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Genetic regulation of iron homeostasis in sideropenic patients with mild COVID-19 disease under a new oral iron formulation: Lessons from a different perspective

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ABSTRACT

Background: Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) needs iron to replicate itself. Coronaviruses are able to upregulate *Chop/Gadd153* and *Arg1* genes, consequently leading to CD8 lymphocytes decrease, degradation of asparagine and decreased nitric oxide (NO), thus impairing immune response and antithrombotic functions. Little is known about regulation of genes involved in iron metabolism in pauci-symptomatic patients with COVID-19 disease or in patients with iron deficiency treated with sucrosomial iron.
Methods: Whole blood was taken from the COVID-19 patients and from patients with sideropenic anemia, treated or not (control group) with iron supplementations. Enrolled patients were: affected by COVID19 under sucrosomal iron support (group A), affected by COVID-19 not under oral iron support (group B), iron deficiency not under treatment, not affected by COVID19 (control group). After RNA extraction and complementary DNA (cDNA) synthesis of *Arg1*, *Hepcidin* and *Chop/Gadd153*, gene expression from the 3 groups was measured by qRT-PCR. M2 macrophages were detected by cytofluorimetry using CD163 and CD14 markers.
Results: Forty patients with COVID-19 (group A), 20 patients with iron deficiency treated with sucrosomial iron (group B) and 20 patients with iron deficiency not under treatment (control group) were enrolled. In all the patients supported with oral sucrosomial iron, the gene expression of *Chop*, *Arg1* and *Hepcidin* genes was lower than in sideropenic patients not supported with iron, M1 macrophages polarization and functional iron deficiency was also lower in group A and B, than observed in the control group.
Conclusions: New oral iron formulations, as sucrosomial iron, are able to influence the expression of genes like *Chop* and *Arg1* and to influence M2 macrophage polarization mainly in the early phase of COVID-19 disease.

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) needs iron to replicate itself (Liu et al., 2020). SARS-CoV-2 links Angiotensin-converting enzyme (ACE) 2 to enter enterocytes, hepatocytes and histiocytes, which contain high intra-cytoplasmatic iron levels (Scialo et al., 2020 Dec). Interestingly, SARS-CoV-2 spike protein shows sequence homology to hepcidin, being able to block enterocyte ferroportin and gut iron absorption (Ehsani, 2020 Oct 16). During an evolutionary arms race, viruses gain the ability to subvert cellular autoprotective mechanisms like cellular stress responses, unfolded protein response (UPR) and autophagy, by blocking the clearance of pathogens including viral particles. This happens mainly through the upregulation of *C/EBP homologous protein (Chop)* gene, also known as growth arrest and DNA damage-inducible gene 153 (*Gadd153*). This same pathway can enhance the susceptibility of the immune system

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dendritic cells (DCs) to SARS-CoV-2 and induce cytokine storm, both related to a higher risk of COVID-19 mortality (Tang et al., 2020 Jul; Cao et al., 2019 Mar 20). Upregulation of *Chop/Gadd153* during Coronavirus induced infectious bronchitis shows an enhancement of cell apoptosis by restricting the activation of the extracellular signal-regulated kinase pathway (Liao et al., 2013). Moreover, this gene induces apoptosis and stimulates hepcidin production in CD8 positive lymphocytes (Caldwell et al., 2018 Apr 1). Of note, iron support is also able to block *Chop* activity, interfering with lymphocytes apoptosis (Siri et al., 2021). On the contrary, iron deficiency is linked to an increased incidence of infections and an impaired immune response, furthermore anemia negatively impacts on patients' survival within the course of COVID-19 (Faghih Dinevari et al., 2021 Feb 10). Recent experimental evidences have shown an increase in the severity of acute lung injury (ALI) in *Hamp* knockout mice after the induction of iron overload (Zhang et al., 2020 Jun). It is now well-established that SARS-CoV-2 infection can lead to an ineffective immune response. Arginase-1 (*Arg1*), which has a critical role in immune cells, can be expressed by myeloid cells as neutrophils and macrophages and it has been associated with the suppression of antiviral immune responses (Pesce JT, Ramalingam TR, Mentink-Kane MM, Wilson MS, El Kasmi KC, Smith AM, T, , 2009 Apr). *Arg1* was shown to be significantly increased (2.3 times) in the whole blood of COVID-19 patients, compared to healthy individuals ($p < 0.01$). Thus, *Arg1* might be a promising marker of the pathogenesis of the disease, and it could be a valuable diagnostic tool (Derakhshani et al., 2021 Mar 4). Remarkably, SARS-CoV-2 is also able to induce *Arg1* expression leading to a degradation of arginine and to a decreased nitric oxide (NO) production, consequently impairing the immune response and antithrombotic activity of NO (Cao et al., 2019 Mar 20; Liao et al., 2013). Further, there is some evidence suggesting that iron induces macrophage polarization in M2 sense. Moreover, M2 macrophages show an anti-inflammatory and anti-COVID-19 activity (Agoro et al., 2018 May 17).

It is also well known that during infections, iron is disputed between the host and the invading pathogen. Biomarkers of iron homeostasis have been assayed in several studies on COVID-19 and some speculative observations on the potential action of specific or supportive treatments of the infections as mediated by interference with iron metabolism have been recently reported (Batiha et al., 2021 Dec; Batiha et al., 2022 Jun). Hyperferritinemia due to an hyperexpression of *Hamp* (Hepcidin Antimicrobial Peptide) gene is quite frequent in subjects with preceding severe or critical COVID-19 disease and the analysis of the mRNA of *Hamp* gene expression in peripheral blood mononuclear cells has shown a correlation with increased ferritin levels and cytokines mRNA expression in these patients. Hyperferritinemia is significantly associated with severe lung involvement at computed tomography scans and with an impaired performance status (Sonnweber et al., 2020). A significant association between ferritin and disease severity and mortality has been also confirmed by a *meta*-analysis from Mahat and colleagues (Mahat et al., 2021 Jul-Sep;11:100727.). Ferritin assay may thus be a useful screening test to evaluate the degree of inflammation in patients with COVID-19.

Hepcidin, on the contrary, is not routinely assayed in clinical practice, high levels of hepcidin have however been reported during the course of severe COVID-19 (Nai et al., 2021 Mar 1). Interestingly, also high serum levels of High-mobility group box 1 (HMGB1) have been detected in patients with COVID-19 patients and directly associated with disease severity, and the risk of acute respiratory distress syndrome (ARDS). SARS-CoV-2 may enhance the release of HMGB1 trough cytolytic effects thus feeding a vicious circle where HMGB1 induces the release of pro-inflammatory cytokines and the up-regulation of ACE2 enzyme (Al-Kuraishy et al., 2022 Jun).

Beyond the interference with iron metabolism, several direct and indirect interactions have been shown between SARS-CoV-2 and erythrocytes, in detail: 1) the erithrocrine fuction of red blood cells during the infection may be altered and lead to inflammatory complications and endothelial dysfunction due to an impaired, dysfunctional, release of molecules like NO and ATP (Al-Kuraishy et al., 2022 Jan-Dec;36:3946320221103151.); 2) the clinical course of COVID-19 may be complicated by haemolytic anemia due to a direct injury or the synthesis of autoantibodies (Al-Kuraishy et al., 2022 Sep). Evaluating the interactions between iron metabolism and COVID-19, in addition to a biological interest, may thus have impact on the management of COVID-19 itself or on future pandemics. For this reason, in the current pilot study, we have analyzed the expression of *Arg1* and *Chop/Gadd153* to explore the relationship between SARS-CoV-2 infection and iron metabolism in sick individuals with mild disease and in individuals with iron deficiency. Up to date, little is known about the genetic regulation of iron metabolism in COVID-19 patients and about the potential effects of sucrosomial iron support in sideropenic patients with a mild form of SARS-CoV-2 infection. Hereby, we report on the results of an Italian multicenter retrospective study aiming i) to investigate the regulatory effects of *Chop/Gadd153*, *Hamp* and *Arg1* genes on iron metabolism in patients with iron deficiency, with or without mild Covid-19 and ii) to study how a new iron formulation such as sucrosomial iron could be able to bypass hepcidin and ferroportin block induced by COVID-19 disease in patients with a mild form of disease.

2. Materials and methods

2.1. Patients and sample collection

For this multicenter and retrospective pilot study, we screened 150 sideropenic patients. Sixty consecutive patients with sideropenic anemia due to gastrointestinal bleeding were enrolled (see Fig. 1, consort flow diagram). All patients enrolled in the study gave their informed consent to use the left-over diagnostic samples for this study. Enrolled patients were treated at the Hematology Units of 3 Italian Centers: Campobasso (Regional Hospital "A. Cardarelli"), Palermo (University Hospital "P. Giaccone") and Meldola (IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori"), from December 2019 to June 2021. The current study was first approved by the Institutional Review Board (IRB) of the AOUP "P. Giaccone" and by the other enrolling centers. Inclusion criteria were: age > 18 years; anemia secondary to a documented gastrointestinal bleeding; Hgb < 10 g/dL; ferritin < 30 ng/ml; total iron binding capacity saturation < 20%; at least one fecal occult blood test positive out of 3; absence of active cancer at gastroscopy and colonoscopy; gastric bleeding (due to ulcer, gastritis, angiodysplasia); small intestine bleeding (due to angiodysplasia); colon bleeding (due to diverticulitis, angiodysplasia, hemorrhoids); iron deficiency diagnosed at least 30 days before the enrollment in the study. Exclusion criteria were: age < 18 years; loss of 1 g Hb/dl in a time < 7 days; hyperthyroidism; severe chronic heart failure; autoimmune disease; inflammatory bowel disease; ischemic/hemorrhagic enterocolitis; active cancer under active treatment or not; helminths infestation; pregnancy/breastfeeding; neoplastic hypermenorrhea and/or menometrorrhagia; severe organ failure.

Forty out of 60 patients were affected by documented mild COVID-19 infection, as defined by the National Institutes of Health (NIH) COVID-19 treatment guidelines (https://www.covid19treatmentguidelines.nih.gov/). Diagnosis of SARS CoV-2 infection was performed according to the guidelines of the Italian Institute of Public Health (Mancini et al., 2020 Dec; Corman et al., 2020 Jan).

Twenty out of 40 patients affected by COVID-19 received daily 60 mg of elemental iron as sucrosomial oral iron (group A), iron therapy was already ongoing from one month at the time of COVID-19 molecular diagnosis, while 20 patients did not receive any iron support (group B). We also enrolled as a control group 20 patients without COVID-19 infection affected by sideropenic anemia due to gastrointestinal bleeding, not receiving any iron support. Demographic data of the enrolled patients and their baseline characteristics (30 days before COVID-19 molecular diagnosis) are reported in Table 1 and Table 2, respectively. For each patient, the following data were recorded at time

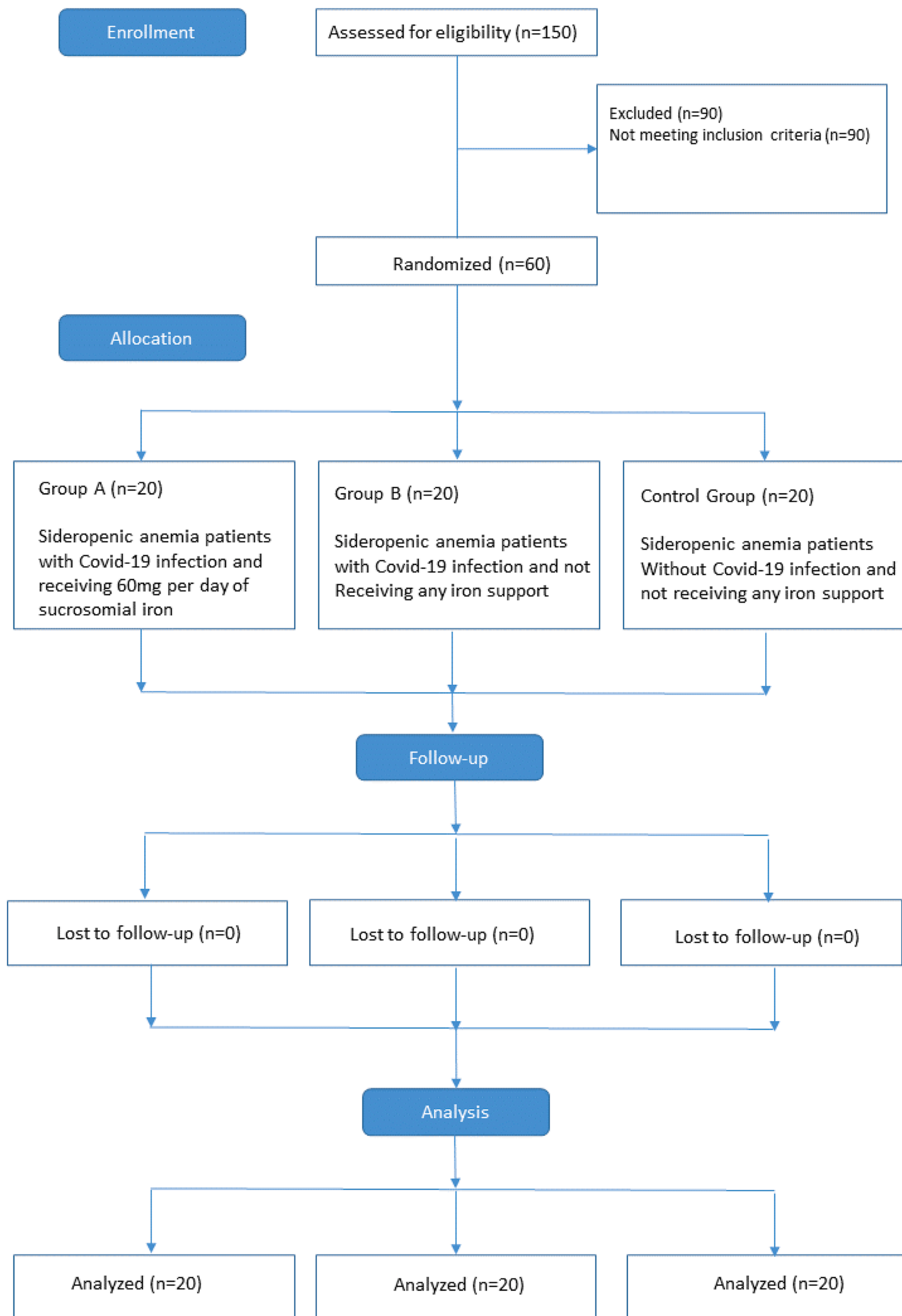

Fig. 1. Consort flow diagram.

Table 1
Demographic characteristics of patients enrolled in the study.

	Sideropenic Patients that DID NOT develope Covid-19	Sideropenic Patients that developed Covid-19	Sideropenic Patients that developed Covid 19 and were Supported With Sucrosomial Iron	P
M/F	11/9	10/10	11/9	n.s.
Median Age (Range)	45 (44–62)	47 (44–63)	47 (44–65)	n.s.
Etnicity	Caucasian	Caucasian	Caucasian	n.s.

Abbreviations M: male. F: female. P: p-value (Kruskal-Wallis test, followed by Tukey test. n.s.: not statically significant.

Table 2
clinical and biological characteristics of patients 30 days before COVID-19 diagnosis.

	Sideropenic Patients that NOT developed COVID-19	Sideropenic Patients that developed COVID-19	Sideropenic Patients that developed COVID-19 while Supported With Sucrosomial Iron	P
Cardiac Comorbidities	No	No	No	n.s.
Lung Comorbidities	No	No	No	n.s.
Other Comorbidities	No	No	No	n.s.
Drug Intake	No	No	No	n.s.
Food Integrators	No	No	No	n.s.
CRP Mg/L(Range)	32 (5–35)	40 (15–95)	35 (18–37)	n.s.
Serum Hepcidin mcg/l (Range)	76 (60–80)	80 (62–85)	78 (70–83)	n.s.
Chop/Gadd153	1	1.2 (1–1.5)	1.3 (1–1.4)	n.s.
Hamp	1(R1-2)	1(R1-2)	1(R1-2)	n.s.
Arg1	1(R1-2)	1.3(R1-2.3)	1(R1-1.5)	n.s.
Lymphocyte	2500/mcl (R1500-3000/mcl)	2300/mcl (R1200-3400/mcl)	2600/mcl (R1600-3000/mcl)	n.s.
M2	0,5%(R0,4–1,2)	0,7%(R0,5–1,1)	0,5%(R0,3–1,3)	n.s.
M1	0,4%(R0,3–1)	0,4%(R0,2–0.9)	0,5%(R0,4–0.6)	n.s.
Ferritin	15 ng/ml (R2-20)	10 ng/ml (*R*3-15)	15 ng/ml (R4-12)	n.s.
Tsat	15 % (R5-20 %)	12 % (R5-14 %)	10 % (R8-12 %)	n.s.
Hb	9 g/dl (R7.8–10)	8.8 g/dl (R8-9.8)	9.1 g/dl (R8.5–10)	n.s.
Hosp	0/20	0/20	0/20	n.s.
Los	0	0	0	n.s.
Transf/Month	2 (R0-2)	3 (R0-3)	1.5 (R0-2)	n.s.
Crp (Mg/L)	10 (5–35)	8 (5–28)	12 (5–30)	n.s.

Abbreviations: *Chop/Gadd153*: *C/EBP homologous protein* gene/growth arrest and DNA damage-inducible gene 153. *Hamp*: Hepcidin Antimicrobial Peptide gene. *Arg1*: arginase 1 gene. CRP: C reactive protein. HB: hemoglobin concentration. HOSP: number of hospitalized patients. LOS: length of stay in hospital (days). M1 and M2: macrophages. NEG: day of first negativity at molecular test for covid19 from the first day of symptoms onset. T0: time of the onset of COVID19 infection. TSAT: transferrin saturation. TRANSF/MONTH: number of hemotransfusion per month. P: p-value (Kruskal-Wallis test, followed by Tukey test). n.s.: not statically significant.

0 (molecular diagnosis of COVID-19 disease) and after 3 months: complete blood count, ferritin levels, transferrin saturation, C reactive protein (CRP), hepcidin serum level, and expression of *Arg1*, *Chop/Gadd153* and *Hamp* genes, duration of hospitalization, number of red cells unit transfusions/month, days needed for negativization of molecular Sars-Cov-2 infection.

Serum Hepcidin was quantified by the validated RadioImmunoAssay (RIA) method as previously described by Grebenchtchikov and Coll. (Grebenchtchikov et al., 2009 Aug).

2.2. RNA isolation and cDNA synthesis

Total RNA was isolated from buffy coat from peripheral blood using the RNeasy Midi Kit (QIAGEN, Hilden, Germany), followed by genomic DNA digestion with TURBO DNA-free (Ambion, Austin, TX). RNA was quantified by NanoDrop™ (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was obtained from 1 ug of DNase-treated RNA employing the SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The cDNA was stored at

−20 ∘C for real-time PCR analyses until its use.

2.3. Real-time PCR

Real-time PCR was performed to assess *Chop/Gadd153*, *Hamp* and *Arg1* mRNA levels. The synthesized cDNA was amplified in duplicate on iCycler iQ5 (Bio-Rad, Hercules,CA) using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA). All assays were measured using the following program: enzyme activation and DNA denaturation at 95 °C for 3 min followed by amplification (35 cycles): 95 °C for 15 *sec*, 60 °C for 30 *sec* in a final volume of 20 μl. At the end of the PCR cycling, melting curves were generated to ascertain the amplification of a single product and the absence of primer dimers. The *Glyceraldehyde-3-Phosphate Dehydrogenase* (*GAPDH*) was used as a housekeeping gene for normalization. The sequences of the used primers are listed in Table 3. Relative expression levels were calculated as follows: 2 (Ct *GADPH* gene- Ct gene of interest) x 1000. Relative expression of tested genes is referred to the expression of the same gene of sideropenic patients unaffected by COVID-19 of the control group.

2.4. Flow cytometry

After PBMCs isolation from peripheral blood using Lymphosep (Biowest, Nuaillé, France), M1 proinflammatory and M2 anti-inflammatory macrophages were assayed by flow-cytometry using the following fluorochrome-conjugated anti-human antibodies: CD68 (PE-Cy7), TLR2 (FITC), CD200R (PE), CD163 (BV605), CD206 (APC-Cy7), CD64 (PE-Dazzle 594), HLADR (PE-Cy5), CCR7 (BV711), CD80 (BV650), CD86 (BV421), CD14 (Pacific Blue) (Biolegend, San Diego, CA) (Mily et al., 2020). Flow cytometry was performed using BD LSR II and BD FACS Aria III flow cytometers (BD Bioscience. Thirty thousand events were acquired from each sample and data were analyzed with FlowJo v.9. software (TreeStar, Ashland, OR).

2.5. Statistical analysis

Sample size was calculated according to the following criteria: first type alpha error 0.01, beta error of the second type 0.05; coefficient of expectation 0.6.A standard normal deviation for α was equal to Zα = 2.5758, a standard normal deviation for β = Zβ = 1.6449C = 0.5 * ln [(1 + r) / (1-r)] = 0.6931. Total sample size was calculated as N = [(Zα + Zβ) / C] 2 + 3 = 40 (Hulley et al., 2013).

Kruskal-Wallis test, followed by Tukey test, was applied using SPC software for Excel, to detect significant differences between the groups of patients. Results are expressed as median plus range of values in each group. Statistical significance was considered at $p \le 0.05$.

3. Results

Our results show that sideropenic infected patients not supported with iron (group B) are characterized by a marked overexpression of *Chop/Gadd153* and *Hamp* genes, compared with not infected sideropenic patients (control group) at disease onset. Also the expression of *Arg1* gene is significantly increased in infected patients (Table 3), patients from the group B also show higher values of ferritin, M1 macrophages and CRP and lower values of TSAT, lymphocytes count and hemoglobin concentration at the time of the COVID-19 molecular diagnosis, if compared to the control group (Table 3). Moreover, in comparison to the control group, patients from group B experience a higher number of hospital admissions and increased transfusion needs. Sideropenic infected patients (groups A and B) express significantly higher levels of CRP than control group (tables 3 and 4) together with higher values of macrophages in M1 form. COVID-19 infection progression decreased absolute lymphocytes count and increased ferritin levels in sideropenic patients with COVID-19. The late phase of SARS-CoV-2 infection shows a significant reduction in the differences between group A and group B and control group (tables 4 and 6), except for the persistence of higher CRP levels and transfusion need in group B. Sideropenic infected patients supported with sucrosomial iron (group A) are characterized by a reduced expression of *Chop/Gadd153*, *Hamp* and *Arg1* genes if compared to the patients from group B, at disease onset. Of note, group A patients show lower ferritin, TSAT, M1 macrophages and CRP values and higher lymphocytes count, M2 macrophages and hemoglobin values if compared to group B patients at disease onset (Table 5). Three months after disease onset, we can still note a higher number of M1 macrophages and a higher monthly transfusion need in the group not receiving sucrosomial iron (Table 3), whereas the expression of *Chop/Gadd153, Hamp* and *Arg1* genes is the same in the two groups, (Table 4). Compared to controls, patients supported with sucrosomial iron showed a higher expression of genes *Chop/Gadd153, Hamp* and *Arg1* (Table 5), gene expression was however remarkably lower than in sideropenic infected patients not supported with iron. After 3 months the only difference was the lower transfusion need in patients supported with sucrosomial iron, although serum hepcidin remained higher. A different pattern of macrophage polarization in M1 and M2 forms in the analyzed groups was observed, with a higher expression of M2 forms in sideropenic patients treated with sucrosomal iron (Fig. 2).

4. Discussion

SARS-CoV-2 virus, as many other coronaviruses (CoVs), needs iron to replicate itself and it carries out several strategies to obtain the iron needed even when its host is sideropenic (Liu et al., 2020). The observed overexpression of *Arg1* gene in infected patients confirms its correlation with coronavirus infection (Derakhshani et al., 2021 Mar 4). *Chop/Gadd153* and *Hamp* genes, both overexpressed in our cohort of infected subjects may allow the virus to obtain the needed iron and they facilitate viral replication (Cao et al., 2019 Mar 20; Kared et al., 2021 Mar 1) and stimulate upregulation of hepcidin (Gáll et al., 2019 Jul 26; Zughaier et al., 2014). As already shown for other viruses, including Hepatitis C (Foka et al., 2016 Aug 17), also SARS-CoV-2 not only promotes hepcidin production by triggering inflammation, but it also exerts a hepcidin mimetic action through the spike protein (Cavezzi et al., 2020 May 28) and enhances hepcidin production by promoting viral persistence. This explains why, in the current pilot study, patients from group B show a higher number of hospital admissions with an increased transfusion need if compared to patients from the control group. The observed decrease in absolute lymphocyte count and increased ferritin levels in sideropenic patients with COVID-19 might be due to a hyperexpression of *Arg1* gene that inhibits immunity against intracellular pathogens, represses T-cell-mediated inflammatory damage (Zea et al., 2005 Apr 15; Tatum et al., 2020 Nov) and degrades arginine. This last molecule (arginine) is a substrate for nitric oxide (NO) production, which can induce antiviral activity against RNA viruses, such as SARS-CoV-2 (Caldwell et al., 2018 Apr 1; Tatum et al., 2020 Nov). Moreover, NO

Table 3

Gene	NM	Forward primer (5′-3′)	Reverse Primer (5′-3′)
Chop/Gadd153	NM_004083.6	GCCTTTCTCCTTCGGGACACTGTCCAGC	CTCGGCGAGTCGCCTCTACTTCCC
Hamp	NM_021175	ATGGCACTGAGCTCCCAGAT	TTCTACGTCTTGCAGCACATCC
Arg1	NM_001244438.2	GGGTTGACTGGAGAGC	CGTGGCTGTCCCTTTGAGAA
Gapdh	NM_002046	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

Table 4

	T0 GROUP B	CONTROL	P	3 MONTHS GROUP B	CONTROL	P
Chop/Gadd153	4	1	0,001	1,5	1	n.s.
Hamp	6 (*R*3-7)	1(R1-2)	0,001	2 (R1-2)	1 (R1-1)	0.05
Arg1	3 (R2-4)	1(R1-2)	0,03	1 (R1-1)	1 (R1-1)	n.s.
Serum Hepcidin mcg/l (Range)	360 (270–450)	10 (6–25)	0,001	290 (260–330)	8 (6–29)	0.001
Lymphocyte	1000/mcl (R850-1500/mcl)	2500/mcl (R1500-3000/mcl)	0,04	2000/mcl (R950-2500/mcl)	2500/mcl (R1500-3000/mcl)	n.s.
M2	0.2 %(R0,2–1.8)	0,5%(R0,4–1,2)	0,05	0.4 %(R0,3–1.5)	0,5%(R0,4–1,2)	n.s.
M1	1.2 %(R1-2,5)	0,4%(R0,3–1)	0,05	0,8%(R0,6–1,8)	0,4%(R0,3–1)	0,04
Ferritin	370 ng/ml (R100-450)	150 ng/ml (R20-200)	0,05	270 ng/ml (R100-300)	150 ng/ml (R20-200)	0.05
Tsat	18 % (R10-28 %)	35 % (R25-40 %)	0,04	28 % (R18-35 %)	35 % (R25-40 %)	n.s.
Hb	8.5 g/dl (R8-9)	10.3 g/dl (R10-11.4)	0,05	12 g/dl (R11-13)	13 g/dl (R12-14)	n.s.
Hosp	3pz/20	0/20	<0,001	0/20	0/20	n.s.
Los	15 day(R7-28)	0	<0,001	0	0	n.s.
Neg	day21(R7-30)	0	<0,001	day 88 (1 pat)	0	<0,001
Transf/Month	3 (R0-4)	2 (R0-2)	0,05	3 (R0-4)	2 (R0-2)	0,05
Crp (Mg/L)	65 (45–95)	10 (5–35)	0.03	22 (15–26)	8 (4–35)	0.05

Table 5

	T0 GROUP A	GROUP B	P	3 MONTHS GROUP A	GROUP B	P
Chop/Gadd153	2	4	0,03	1	1,5	n.s.
Hamp	3(R1-4)	6 (*R*3-7)	0,01	1 (R1-1)	2 (R1-2)	n.s.
Arg1	2(R1-3)	3 (R2-4)	0,05	1 (R1-1)	1 (R1-1)	n.s.
Serum Hepcidin mcg/l (Range)	150 (140–220)	360 (270–450)	0,001	120 (110–150)	290 (260–330)	0.001
Lymphocyte	1800/mcl (R1200-2800/mcl)	1000/mcl (R850-1500/mcl)	0,04	2800/mcl (R18500-3500/mcl)	2000/mcl (R950-2500/mcl)	n.s.
M2	0,6%(R0,4–0,8)	0.2 %(R0,2–1.8)	0,02	0,5%(R0,4–1,2)	0.4 %(R0,3–1.5)	n.s.
M1	0,4%(R0,3–1)	1.2 %(R1-2,5)	0,02	0,4%(R0,3–1)	0,8%(R0,6–1,8)	0,05
Ferritin	120 ng/ml (R20-200)	370 ng/ml (R100-450)	0,04	150 ng/ml (R20-250)	200 ng/ml (R100-300)	n.s.
Tsat	30 % (R25-40 %)	15 % (R10-28 %)	0,03	35 % (R25-40 %)	28 % (R18-35 %)	n.s.
Hb	12 g/dl (R11-13)	10 g/dl (R8-11)	0,03	13 g/dl (R12-14)	12 g/dl (R11-13)	n.s.
Hosp	0/20	3pz/20	<0,001	0/20	0/20	n.s.
Los	0	15 day(R7-28)	<0,001	0	0	n.s.
Neg	day14(R7-18)	day21(R7-30)	0,04	0	day 88 (1 pat)	<0,001
Transf/Month	0 (R0-0)	3 (R0-2)	<0,001	0 (R0-0)	3 (R0-2)	<0,001
Crp (Mg/L)	30 (20–37)	65 (45–95)	0.04	20 (18–25)	22 (15–26)	n.s.

Table 6

	T0 GROUP A	CONTROL	P	3 MONTHS GROUP A	CONTROL	P
Chop/Gadd153	2	1	0,02	1	1	n.s.
Hamp	3(R1-4)	1(R1-2)	0,01	1 (R1-1)	1 (R1-1)	n.s.
Arg1	2(R1-3)	1(R1-2)	0,03	1 (R1-1)	1 (R1-1)	n.s.
Serum Hepcidin mcg/l (Range)	150 (140–220)	10 (6–25)	0,001	120 (110–150)	8 (6–29)	0.001
Lymphocyte	1800/mcl (R1200-2800/mcl)	2500/mcl (R1500-3000/mcl)	n.s.	2800/mcl (R18500-3500/mcl)	2500/mcl (R1500-3000/mcl)	n.s.
M2	0,6%(R0,4–0,8)	0,5%(R0,4–1,2)	n.s.	0,5%(R0,4–1,2)	0,5%(R0,4–1,7)	n.s.
M1	0,4%(R0,3–1)	0,4%(R0,3–1)	n.s.	0,4%(R0,2–1,1)	0,4%(R0,3–1)	n.s.
Ferritin	120 ng/ml (R20-200)	150 ng/ml (R20-200)	n.s.	150 ng/ml (R20-250)	150 ng/ml (R20-200)	n.s.
Tsat	30 % (R25-40 %)	35 % (R25-40 %)	n.s.	35 % (R25-40 %)	35 % (R25-40 %)	n.s.
Hb	12 g/dl (R11-13)	13 g/dl (R12-14)	n.s.	13 g/dl (R12-14)	13 g/dl (R12-14)	n.s.
Hosp	0/20	0/20	n.s.	0/20	0/20	n.s.
Los	0	0	n.s.	0	0	n.s.
Neg	day14(R7-18)	0	<0,001	0	0	n.s.
Transf/Month	0 (R0-0)	2 (R0-2)	<0,001	0 (R0-0)	2 (R0-2)	<0,001
Crp (Mg/L)	30 (20–37)	10 (5–35)	0.04	20 (18–25)	8 (4–35)	0.03

Abbreviations: *Chop/Gadd153*: *C/EBP homologous protein* gene/growth arrest and DNA damage-inducible gene 153. *Hamp*: Hepcidin Antimicrobial Peptide gene. *Arg1*: arginase 1 gene. CRP: C reactive protein. HB: hemoglobin concentration. HOSP: number of hospitalized patients. LOS: length of stay in hospital (days). M1 and M2: macrophages. NEG: day of first negativity at molecular test for covid19 from the first day of symptoms onset. T0: time of the onset of COVID19 infection. TSAT: transferrin saturation. TRANSF/MONTH: number of hemotransfusions per month.

affects cellular iron homeostasis by the up-regulation of the expression of ferroportin-1 (*Fpn1*), the major cellular iron exporter in human cells (Nairz et al., 2013 May 6). COVID-19 infection determines *Arg-1* overexpression, NO and *Fpn1* expression reduction determining an increased iron content that can be used by SARS-CoV-2 virus for its own replication (Liu et al., 2020). C- reactive protein is involved in polarization of macrophages in M1 form, this supports the observed overexpression of PCR and M1 macrophages in infected patients (Devaraj and Jialal, 2011 Jun). Moreover, it seems that classically activated M1 alveolar lung macrophages (ALM) facilitate COVID-19 spread and alternatively activated M2 ALM limit its spread with a higher endosomal pH but a lower lysosomal pH, thus delivering the virus to lysosomes for degradation (Lv

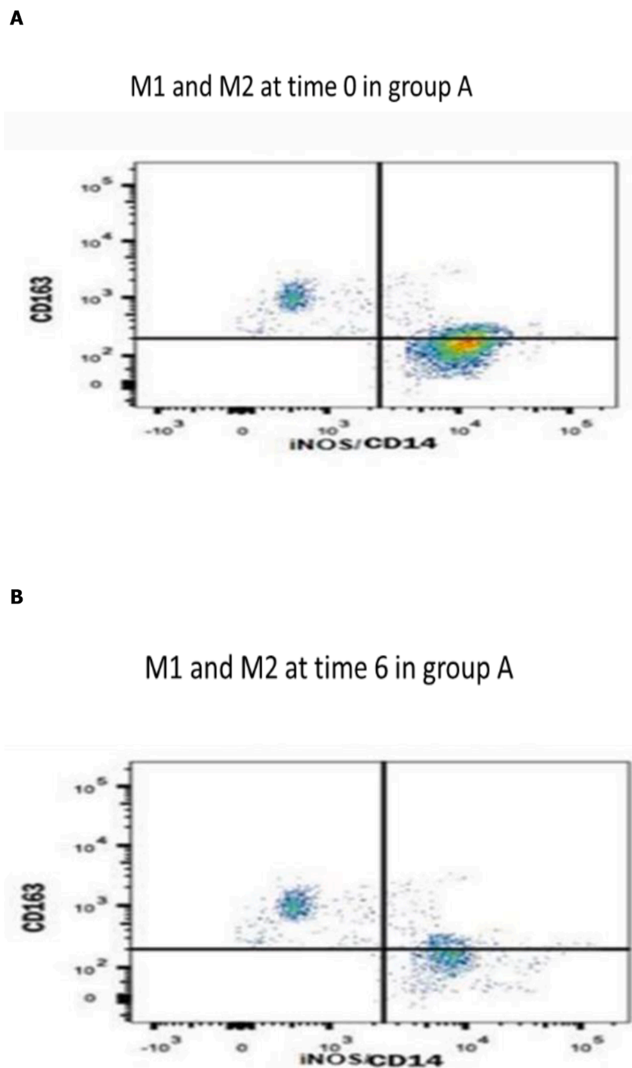

Fig. 2. Figure 2f: Representative dot plots and quadrant gating showing subset frequencies of M1- and M2-polarized cells using CD163 and CD14 markers. (A) the clouds in the right lower part and in the left upper part of the cytogram represent the number of M1 and M2 macrophages respectively in COVID-19 patient without sucrosomial iron support. (B) the clouds in the right lower part and in the left upper part of the cytogram represent the number of M1 and M2 macrophages respectively in COVID-19 patients receiving sucrosomial iron support.

et al., 2021 Apr 13). Our data suggest that the acute and late phases of COVID-19 infection may be biologically very different in iron deficient individuals not supported with iron. Not infected sideropenic patients from the current study have experienced a lower number of hospital admissions with a lower monthly transfusion need, a shorter length of hospital stays and a shorter interval of the first negativity at molecular test for COVID-19 from the first day of symptoms onset. Probably this is due to the iron deficiency in anemic premenopausal woman leading to a reduction of absolute counts of peripheral blood T lymphocytes with an impaired cell-mediated immunity and a secondary higher predisposition to infection (Reza Keramati et al., 2011 Jan). Moreover, iron chelation might induce increased *Chop/Gadd153* gene expression with apoptosis in Jurkat T-lymphocytes (Pan et al., 2004 Apr 1). It might be possible that iron support might have the opposite effect on *Chop/Gadd153* gene expression, with mitigation of *Hamp* gene expression (Gáll et al., 2019 Jul 26) and increase of lymphocytes count, with a better antiviral immunity (Cao et al., 2019 Mar 20). Increased cell iron loading polarizes resting macrophages to M2-like phenotype and dampens pro-inflammatory immune responses, while iron deficiency exerts the opposite effect (Agoro et al., 2018 May 17). Sucrosomial iron is a macro-assembly in which a sucrester shell contains a liposome rich in phosphatidylcholine and phosphatidylethanolamine, containing pyrophosphate iron. It is able to bypass usual systems of iron absorption as ferroportin or duodenal iron transporter and is absorbed also during hepcidin production (Gómez-Ramírez et al., 2018 Oct 4). Phosphatidylcholine and phosphatidylethanolamine of sucrosomial iron probably are able to polarize macrophages from M1 proinflammatory form to M2 anti-inflammatory, anti-COVID-19 form (Gómez-Ramírez et al., 2018 Oct 4; Cruz-Leal et al., 2014 Jun; Trial et al., 2016 Jun 20; Tian et al., 2020 Jul 1) as supported by our results.

5. Conclusions

COVID-19 genetically controls iron metabolism in infected patients, nevertheless our knowledge on the mechanisms of SARS-CoV2 in iron metabolism regulation is quite poor in mild COVID-19 patients with iron deficiency. In subjects with iron deficiency, COVID-19 modulates genes expression to have the maximum quantity of iron to replicate itself.

Until now the dogma in the management of infections was to take advantage of iron chelation and to avoid iron supplementation, useful in COVID-19 replication (Liu et al., 2020; Perricone et al., 2020 Aug; Edeas et al., 2020 Aug).

In our opinion, this dogma should be revised because iron (mainly new oral formulation of sucrosomial iron) helps the immune system of iron deficient patients to fight against COVID-19 infection and to modulate the expression of some genes involved in SARS-CoV-2 replication (Derakhshani et al., 2021 Mar 4; Agoro et al., 2018 May 17; Pan et al., 2004 Apr 1; Bowlus, 2003 Mar). For these reasons, iron support with sucrosomial iron might be useful in improving the immune response against SARS-CoV-2 and to interfere with the genetic control of the virus in iron metabolism in the initial phase of the disease in iron deficient patients affected by mild COVID-19, according to the results of the pilot study, we thus recommend sucrosomial iron supplementation to iron deficient patients, even with active SSARS-CoV-2 infection.

CRediT authorship contribution statement

Giulio Giordano: Conceptualization, Investigation, Project administration, Writing – original draft. **Maria Teresa Bochicchio:** Methodology, Data curation, Writing – original draft. **Giovanna Niro:** Resources, Data curation, Formal analysis. **Alessandro Lucchesi:** Investigation, Writing – review & editing, Visualization. **Mariasanta Napolitano:** Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Agoro, R., Taleb, M., Quesniaux, V.F.J., Mura, C., 2018 May 17. Cell iron status influences macrophage polarization. PLoS One. 13 (5), e0196921.

Al-Kuraishy, H.M., Al-Gareeb, A.I., Kaushik, A., Kujawska, M., Batiha, G.E., 2022 Sep. Hemolytic anemia in COVID-19. Ann Hematol. 101 (9), 1887–1895. https://doi.org/10.1007/s00277-022-04907-7.].

Al-Kuraishy HM, Al-Gareeb AI, Onohuean H, El-Saber Batiha G. COVID-19 and erythrocrine function: The roller coaster and danger. Int. J. Immunopathol. Pharmacol. 2022 Jan-Dec;36:3946320221103151. 10.1177/03946320221103151.

Al-Kuraishy HM, Al-Gareeb AI, Alkazmi L, Habotta OA, Batiha GE. High-mobility group box 1 (HMGB1) in COVID-19: extrapolation of dangerous liaisons. Inflammopharmacology. 2022 Jun;30(3):811-8 20. 10.1007/s10787-022-00988-y.

Batiha, G.E., Shaheen, H.M., Al-Kuraishy, H.M., Teibo, J.O., Akinfe, O.A., Al-Garbee, A.I., et al., 2021 Dec. Possible mechanistic insights into iron homeostasis role of the action of 4-aminoquinolines (chloroquine/hydroxychloroquine) on COVID-19 (SARS-CoV-2) infection. Eur. Rev. Med. Pharmacol. Sci. 25 (23), 7565–7584. https://doi.org/10.26355/eurrev.

Batiha, G.E., Al-Gareeb, A.I., Qusti, S., Alshammari, E.M., Kaushik, D., Verma, R., et al., 2022 Jun. Deciphering the immunoboosting potential of macro and micronutrients in COVID support therapy. Environ. Sci. Pollut. Res. Int. 29 (29), 43516–43531. https://doi.org/10.1007/s11356-022-20075-7.

Bowlus, C.L., 2003 Mar. The role of iron in T cell development and autoimmunity. Autoimmun. Rev. 2 (2), 73–78. https://doi.org/10.1016/s1568-9972(02)00143-x.

Caldwell, R.W., Rodriguez, P.C., Toque, H.A., Narayanan, S.P., Caldwell, R.B., 2018 Apr 1. Arginase: A Multifaceted Enzyme Important in Health and Disease. Physiol. Rev. 98 (2), 641–665. https://doi.org/10.1152/physrev.00037.2016.

Cao, Y., Trillo-Tinoco, J., Sierra, R.A., Anadon, C., Dai, W., Mohamed, E., et al., 2019 Mar 20. ER stress-induced mediator C/EBP homologous protein thwarts effector T cell activity in tumors through T-bet repression. Nat. Commun. 10 (1), 1280. https://doi.org/10.1038/s41467-019-09263-1. Erratum. In: Nat Commun. 2019 Aug 15;10(1):3680.

Cavezzi, A., Troiani, E., Corrao, S., 2020 May 28. COVID-19: hemoglobin, iron, and hypoxia beyond inflammation. A narrative review. Clin. Pract. 10 (2), 1271. https://doi.org/10.4081/cp.2020.1271.

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3): 2000045. 10.2807/1560-7917.ES.2020.25.3.2000045. Erratum in: Euro Surveill. 2020 Apr;25(14): Erratum in: Euro Surveill. 2020 Jul;25(30): Erratum in: Euro Surveill. 2021 Feb;26(5).

Cruz-Leal, Y., Lucatelli Laurindo, M.F., Osugui, L., Luzardo Mdel, C., López-Requena, A., et al., 2014 Jun. Liposomes of phosphatidylcholine and cholesterol induce an M2-like macrophage phenotype reprogrammable to M1 pattern with the involvement of B-1 cells. Immunobiology. 219 (6), 403–415. https://doi.org/10.1016/j.imbio.2014.01.006.

Derakhshani, A., Hemmat, N., Asadzadeh, Z., Ghaseminia, M., Shadbad, M.A., Jadideslam, G., et al., 2021 Mar 4. Arginase 1 (Arg1) as an Up-Regulated Gene in COVID-19 Patients: A Promising Marker in COVID-19 Immunopathy. J Clin Med. 10 (5), 1051. https://doi.org/10.3390/jcm10051051.

Devaraj, S., Jialal, I., 2011 Jun. C-reactive protein polarizes human macrophages to an M1 phenotype and inhibits transformation to the M2 phenotype. Arterioscler Thromb Vasc Biol. 31 (6), 1397–1402. https://doi.org/10.1161/ATVBAHA.111.225508.

Edeas, M., Saleh, J., Peyssonnaux, C., 2020 Aug. Iron: Innocent bystander or vicious culprit in COVID-19 pathogenesis? Int J Infect Dis. 97, 303–305. https://doi.org/10.1016/j.ijid.2020.05.110.

Ehsani, S., 2020 Oct 16. COVID-19 and iron dysregulation: distant sequence similarity between hepcidin and the novel coronavirus spike glycoprotein. Biol Direct. 15 (1), 19. https://doi.org/10.1186/s13062-020-00275-2.

Faghih Dinevari, M., Somi, M.H., Sadeghi Majd, E., Abbasalizad Farhangi, M., Nikniaz, Z., 2021 Feb 10. Anemia predicts poor outcomes of COVID-19 in hospitalized patients: a prospective study in Iran. BMC Infect Dis. 21 (1), 170. https://doi.org/10.1186/s12879-021-05868-4.

Foka, P., Dimitriadis, A., Karamichali, E., Kyratzopoulou, E., Giannimaras, D., Koskinas, J., et al., 2016 Aug 17. Alterations in the iron homeostasis network: A driving force for macrophage-mediated hepatitis C virus persistency. Virulence. 7 (6), 679–690. https://doi.org/10.1080/21505594.2016.1175700.

Gáll, T., Balla, G., Balla, J., 2019 Jul 26. Heme, Heme Oxygenase, and Endoplasmic Reticulum Stress-A New Insight into the Pathophysiology of Vascular Diseases. Int J Mol Sci. 20 (15), 3675. https://doi.org/10.3390/ijms20153675.

Gómez-Ramírez, S., Brilli, E., Tarantino, G., Muñoz, M., 2018 Oct 4. Sucrosomial® Iron: A New Generation Iron for Improving Oral Supplementation. Pharmaceuticals (Basel). 11 (4), 97. https://doi.org/10.3390/ph11040097.

Grebenchtchikov, N., Geurts-Moespot, A.J., Kroot, J.J., den,, 2009 Aug. Heijer M, Tjalsma H, Swinkels DW, et al High-sensitive radioimmunoassay for human serum hepcidin. Br J Haematol. 146 (3), 317–325. https://doi.org/10.1111/j.1365-2141.2009.07758.x.

Hulley, S. B., Cummings, S. R., Browner, W. S., Grady, D. G., & Newman, T. B. (2013). Designing Clinical Research: An Epidemiologic Approach (2nd ed.). Philadelphia: Lippincott, Williams & Wilkins.Appendix 6C, page 79.

Kared, H., Redd, A.D., Bloch, E.M., Bonny, T.S., Sumatoh, H., Kairi, F., et al., 2021 Mar 1. SARS-CoV-2-specific CD8+ T cell responses in convalescent COVID-19 individuals. J Clin Invest. 131 (5), e145476.

Liao, Y., Fung, T.S., Huang, M., Fang, S.G., Zhong, Y., Liu, D.X., 2013. Upregulation of CHOP/GADD153 during Coronavirus Infectious Bronchitis Virus Infection Modulates Apoptosis by Restricting Activation of the Extracellular Signal-Regulated Kinase Pathway. J. Virol. https://doi.org/10.1128/jvi.00626-13.

Liu, W., Zhang, S., Nekhai, S., Liu, S., 2020. Depriving Iron Supply to the Virus Represents a Promising Adjuvant Therapeutic Against Viral Survival. Curr Clin Microbiol Rep. 7 (2), 13–19. https://doi.org/10.1007/s40588-020-00140-w.

Lv, J., Wang, Z., Qu, Y., Zhu, H., Zhu, Q., Tong, W., et al., 2021 Apr 13. Distinct uptake, amplification, and release of SARS-CoV-2 by M1 and M2 alveolar macrophages. Cell Discov. 7 (1), 24. https://doi.org/10.1038/s41421-021-00258-1.

Mahat RK, Panda S, Rathore V, Swain S, Yadav L, Sah SP. The dynamics of inflammatory markers in coronavirus disease-2019 (COVID-19) patients: A systematic review and meta-analysis. Clin Epidemiol Glob Health. 2021 Jul-Sep;11:100727. 10.1016/j.cegh.2021.100727.

Mancini, F., Barbanti, F., Scaturro, M., Errico, G., Iacobino, A., Bella, A., 2020 Dec. on behalf ISS COVID-19 study group. Laboratory management for SARS-CoV-2 detection: a user-friendly combination of the heat treatment approach and rt-Real-time PCR testing. Emerg. Microbes Infect. 9 (1), 1393–1396. https://doi.org/10.1080/22221751.2020.1775500.

Mily A, Kalsum S, Loreti MG, Rekha RS, Muvva JR, Lourda M, et al.Polarization of M1 and M2 Human Monocyte-Derived Cells and Analysis with Flow Cytometry upon Mycobacterium tuberculosis Infection. J Vis Exp. 2020 Sep 18;(163). 10.3791/61807. Erratum in: J Vis Exp. 2020 Oct 14;(164).

Nai, A., Lidonnici, M.R., Federico, G., Pettinato, M., Olivari, V., Carrillo, F., et al., 2021 Mar 1. NCOA4-mediated ferritinophagy in macrophages is crucial to sustain erythropoiesis in mice. Haematologica. 106 (3), 795–805. https://doi.org/10.3324/haematol.2019.241232.

Nairz, M., Schleicher, U., Schroll, A., Sonnweber, T., Theurl, I., Ludwiczek, S., et al., 2013 May 6. Nitric oxide-mediated regulation of ferroportin-1 controls macrophage iron homeostasis and immune function in Salmonella infection. J Exp Med. 210 (5), 855–873. https://doi.org/10.1084/jem.20121946.

Pan, Y.J., Hopkins, R.G., Loo, G., 2004 Apr 1. Increased GADD153 gene expression during iron chelation-induced apoptosis in Jurkat T-lymphocytes. Biochim Biophys Acta. 1691 (1), 41–50. https://doi.org/10.1016/j.bbamcr.2003.12.003.

Perricone, C., Bartoloni, E., Bursi, R., Cafaro, G., Guidelli, G.M., Shoenfeld, Y., Gerli, R., 2020 Aug. COVID-19 as part of the hyperferritinemic syndromes: the role of iron depletion therapy. Immunol Res. 68 (4), 213–224. https://doi.org/10.1007/s12026-020-09145-5.

PPesce JT, Ramalingam TR, Mentink-Kane MM, Wilson MS, El Kasmi KC, Smith AM, Tet al. Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. PLoS Pathog. 2009 Apr;5(4):e1000371. 10.1371/journal.ppat.1000371.

Reza Keramati, M., Sadeghian, M.H., Ayatollahi, H., Mahmoudi, M., Khajedaluea, M., Tavasolian, H., et al., 2011 Jan. Peripheral Blood Lymphocyte Subset Counts in Pre-menopausal Women with Iron-Deficiency Anaemia. Malays. J. Med. Sci. 18 (1), 38–44.

Scialo, F., Daniele, A., Amato, F., Pastore, L., Matera, M.G., Cazzola, M., et al., 2020 Dec. ACE2: The Major Cell Entry Receptor for SARS-CoV-2. Lung. 198 (6), 867–877. https://doi.org/10.1007/s00408-020-00408-4.

Siri M, Dastghaib S, Zamani M, Rahmani-Kukia N, Geraylow KR, Fakher S,et al Autophagy, Unfolded Protein Response, and Neuropilin-1 Cross-Talk in SARS-CoV-2 Infection: What Can Be Learned from Other Coronaviruses. Int J Mol Sci. 2021 Jun 1; 22(11):5992. 10.3390/ijms22115992.

Sonnweber T, Boehm A, Sahanic S, Pizzini A, Aichner M, Sonnweber B, et al Persisting alterations of iron homeostasis in COVID-19 are associated with non-resolving lung pathologies and poor patients' performance: a prospective observational cohort study. Respir Res. 2020 Oct 21;21(1):276. 10.1186/s12931-020-01546-2.

Tang, Y., Liu, J., Zhang, D., Xu, Z., Ji, J., Wen, C., 2020 Jul. Cytokine Storm in COVID-19: The Current Evidence and Treatment Strategies. Front Immunol. 10 (11), 1708. https://doi.org/10.3389/fimmu.2020.01708.

Tatum, D., Taghavi, S., Houghton, A., Stover, J., Toraih, E., Duchesne, J., 2020 Nov. Neutrophil-to-Lymphocyte Ratio and Outcomes in Louisiana COVID-19 Patients. Shock. 54 (5), 652–658. https://doi.org/10.1097/SHK.0000000000001585.

Tian, Y., Liu, Y., Xue, C., Wang, J., Wang, Y., Xu, J., et al., 2020 Jul 1. The exogenous natural phospholipids, EPA-PC and EPA-PE, contribute to ameliorate inflammation and promote macrophage polarization. Food Funct. 11 (7), 6542–6551. https://doi.org/10.1039/d0fo00804d.

Trial, J., Cieslik, K.A., Entman, M.L., 2016 Jun 20. Phosphocholine-containing ligands direct CRP induction of M2 macrophage polarization independent of T cell polarization: Implication for chronic inflammatory states. Immun Inflamm Dis. 4 (3), 274–288. https://doi.org/10.1002/iid3.112.

Zea, A.H., Rodriguez, P.C., Atkins, M.B., Hernandez, C., Signoretti, S., Zabaleta, J., et al., 2005 Apr 15. Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion. Cancer Res. 65 (8), 3044–3048. https://doi.org/10.1158/0008-5472.CAN-04-4505.

Zhang V, Ganz T, Nemeth E, Kim A. Iron overload causes a mild and transient increase in acute lung injury. Physiol Rep. 2020 Jun;8(12):e14470. 10.14814/phy2.14470.

Zughaier, S.M., Stauffer, B.B., McCarty, N.A., 2014. Inflammation and ER stress downregulate BDH2 expression and dysregulate intracellular iron in macrophages. J. Immunol. Res. 2014, 140728 https://doi.org/10.1155/2014/140728.