.025**
	15 [2-151]	8 [1-78]	**<0.001**		
ICU Admission	58 (18.0%)			8 (7.0 %)	**0.003**
	45 (27.6 %)	13 (8.1%)	**<0.001**		
Length of ICU stay (days)	22 [1 - 80]			14.5[3-54]	0.216
	23 [3-80]	17 [0-57]	**<0.001**		
Death	42 (13.0 %)			7 (6.0 %)	**0.031**
	25 (15.3 %)	17 (10.7 %)	0.142		

Furthermore, patients with IDA had shorter hospital stays both in general admission (median of 8.0 vs. 15.0 days, p<0.001) and ICU admission (median of 17.0 vs. 23.0 days, p<0.001).
Women were more likely to be anemic than men (80% vs. 67%, p=0.002). IDA was also more frequent in women than in men (58.0% vs. 38.0%, p=0.001). However, men were more prone to hospitalization than women (68.9% vs. 59.8%; p=0.030) with longer hospital stays (13.0 vs. 9.0 days, p=0.001).

In addition, men were more frequently admitted to the ICU (22.3% vs. 8.7%; p<0.001) with longer ICU stays (6.7 vs. 1.9 days, p<0.001), and higher mortality (15.0% vs. 7.8%; p=0.013) than women (see Table 2). Age did not differ significantly between sexes (70 vs. 67.5 years, p=0.143).

Table 2: Distribution of demographic and outcome variables according to sex.

	Men n (%)	Women n (%)	p
	206 (47.4%)	229 (52.6%)	
Age (years)	70	67.5	0.143
Anemia	138 (67.0%)	184 (80.0%)	**0.002**
IDA*	53 (38.0%)	106 (58.0%)	**0.001**
G. Admission	142 (68.9%)	137 (59.8%)	**0.03**
ICU	46 (22.3%)	20 (8.7%)	**<0.001**
Death	31 (15.0%)	18 (7.8%)	**0.013**

Tables 3 and 4 present the key biochemical and hematological parameters that have a known impact on the prognosis of COVID-19. As expected, patients with anemia displayed decreased levels of iron and hemoglobin, along with reduced lymphocyte count. Otherwise, these patients exhibited elevated levels of D-dimer, CRP, procalcitonin, and LDH (Table 3).

Table 3: Comparison of biochemical and hematological variables between non-anemic patients and those with anemia (all kind).

	Anaemic patients	Non anaemic patients	p
	Median (IQR)	Median (IQR)	
Leukocytes (1x10^9 /L)	6.6 [0.4 - 79.2]	6.4 [2.3 - 37.2]	0.717
Neutrophils (1x10^9 /L)	4.6 [0.1 - 28.0]	4.1 [1.1 - 69.9]	0.166
Lymphocytes (1x10^9 /L)	0.9 [0.1 - 74.9]	1.3 [0.2 - 3.8]	**<0.001**
Monocytes (1x10^9 /L)	0.5 [0 - 1.7]	0.6 [0.1 - 3.0]	**0.023**
COHb (%)	1.6 [0.2 - 4.1]	1.2 [0 - 6]	0.309
Hb (g/L)	112 [60 - 119]	149 [119 - 191]	**<0.001**
MCHC (g/L)	315 [181 - 395]	338 [260 - 355]	**<0.001**
MCV (1x10^ - 15 L)	87.3 [59.5 - 122.0]	90.5 [79.0 - 105.4]	**<0.001**
D Dimer max (ng/mL)	19.90 [2.23 - 1216.23]	7.05 [0.76 - 530.46]	**<0.001**
Procalcitonin Max (ng/mL)	0.2 [0.02 - 156.4]	0.1 [0.02 - 3.5]	**<0.001**
LDHo (U/L)	185.0 [5.0 - 612.0]	165.0 [76.0 - 449.0]	**0.029**
LDHmax (U/L)	317.0 [17.0 - 1072.0]	250.0 [88.0 - 642.0]	**0.001**
CRPo (mg/L)	74 [0 - 441]	33 [5.5 - 305]	**<0.001**
CRPmax (mg/L)	14 [7 - 470]	77 [5 - 305]	**<0.001**
Ferritino (µg/L)	302.5 [1.2 - 4021.0]	350.0 [10.0 - 2808.0]	0.305
Ferritinmax (µg/L)	743.0 [7.0 - 15472.0]	456.0 [87.0 - 2808.0]	0.333
Fe (µmol/L)	3.8 [0 - 30.9]	6.2 [0.9 - 26.3]	**<0.001**
Femin (µmol/L)	3.0 [0.9 - 11.4]	3.4 [0.5 - 9.7]	0.509
Femax (µmol/L)	12.9 [2.3 - 56.6]	16.6 [6.8 - 25.9]	0.077
Transferrin (g/L)	1.89 [0.83 - 4.56]	2.02 [1.20 - 3.51]	0.287
Transferrin Saturation (%)	7 [1 - 63]	12 [2 - 41]	**0.001**

On their part, patients with iron deficiency anemia (IDA) demonstrated even lower levels of iron and hemoglobin, accompanied by decreased erythrocyte count and mean corpuscular hemoglobin concentration (MCHC), along with higher lymphocyte count. Intriguingly, patients with IDA showcased lower levels of D-dimer, higher lymphocyte count, and less pronounced signs of an inflammatory process, as reflected by lower CRP and ferritin levels (Table 4).

Table 4: Comparison of biochemical and hematological variables between patients with anemia (IDA vs. non-IDA patients).

	IDA	Non-IDA	p
	Median (IQR)	**Median (IQR)**	
Leukocytes (1x10^9 /L)	6.2 [1.1 - 33.9]	6.9 [0.4 - 79.2]	0.053
Neutrophils (1x10^9 /L)	3.7 [0.3 - 28.0]	5.2 [0.1 - 18.3]	**0.002**
Lymphocytes (1x10^9 /L)	1.2 [0.1 - 17.9]	0.8 [0.1 - 74.9]	**<0.001**
Monocytes (1x10^9 /L)	0.5 [0 - 2.1]	0.5 [0 - 1.7]	0.853
COHb (%)	1.3 [0.4 - 3.2]	1.7 [0.2 - 4.1]	0.056
Hb (g/L)	109 [60 - 119]	114 [68 - 119]	**<0.001**
MCHC (g/L)	248 [181 - 338]	331 [279 - 395]	**<0.001**
MCV (1x10^ - 15 L)	75.7 [59.5 - 84.8]	96.2 [83.2 - 122.0]	**<0.001**
D Dimer max (ng/mL)	14.18 [2.23 - 872.28]	25.45 [2.47 - 1216.23]	**<0.001**
Procalcitonin Max (ng/mL)	0.105 [0.02 - 18.41]	0.26 [0.03 - 156.47]	**0.056**
LDHo (U/L)	219.0 [81.0 - 612.0]	165.0 [5.0 - 451.0]	**<0.001**
LDHmax (U/L)	338.0 [132.0 - 1072.0]	304,5 [17.0 - 780.0]	0.151
CRPo (mg/L)	55[0 - 253]	84 [4 - 441]	**<0.001**
CRPmax (mg/L)	114 [16 - 305]	168 [7 - 470]	**<0.001**
Ferritino (µg/L)	115.0 [2.0 - 1651.0]	593.0 [75.0 - 4021.0]	**<0.001**
Ferritinmax (µg/L)	301.0 [7.0 - 4752.0]	1026.0 [85.0 - 15472.0]	**<0.001**
Fe (µmol/L)	0.29 [0.04 - 1.79]	0.45 [0.0 - 3.09]	**<0.001**
Femin (µmol/L)	0.29 [0.04 - 1.79]	0.45 [0.09 - 3.09]	0.637
Femax (µmol/L)	1.12 [0.23 - 5.66]	1.38 [0.34 - 3.85]	0.058
Transferrin (g/L)	2.37 [1.06 - 4.18]	1.70 [0.83 - 4.56]	**<0.001**
Transferrin Saturation (%)	5 [1 - 30]	10 [1 - 63]	**<0.001**

In view of the age differences between anemic patients and control subjects, as well as between patients with different types of anemia we performed further analyses to compare homogeneous age groups. In the inspection of subjects older than 70 years (n=239), no significant differences in age were observed between anemic and non-anemic patients (83 vs 81 years, p=0.166); however, significant differences in length of hospital stay (9 vs 5, p=0.004) and frequency of admission (79% vs 65%, p=0.031) remain. Within the group of anemic patients, a similar analysis was performed, selecting patients over 50 years of age (n=256). No significant differences in age were found between iron-deficient and non-iron-deficient patients (77 vs 78 years, p=0.080), but there were significant differences in the frequency of admission (65.4% vs 88.5%) and the length of ward (6 vs 13) and ICU (2 vs 7) stays (p<0.001 for both variables). No significant differences in mortality were observed for any of these groups (p=0.415).

Regression and Multivariate analysis

To examine comprehensively the impact of age, comorbidities, as well as other demographic and analytical variables, on disease progression, we performed logistic regression analyses, with ward admission (Table 5) and transfer to the ICU and/or death (Table 6) as outcome variables. Consistent with previous findings, both sex and age were significant factors that influenced disease severity and progression. The presence of comorbidities also had a clear impact on disease evolution, with COPD patients having a 5-fold increased probability of admission to ward (OR=5.098 (95%CI 1.771–14.672); p=0.003), obese patients having around a 3-fold increase (OR=3.006 (95%CI 1.637–6.609); p=0.006), and patients with heart disease (OR=3.121 (95%CI 1.895–5.139); p<0.001) or arterial hypertension (OR=3.166 (95%CI 2.107–4.756); p<0.001) having approximately a 3-fold increase in probability of ward admission (Table 5). In general terms, the presence of any comorbidity implied a 4.5-fold increase in risk. At the analytical level, Hb and MCV values were significant predictors of disease progression (p<0.001), as well as the lymphocyte/neutrophil ratio (LNR) (OR=0.96 (95%CI 0.94–0.98); p<0.001), and the level of inflammatory parameters such as CRP (OR=1.1 (95%CI 1.025–1.194); p=0.01) or ferritin

(OR=1.001 (95%CI 1.000–1.003); p=0.015) at the beginning of the episode. The presence of anemia was a significant negative prognostic factor for hospitalization (OR=3.437 (95%CI 2.203–5.364); p<0.001), whereas among anemic patients, having iron deficiency anemia was a protective factor (OR=0.204 (95%CI 0.118–0.352); p<0.001). (Table 5)

A multivariate analysis was performed to include the most significant variables with an influence on ward admission. Two models were developed, with the first including the presence or lack of anemia and the second exploring the influence of the type of anemia (iron deficiency or not). In Model I, only sex lost its independent significance when anemia was included as a variable (Table 5). However, when iron deficiency was included in the model (Model II), only iron deficiency (OR=0.315 (95%CI 0.173–0.574); p<0.001), the presence of comorbidities, and age maintained their independent statistical significance. The presence of anemia was a significant negative prognostic factor (adjusted OR=3.733 (95%CI 2.270–6.138); p<0.001), while iron deficiency acted as a protective factor (adjusted OR=0.315 (95%CI 0.173–0.574); p<0.001).

Table 5: Logistic regression analysis of demographic and analytical variables and comorbidities as determinants of admission to the ward.

	Individual logistic regressions		
	OR	**95%CI**	**p**
Demographic data			
Age	1.041	1.029 - 1.054	<0.001
Sex	1.49	1.003 - 2.214	0.048
Comorbidities			
Obesity	3.006	1.367 - 6.609	0.006
Diabetes	2.577	1.547 - 4.293	<0.001
HBP	3.166	2.107 - 4.756	<0.001
Oncologic	2.067	1.052 - 4.063	0.035
CKD	2.579	1.535 - 4.333	<0.001
COPD	5.098	1.771 - 14.672	0.003
Smoker	2.04	1.206 - 3.452	0.008
Cardiopathy	3.121	1.895 - 5.139	<0.001
Comorbidities	4.504	2.780 - 7.296	<0.001
Laboratory parameters			
Haemoglobin	0.831	0.758 - 0.910	<0.001
MCV	1.038	1.019 - 1.057	<0.001
Neutrophils	1.07	1.004 - 1.139	0.037
Lymph/Neut Ratio	0.962	0.941 - 0.984	0.001
CRP	1.106	1.025 - 1.194	0.01
Ferritin	1.001	1.000 - 1.003	0.015
Transferrin	0.987	0.982 - 0.992	<0.001
Presence and type of anemia			
Anaemia	3.437	2.203 - 5.364	<0.001
Iron deficit anaemia	0.204	0.118 - 0.352	<0.001

*Inflammatory parameters correlated with the L/N Ratio. Erythrocyte parameters are reflected in the anemia and iron deficiency classification.

In cases with a worse clinical course, which required ICU admission or resulted in death (Table 6), anemia remained associated with poor prognosis (OR=2.715 (95%CI 1.447–5.093); p=0.002), while iron deficiency maintained its protective role (OR=0.329 (95%CI 0.193–0.561); p<0.001). Comorbidities continued to play a significant role (OR=2.87 (95%CI 1.43–5.79), p=0.003), although some individual comorbidities (diabetes, cancer, renal failure, and heart disease) show no significance. Age (OR=1.015 (95%CI 1.002–1.028); p=0.027) and, particularly, sex (OR=2.677 (95%CI 1.669–4.293); p<0.001) strongly influenced admission to the ICU and/or death. At the analytical level, the protective role of a high LNR (lymphocyte/neutrophil ratio) (OR=0.904 (95%CI 0.868–0.942); p<0.001) and high Hb values (OR=0.840 (95%CI 0.751–0.940); p=0.002) stood out. Parameters associated with hemolysis during the episode, such as maximum carboxyhemoglobin in non-smokers (COHb) and maximum LDH value, were associated with worse clinical outcomes, with maximum COHb (OR=2.272 (95%CI 1.353–3.813); p=0.002) being particularly noteworthy.

The multivariate analysis included the most significant variables with an influence on ICU admission or death. In Model I, which included the presence of anemia, not statistically significant and independent association was found (OR=2.575 (95%CI 0.900–7.367), p=0.078) for anemia or for the presence of comorbidities on the outcome. Instead, smoking (OR=3.335 (95%CI 1.200–9.266); p=0.021), sex (OR=2.303 (95%CI 1.011–5.246); p=0.047), and high COHb values (OR=2.092 (95%CI 1.179–3.712); p=0.012) were found to be significant risk factors for worse prognosis (Table 6, Model I). Conversely, in Model II, which focused on the role of iron deficiency, patients with elevated COHb values still had an increased risk (OR=2.822 (95%CI 1.352–5.887); p=0.006), while the presence of iron deficiency was independently associated with a protective effect (OR=0.274 (95%CI 0.097–0.769); p=0.014) (Table 6, Model II).

Discussion

There is still much to learn about the pathophysiology of SARSCoV-2 virus infection and the determinants that govern the progression of COVID-19 disease. While risk factors such as age, comorbidities, and male sex have been explored, there are still numerous aspects left to be discovered and understood. One of the theories that explains the pathophysiology of the disease focuses on the detrimental effects of the virus on erythrocytes and the hemoglobin molecule, which can lead to anemia and tissue hypoperfusion, among other consequences.

Several studies have confirmed the prevalence of anemia among COVID-19 patients and have evaluated the impact of anemia on the prognosis of the disease. Nonetheless, given the crucial role of iron in the immune response and inflammation and the possibility of iron release as protective factor a consequence of hemoglobin destruction, some researchers have hypothesized that this could be an important determinant of cytokine storm and pathological inflammation. Hence, we aimed to investigate the influence of anemia prior to infection on the development of COVID-19 disease, and whether the presence of iron deficiency anemia with low blood iron reserves has a significant impact on the progression of the disease.

Our study demonstrates that patients with iron deficiency anemia who contract the SARS-CoV-2 virus tend to exhibit less severe disease and consequently, have a more favorable prognosis in comparison to other patients with anemia. Presence of IDA was a protective factor (OR=0.204) for hospitalization as well as for ICU admission or death (OR=0.329). This remains an independent protective factor after adjusting for other clinical relevant determinants as sex, age or presence of comorbilities. While numerous studies have been conducted on the prognosis and outcome of COVID-19 patients with anemia, to the best of our knowledge, none have compared the outcome of patients with iron deficiency anemia to that of other causes of anemia.

Table 6: Logistic regression analysis of demographic and analytical variables and comorbidities as determinants of ICU admission or death.

	Individual logistic regressions		
	OR	**95%CI**	**pa**
Demographic data			
Age	1.015	1.002 - 1.028	0.027
Sex	2.677	1.669 - 4.293	<0.001
Comorbidities			
HBP	1.623	1.013 - 2.601	0.044
COPD	3.379	1.692 - 6.746	0.001
Smoker	3.387	2.053 - 5.590	<0.001
Comorbidities	2.877	1.430 - 5.790	0.003
Laboratory parameters			
Haemoglobin	0.84	0.751 - 0.940	0.002
MCV	1.033	1.01 - 1.055	0.004
Leukocytes0	1.035	0.997 - 1.074	0.068
Lymph/Neut Ratio	0.904	0.868 - 0.942	<0.001
LDH max	1.002	1.001 - 1.004	0.011
CRP max	1.11	1.073 - 1.148	<0.001
Procalcitonin max	1.065	0.995 - 1.139	0.07
COHb max	2.272	1.353 - 3.813	0.002
Anaemia and type			
Anaemia	2.715	1.447 - 5.093	0.002
Ferropenia	0.329	0.193 - 0.561	<0.001

*Inflammatory parameters correlated with the L/N Ratio. Erythrocyte parameters are reflected in the anemia and iron deficiency classification.

This comparison is particularly noteworthy as iron supplementation is often recommended as a treatment for anemic patients with SARS-CoV-2 pneumonia, which could be detrimental based on our findings [13].

Our results diverge from certain articles that report lower iron levels in severe COVID-19 patients than in those with milder disease [14,15]. A notable limitation of those studies is that they assess baseline iron reserves using parameters, such as ferritin, which are influenced by inflammatory states. Furthermore, they do not distinguish between anemic and non-anemic patients and rely on a limited sample size.

The majority of the pathophysiological mechanisms associated with SARS-CoV-2 infection pertain to the infectious and inflammatory impacts of the coronavirus. The repercussions on pulmonary function, which leads to a decrease in gas exchange capacity, have been of particular interest. However, systemic inflammatory effects have also been documented, implying that the virus's effects are observable in organs such as the liver, gastrointestinal tract, and kidneys. Other pathophysiological pathways, including hemoglobin dysfunction due to viral action and iron overload at the tissue level, have been postulated by some studies [16,17]. Several pieces of evidence indicate that the virus can affect hemoglobin by binding to the net hemoglobin chain, resulting in its denaturation and the dissociation of iron and porphyrin, thereby releasing Fe into circulation [17–19].

Numerous investigations in scientific literature explore the role of iron during infection. The outcomes of such studies display substantial variation, and an overarching consensus appears elusive [4,15,20].

Analogously, the case for anemia also lacks uniformity although a higher concurrence amongst various publications indicates that hemoglobin (Hb) deficiency correlates with poorer prognoses [3,5,6]. Clearly, lower Hb values upon admission are linked to increased disease severity. Lanser et al. observed a more pronounced decrease in Hb in severely affected patients, with worse outcomes for those presenting with anemia prior to admission [21]. It should be noted, however, that this study did not differentiate between different types of pre-existing anemia. On the other hand, certain investigations assert that survival is not correlated to hemoglobin levels but rather to parameters typically associated with hemolysis, such as red blood cell distribution width (RDW) or lactate dehydrogenase (LDH) [3]. Some researchers postulate that ferritin may influence cytokine release by macrophages, potentially establishing a detrimental feedback loop in which altered iron metabolism impairs immune response regulation, thereby exacerbating inflammation [16,22]

Anemia is a prevalent comorbidity in patients with COVID-19, it may be a previous condition or resulting from the virus-induced hyperinflammatory state combined with its hemolytic capacity. Excluding the study of Lanser and cols. none of the reported investigations analyze Hb values before infection, neither assess red blood cell parameters to determine iron status [3,4,19,21] as we did in the present study. Existing articles exploring the role of iron in COVID-19 infection have classified patients with iron deficiency based on ferritin, transferrin, and transferrin saturation index values. These parameters require cautious interpretation in the context of patients with inflammatory states, as ferritin serves as an acute-phase reactant and transferrin acts as a negative acute-phase reactant. Consequently, the inflammatory state present in COVID-19 patients may lead to an overestimation of the patient's iron stores during the initial stages and throughout the infection. The distinctive advantage of our work lies in the characterization of iron deficiency in our patients based on erythrocyte-derived parameters (MCV and MCHC). These parameters remain unaffected by the patient's inflammatory state and offer greater temporal stability than biochemical parameters, rendering a low MCV value an accurate indicator of systemic and sustained medium-term iron deficiency.

Consistent with findings from other studies, patients with anemia demonstrated worse prognoses, higher admission rates, and elevated morbidity and mortality rates, consistent with findings from other studies. Furthermore, our research reveals better outcomes in patients with iron deficiency anemia compared to those with non-iron deficiency anemia. Patients with pre-existing iron deficiency anemia displayed fewer and shorter ward and ICU admissions relative to patients with other anemia etiologies.

In our study, peak COHb levels were significantly associated with poorer prognoses (OR 2.27, p=0.002). During hemoglobin degradation, a carbon monoxide (CO) molecule is produced, which, in instances of pronounced hemolysis, results in elevated carboxyhemoglobin (COHb) levels. This parameter has been minimally investigated in COVID-19 patients, but it seems to correlate with increased severity [23], supporting the theory of hemolysis and its connection to COVID-19 severity. Numerous studies have demonstrated that other parameters related to hemolytic processes, such as LDH, RDW, and bilirubin, are elevated in COVID-19 patients, particularly in more severe cases. Our study also identified an increase in COHb values, which would be more specifically associated with a hemolytic process.

This mechanism would explain why iron overload plays a detrimental role in the prognosis of COVID-19 patients. The differences in disease severity found in our study, expressed as frequency and duration of admission, requirement for intensive care and mortality, are in line with this hypothesis. Nonetheless, non-anemic patients have a better outcome after infection than iron deficient patients. This could be explained by the fact that the morbidity and mortality of infection is closely related to tissue hypoperfusion, secondary to desaturation, which probably plays a more important role than oxidative stress.

Given these results, it would be interesting to consider treatment aimed at controlling Fe ion metabolism in those cases in which the inflammatory alteration has already begun, in order to halt oxidative stress [6,24]. Some authors suggest that iron depletion using chelators may be beneficial for the patient [25]. However, there are no interventional clinical studies in which patients have been treated with chelation, except for very small cohorts in which the assessment of chelation was not the main objective [26]. In another case report a patient with marked IDA and severe COVID infection improved after administration of erythropoietin [1,27]. The author speculates on the therapeutic mechanisms that influenced the outcome and includes that the improvement in this case may have been due not only to an increase in Hb, but also to an iron sequestration by the bone marrow that counteracts the inflammatory effect of the virus.

Our study has limitations. Primarily, it is a retrospective study and both the analytical data and the presence of comorbidities were extracted from the data in the medical record and the admission episode. Therefore, not all analytical data was collected from all patients. Although all patients were unvaccinated, the group is not homogeneous in terms of SARS-CoV-2 variants, and viral genotype was not taken into account. The groups were also not homogeneous in terms of age, although age-adjusted regression analysis and age-homogeneous subgroup analyses compensate for this shortcoming. In terms of strengths, we can mention a larger sample size than other studies on the role of iron in anemia and COVID-19, and a better assessment of iron deficiency anemia by using data on erythrocyte parameters such as MCV instead of other parameters that may be influenced by the inflammatory process.

Conclusion

In summary, we have found that patients with anemia have a worse prognosis in terms of hospitalization, requirement for intensive care, and mortality than other patients with COVID-19, even after adjusting for age, sex, and the presence of other comorbidities. Interestingly, among anemic patients, those with iron deficiency anemia exhibit a more favorable outcome, with a lower proportion of hospital admissions, need for intensive care, and mortality compared to other anemic patients. This association remains significant even after adjusting for the relevant confounding factors. In line, we have found that patients with iron deficiency anemia have a lower inflammatory state, as evidenced by lower levels of C-reactive protein (CRP), ferritin, and carboxyhemoglobin (COHb). Iron metabolism appears to play a crucial role in the pathogenesis of SARS-CoV-2, potentially through hemolytic effects on hemoglobin and interference with iron regulatory mechanisms. Considering the potential role of iron depletion, it would be valuable to

conduct further studies investigating the potential benefits of incorporating iron depletion strategies as adjunctive treatments to the existing therapeutic approaches. Our findings highlight the importance of evaluating the iron status, particularly iron deficiency anemia, in patients with COVID-19, as it is associated with a more favorable prognosis.

Ethics Approval

The study "Role of Hematic Iron and Anemia in SARS-CoV-2 Pathogenesis" was reviewed and approved by the Research Ethics Committee of Cantabria (approval code: 2023.416). The research was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Given the retrospective nature of the study, which involved the analysis of anonymized data previously collected during routine clinical care, obtaining informed consent from participants was not feasible. The confidentiality and privacy of all participants were safeguarded throughout the study, and no identifiable information was used or disclosed.

Author contributions

Guillermo Velasco de Cos: Conceptualization, formal analysis, investigation, methodology, project administration, visualization, writing original draft, and writing review & editing. Armando Raul Guerra Ruiz: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, visualization, validation, and writing review & editing. Rafael José García Martinez and Sarai Torres Robledillo: Investigation. María José Muruzabal Sitges: Supervision, visualization, and writing review & editing. Bernardo Alio Lavín Gómez, Seila Hernández Vicente, and David Ruiz Ochoa: Supervision and writing review & editing. María Teresa García Unzueta: Supervision.

Disclosures

Conflict of interests

The author does not have any conflict of interest to disclose in this study.

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Data availability

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

Abbreviations

IDA: Iron deficiency anemia
COHb: Carboxyhaemoglobin
LDH: Lactate dehydrogenase
Hb: Hemoglobin molecule
ROS: Reactive oxygen species
ICU: Intensive care unit
MCV: Mean corpuscular volume
MCHC: Mean corpuscular haemoglobin concentration
COPD: Chronic obstructive pulmonary disease
CRP: C-reactive protein
RDW: Red blood cell distribution width
CO: Carbon monoxide

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