Received: 20 June 2022 | Revised: 19 July 2022 | Accepted: 27 July 2022

DOI: 10.1002/cbf.3735

RESEARCH ARTICLE

CELL BIOCHEMISTRY & FUNCTION WILEY

Systemic redox imbalance in severe COVID-19 patients

Isabelle A. L. Postiga | Paula C. Teixeira | Carla Andretta Moreira Neves | Paulo Santana Filho | Bruna Marmett | Roseana Carvalho | Alessandra Peres | Liane Rotta | Claudia Elizabeth Thompson | Gilson P. Dorneles | Pedro R. T. Romão

Graduate Program in Health Sciences, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil

Correspondence
Pedro R. T. Romão, Universidade Federal de Ciências da Saúde de Porto Alegre, Rua Sarmento Leite, 245, Porto Alegre 90050-170, Brazil.
Email: pedror@ufcspa.edu.br

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: Finance Code 001; Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul

Abstract

The aim of this study was to evaluate the systemic redox state and inflammatory markers in intensive care unit (ICU) or non-ICU severe COVID-19 patients during the hospitalization period. Blood samples were collected at hospital admission (T1) (Controls and COVID-19 patients), 5–7 days after admission (T2: 5–7 days after hospital admission), and at the discharge time from the hospital (T3: 0–72 h before leaving hospital or death) to analyze systemic oxidative stress markers and inflammatory variables. The reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP) were analyzed in peripheral granulocytes and monocytes. THP-1 human monocytic cell line was incubated with plasma from non-ICU and ICU COVID-19 patients and cell viability and apoptosis rate were analyzed. Higher total antioxidant capacity, protein oxidation, lipid peroxidation, and IL-6 at hospital admission were identified in both non-ICU and ICU COVID-19 patients. ICU COVID-19 patients presented increased C-reactive protein, ROS levels, and protein oxidation over hospitalization period compared to non-ICU patients, despite increased antioxidant status. Granulocytes and monocytes of non-ICU and ICU COVID-19 patients presented lower MMP and higher ROS production compared to the healthy controls, with the highest values found in ICU COVID-19 group. Finally, the incubation of THP-1 cells with plasma acquired from ICU COVID-19 patients at T3 hospitalization period decreased cell viability and apoptosis rate. In conclusion, disturbance in redox state is a hallmark of severe COVID-19 and is associated with cell damage and death.

KEYWORDS
apoptosis, inflammation, oxidative stress, reactive oxygen species, SARS-CoV-2

1 | INTRODUCTION

In the beginning of 2020, the severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2) rapidly spread worldwide and became a pandemic affecting the population.[1] Individuals infected might manifest the disease in different degrees, including asymptomatic cases or report mild to severe symptoms, and around of 20% are considered severe cases that require hospitalization or intensive care.[2,3] The most common symptoms are like other viral infections that commit the respiratory system, as flu-like syndrome, fever,

Isabelle A. L. Postiga and Paula C. Teixeira are considered the first authors of this study.

cough, fatigue, coryza, sore throat. However, most severe cases are associated with complications in pulmonary, cardiovascular, and neurological systems that lead to the necessity to intensive care and increased mortality. In this line, a disturb in both cellular and systemic host metabolism could contribute to viral replication and expression of pro-inflammatory cytokines.[4,5]

The host response to the infection driven the severity of the diseases, being observed that more severe cases are associated with increased immune response of the host that cause activation of multisystemic inflammatory responses and respiratory dysfunction.[6,7] The SARS-CoV-2 pathogenesis had as pivot mechanism the redox system and the pro-inflammatory cytokines release and is related to hypoxia and oxidative stress.[8] The immune innate response begins when the virus enters into the airways and it activates macrophages and dendritic cells that release inflammatory cytokines and produce reactive oxygen species (ROS). The viral replication and the multisystemic pro-inflammatory environment contributes to the constant production of ROS, due to respiratory burst activity of immune cells (macrophages and neutrophils mainly), alterations in the activity of enzymes as NADPH oxidase, xanthine oxidase, and respiratory chain in the mitochondria.[9,10] In this scenario, the virus acts diminishing the antioxidant defense through the transcriptor factor nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) inhibition, which is responsible for the increase of antioxidant enzymes activity. Consequently, the increased production of ROS and the inhibition of antioxidant defense culminates in impaired redox balance, affecting membrane lipids and cytoplasmic proteins.[11] Moreover, the effects of ROS mechanism on pulmonary cells might have an important role in hypoxic respiratory failure diagnosed in severe cases of SARS-CoV-2 infection.[6]

Therefore, oxidative stress is directly related to the severity of the infection caused by SARS-CoV-2 and the follow-up of alterations in redox status biomarkers (with clinical relevance) could be used to identify high-risk patients and to contribute to the identification of possible targets for therapeutic approaches. The aim of this study was to evaluate the systemic markers of oxidative stress and IL-6 in patients infected with SARS-CoV-2 with different severity degrees during the hospitalization period.

Significance statement

COVID-19 induces a strong inflammatory response that disturbs immunological response resulting in multiorgan impairment and systemic damage on several tissues. This study provided information regarding the redox state in SARS-CoV-2 severely infected patients over hospitalization. In summary, the progression of the hospitalization increased protein oxidation and reactive oxygen species (ROS) generation in intensive care unit (ICU) COVID-19 patients. Furthermore, we showed that peripheral leukocytes produce a higher amount of ROS during severe SARS-CoV-2. Finally, the alterations in plasma obtained from ICU COVID-19 at the end of the hospitalization resulted in lower cell viability and higher apoptosis rate. These findings suggest an interesting dynamic in the biomarkers with a potential use in clinical management of the disease.

2 | METHODS

2.1 | Study population

This is a clinical cohort study that enrolled 30 hospitalized patients with positive diagnosis for SARS-CoV-2 reverse transcription–polymerase chain reaction (RT-PCR) test. Hospitalized patients were recruited after admission to the COVID-19 Unit of Hospital São Camilo (Esteio/RS, Brazil) between June and December of 2020. The present study was approved by the Ethics Committee of UFCSPA (CAAE: 38886220.0.0000). We use the diagnoses criteria for COVID-19 from World Health Organization interim guidance and Diagnosis and Treatment Guideline for Novel Coronavirus Pneumonia, being considered positive to SARS-CoV-2 infection patients with positive result for at least two nucleic acid tests for SARS-CoV-2. The patient's electronic medical records were used to collect clinical and sociodemographic data upon admission to the unit. Additionally, we included as control a group of 12 individuals with age and sex-matched admitted to hospital with pneumonia that tested negative to SARS-CoV-2 RT-PCR. All patients or those legally responsible for the patients received the explanation of the research objectives and procedures and informed consent to participate in the study. All authors sign an agreement to preserve patients and staff anonymity regarding the use of the present data.

2.2 | Blood collection

Blood samples of controls (n = 12) and COVID-19 patients (n = 29; 18 non-intensive care unit [ICU] and 11 ICU COVID-19) were collected right after hospital admission (T1) from the antecubital vein into 4 ml tubes with EDTA as anticoagulant. Further samples of blood were collected from non-ICU COVID-19 and ICU COVID-19 patients during the hospitalization, 5–7 days after admission (T2: 5–7 days after hospital admission), and at the discharge time from the hospital (T3: 0–72 h before leaving hospital or death). Blood was centrifuged (2000g, 10 min) to obtained plasma samples, which was aliquoted and immediately kept at −80°C until analysis.

2.3 | Thiol concentration

The thiol concentration measure was based in the interaction between thiol group and 5-5′-dithiobis 2-nitrobenzoic acid (DTNB). The analytical method was previously described by Ellman.[12] Briefly, we used 50 µl of sample, 25 µl of phosphate saline solution

(10 mmol/L, pH 7.4), and 25 µl of DTNB in a plate of 96 wells. The assay was incubated for 30 min, protected from light, and then read on a microplate reader (Spectra Max 250; Molecular Devices) at 412 nm. The results were expressed in µmol/ml.

2.4 | Nonenzymatic antioxidant status

The nonenzymatic antioxidant status in plasma was assessed through the reduction of the 3-[4,5-dimethylthiazol-2-yl]−2,5-diphenylte trazolium bromide (MTT) by molecules with antioxidant action, according Medina et al.[13] In a test tube, 100 µl of sample and 20 µl of MTT were added with 380 µl of phosphate-saline solution (10 mmol/L, pH 7.4). This solution was incubated in a water bath for 60 min at 37°C, protected from light. Then, 1 ml of a solution of isopropyl alcohol with hydrochloric acid (0.4 N) was added and centrifuged at 3500 rpm for 10 min at room temperature. The reaction supernatant was transferred to a 96-well plate and analyzed in the microplate reader (Spectra Max 250; Molecular Devices) at 570nm. The evaluation of the antioxidant status was expressed according to the absorbance obtained.

2.5 | Total antioxidant capacity (TAC)

The TAC was evaluated based on the oxidation of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) according to the method reported by Miller et al.[14] In this assay, a solution of ABTS (7 mM) with potassium persulfate (2.4 mM) was prepared, kept protected from light, and later diluted in methanol. This final solution was reacted with 10 µl of sample of plasma in a 96-well plate. The calibration curve was made using Trolox reagent. The antioxidant capacity was determined by reading on a microplate reader (Spectra Max 250; Molecular Devices) at 740 nm. Data were expressed in µmol/L.

2.6 | Nitrite levels

The nitrite levels in plasma was determined by the Griess method.[15] Briefly, a Griess solution was prepared with 0.2% sulfanilic acid, 0.2% *N*-(1-naphthyl) ethylenediamine in 5% phosphoric acid. First, we performed the deproteinization of the sample by adding 200 µl of sample with 20 µl of zinc chloride (1 M), followed by centrifugation at 3500 rpm for 10 min. Then, 50 µl of sample was pipetted, which was reacted with the Griess solution, followed by the addition of 50 µl of vanadium (150 µM). Finally, this solution was incubated for 60 min at 37°C. The standard curve was performed using sodium nitrate ($NaNO_2$). The analysis was conducted in a 96-well plate using a microplate reader (Spectra Max 250; Molecular Devices) at 540 nm. Data were expressed in µmol/L.

2.7 | ROS

The ROS content was evaluated in the plasma through the fluorescence intensity of the redox-sensitive dye 2′,7′-dichlorodihydrofluorescein diacetate (DCFH, 100 µM; Sigma-Aldrich). DCFH is permeable through the cell membrane and it undergoes the process of deacetylation by intracellular esterases enzymes, forming the intermediate compound 2′,7′-dichlorodihydrofluorescein in cytoplasm, in which reacts with ROS forming oxidized 2′,7′ dichlorofluorescein (DCF), a fluorescent molecule. The analysis was performed with excitation and emission wavelengths of 480 and 535 nm, respectively, using SpectraMax M2e (Molecular Devices).

2.8 | Protein oxidation

The evaluation of protein and amino acid oxidation was performed using the method proposed by Witko-Sarsat et al.[16] This method determines the levels of advanced oxidation protein products (AOPP) in plasma. Briefly, 50 µl of plasma was used, which was diluted in 450 µl of phosphate-saline solution (10 mmol/L, pH 7.4). In the final solution was added 50 µl of potassium iodide (1.16 ml/L) followed by 100 µl of citric acid (0.2 mol/L). The control was done with a Bovine serum albumin solution. The absorbance was measured in a microplate reader (Spectra Max 250; Molecular Devices) with an absorbance of 340 nm. The result was calculated by a standard curve with chloramine equivalents and was expressed in µmol/L.

2.9 | Lipid peroxidation

Lipid peroxidation was evaluated by the thiobarbituric acid reactive substances (TBARS) method.[17] This test measures the concentrations of malondialdehyde (MDA), products formed from the oxidation of lipids, through their reactivity with thiobarbituric acid. For this assay, 200 µl of plasma reacted with 250 µl of acetic acid (2.5 M, pH 3.4), 50 µl of sodium dodecyl sulfate (8%), and 250 µl of thiobarbituric acid (0.8%). This mixture was vortexed and then incubated in a water bath for 60 min at 100°C. Afterwards, the samples were centrifuged at 3500 rpm for 10 min at 4°C. The supernatant was transferred to a 96-well plate and analyzed in the microplate reader (Spectra Max 250; Molecular Devices) at 532nm. The result was expressed in nmol/ml.

2.10 | C-reactive protein and IL-6 analyzes

C-reactive protein (CRP) levels were determined using automated equipment that uses the principle of chemiluminescence to determine plasma CRP levels. The IL-6 concentration (Invitrongen Life Sciences, EUA) was analyzed by enzyme linked immunosorbent assay according to manufacturer's instructions for procedure using a

microplate reader (EzBiochrom). The variability coefficient of variability was was <7.5%. The detection limit was 2–200 pg/ml.

2.11 | ROS measurement in peripheral leukocytes

ROS production and mitochondrial membrane potential (MMP) were analyzed in peripheral granulocytes and monocytes from healthy controls ($n = 8$), non-ICU COVID-19 ($n = 8$), and ICU COVID-19 ($n = 8$) patients at hospital admission. The MMP using the fluorescent dye rhodamine 123 (Rh 123; Sigma-Aldrich®) as previously described. Briefly, whole blood (100 µl) was incubated with 500 µl of Rh 123 (1 µg/ml) for 10 min at 37°C. After being washed, the cells were resuspended in 0.5 ml of phosphate-buffered saline (PBS). The generation of ROS was quantified by the cell-permeant dye 2′,7′-DCF diacetate (DCF-DA; Sigma-Aldrich), which becomes fluorescent after oxidation to DCF. Whole blood was incubated with 0.5 ml of 10 µM DCF-DA for 30 min at room temperature. After being washed with PBS, the cells were resuspended in 0.5 ml PBS. The analysis of MMP and ROS production was conducted using BD FACSCalibur (Becton-Dickinson) flow cytometer and CellQuest Pro software (Joseph Trotter; Scripps Research Institute) using the blue argon-ion 488 nm laser with the FL1 filter channel. A minimal of 30.000 events/tubes was acquired, and granulocytes and monocytes were identified and gated according to each forward scatter (FSC) and side scatter (SSC) profiles.

2.12 | Cell viability and apoptosis in THP-1 monocytic cell line

THP-1 human monocyte cells (ATCC TIB-202) (2×10^5 cells/well) were cultured in RPMI media (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin and streptomycin (both from Sigma-Aldrich) in 96-well plates for 24 h. Afterwards, the media plus FBS were removed; cells were washed with PBS and incubated with RPMI supplemented with 10% plasma of non-ICU ($n = 8$) and ICU ($n = 8$) COVID-19 patients for 15 h. Cell viability was measured using MTT (Sigma-Aldrich). After the treatment, MTT (20 µl, 5 mg/ml) was added, and incubations were continued for 4 h after that. The purple formazan was solubilized and the absorbance at 570 nm determined using a Spectramax M2 microplate reader (Molecular Devices). The evaluation of apoptosis rate was performed by using Annexin-V+ FITC following manufacturer's recommendations (Biolegend) in BD FACScalibur flow cytometer (Becton-Dickinson). THP-1 were identified and gated according to each FSC and SSC profile.

2.13 | Statistical analysis

The data normality was tested using the Kolgomorov–Smirnov test. Parametric data was presented as mean ± standard deviation (SD)/percentile 2.5–97. Participant characteristics, biochemical variables of oxidative stress, and IL-6 were compared among groups using one-way analysis of variance followed by Bonferroni post hoc test for multiple comparisons. Comparison between qualitative variables was performed using a χ^2 test. The evolution of CRP, oxidative stress variables and IL-6 were analyzed by a mixed generalized linear model followed by Bonferroni post hoc test correction for multiple comparisons. A p-value ≤ .05 were considered statistically significant. SPSS 22.0 software (IBM Inc.) was used for statistical analysis.

3 | RESULTS

In the present study, we investigated the redox status of non-ICU COVID-19 patients and ICU COVID-19 patients along hospitalization. First, we identified the symptoms and undergoing health conditions. Then, we compared oxidative stress markers and IL-6 concentration, as a hypercitokenemia indicator, in healthy individuals (control group), non-ICU COVID-19 patients, and ICU COVID-19 patients. Finally, we assessed the progression of the diseases evaluating C-reactive protein, redox biomarkers, and IL-6 at the moment of the hospital admission (T1), during hospitalization (T2), and at 0–72 h before the outcome (T3).

3.1 | Sociodemographic variables and the description of symptoms and underlying conditions of non-ICU and ICU patients

Sociodemographic characteristics are illustrated in Table 1. Briefly, 51 individuals were enrolled in our study and divided in healthy individuals, control group with negative PCR test for SARS-CoV-2 infection ($n = 12$), and patients hospitalized with COVID-19, which tested positive to SARS-CoV-2 infection ($n = 29$, 82.35%). Then, patients diagnosed with SARS-CoV-2 infection were divided according to the severity of the disease into non-ICU COVID-19 patients ($n = 18$, 27.45%) and ICU COVID-19 patients ($n = 11$, 54.9%). ICU patients were older than healthy individuals ($p < .05$), non-ICU and ICU patients demonstrated higher body mass and BMI compared to healthy individuals ($p < .05$). Mortality was observed only in the ICU group (46%).

The most common clinical symptoms observed included fever (non-ICU: 80%; ICU: 72.72%); respiratory failure (non-ICU: -; ICU: 80%); cough (non-ICU: 46.66%; ICU: 61.81%); dyspnea (non-ICU: 60%; ICU: 16.36%); body ache (non-ICU:26.6%; ICU: 21.81%); and sore throat (non-ICU: 40%; ICU: 18.8%);. Some underlying medical condition of the patients comprised hypertension (non-ICU: 25.4%; ICU: 29.7%); diabetes mellitus (non-ICU: 23.7%; ICU: 19.8%); neurological disease (non-ICU: 21%; ICU: 22.5%).

The comparison of healthy individuals, non-ICU COVID-19 patients, and ICU COVID-19 patients demonstrated no difference in the thiol content ($p = .4145$, Figure 1A) and in antioxidant status

TABLE 1 Sociodemographic and clinical characteristics of the patients

COVID-19			
	Healthy controls (*n* = 12)	**Non-ICU (*n* = 18)**	**ICU (*n* = 11)**
Age (years)	57.55 (38.71–76.29)	59.57 (49.41–69.72)	65.50 (61.60–69.39)[a]
Sex (female, %)	33.3	57.1	44.2
Body mass (kg)	61.75 (38.20–85.29)	86.76 (79.52–94.01)[a]	89.23 (81.23–96.45)[a]
BMI (kg/m^2)	23.66 (19.48–34.85)	29.56 (26.57–31.65)[a]	30.49 (27.83–33.15)[a]
Hospitalization (days)		7.33 (1–14.92)	20.53 (16.51–24.55)[a]
Death/mortality (%)		-	46.2
Symptoms (%)			
Fever		80.00%	72.72%
Respiratory failure			80.00%
Cough		46.66%	61.81%
Dyspnea		60.00%	16.36%
Body aches		26.66%	21.81%
Sore throat		40.00%	18.18%
Flu-like syndrome		13.33%	16.36%
Fatigue		20.00%	9.09%
Myalgia		20.00%	5.45%
Nausea or vomiting		20.00%	5.45%
Headache		13.33%	7.27%
Coryza		13.33%	7.27%
Diarrhea		6.66%	7.27%
Underlying medical conditions			
Hypertension		25.4	29.7
Diabetes mellitus		23.7	19.8
Neurological diseases		21	22.5
Cardiovascular diseases		7.8	12.7
Rheumatic diseases		1.2	1.2
Cancer		2.4	
Chronic kidney disease			2.4

Note: Data are presented as mean ± confidence intervals 95%.
Abbreviations: BMI, body mass index; ICU, intensive care unit.
[a]Statistical difference compared to healthy controls ($p < .05$).

($p = .3689$, Figure 1B). However, TAC concentration showed higher levels in the non-ICU COVID-19 patients and ICU COVID-19 patients compared to healthy individuals ($p < .001$, Figure 1C), while non-ICU COVID-19 patients had higher nitrite concentration compared to ICU COVID-19 patients ($p < .001$, Figure 1D). ROS concentration did not differ among the groups ($p = .2562$, Figure 1E). The non-ICU COVID-19 patients and ICU COVID-19 patients demonstrated higher protein oxidation ($p < .001$, Figure 1F) and TBARS concentration compared to healthy individuals ($p < .001$, Figure 1G). IL-6 concentration was higher in non-ICU ($p < .001$, Figure 1H) and ICU patients ($p < .05$, Figure 1H) compared to healthy individuals.

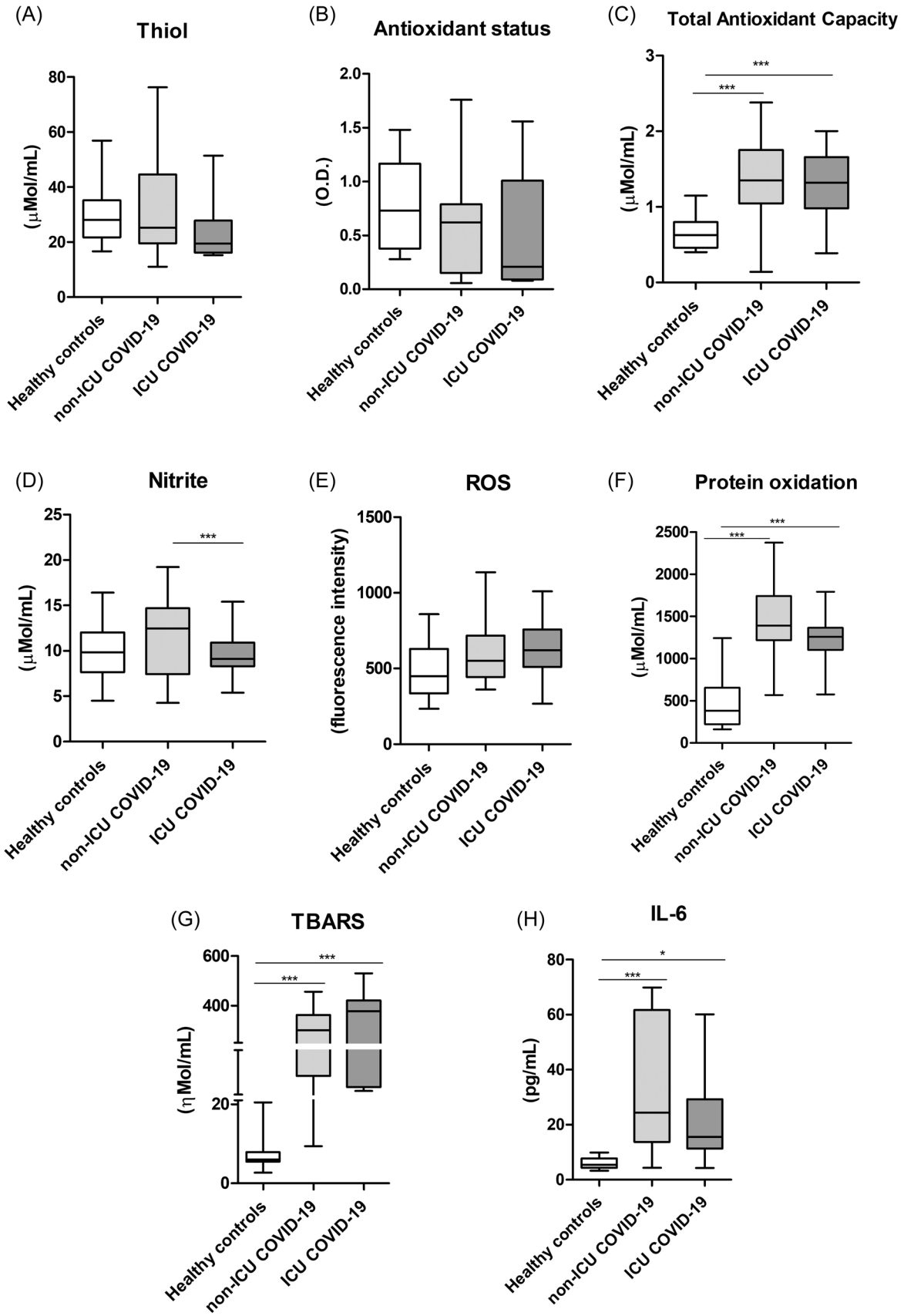

FIGURE 1 Biological markers of oxidative status and IL-6 in healthy individuals, non-ICU COVID-19 patients, and ICU COVID-19 patients. (A) Thiol concentration. (B) Antioxidant status. (C) TAC. (D) Nitrite concentration. (E) ROS concentration. (F) Protein oxidation (AOPP). (G) TBARS. (H) IL-6. Data are presented as mean ± SD. Difference among the groups were verified one way ANOVA followed by Bonferroni post hoc test. *$p < .05$; ***$p < .001$. ANOVA, analysis of variance; ICU, intensive care unit; ROS, reactive oxygen species; TAC, total antioxidant capacity.

3.2 | Redox status variables analyzed during the hospitalization period of non-ICU and ICU patients infected with SARS-CoV-2

The oxidative status of patients was also evaluated according to the progression of the hospitalization in both, non-ICU patients (open spheres) and ICU patients (solid spheres). As observed in Figure 2, lower C-reactive protein concentration was found at T3 compared to T1 in the non-ICU COVID-19 patients, while higher levels were detected at T1, T2, and T3 of ICU COVID-19 patients compared to the same periods in the non-ICU COVID-19 patients ($p < .01$, Figure 2A). The thiol content showed lower concentration in T3 of non-ICU group compared to T1, and ICU group at T1 had lower thiol concentration compared to non-ICU at T1 ($p < .05$, Figure 2B). The antioxidant status was higher at T1 and T3 in the ICU group compared to the same periods in the non-ICU group ($p < .05$, Figure 2C). Non-ICU COVID-19 patients demonstrated higher TAC concentration at T3 compared to T1, and the ICU COVID-19 patients at T3 had lower TAC concentration compared to T1 and the same period of non-ICU COVID-19 patients ($p < .05$, Figure 2D). The nitrite concentration was lower at T3 in ICU COVID-19 patients compared to non-ICU patients at the same period ($p < .05$, Figure 2E), and ICU patient demonstrated lower nitrite at T2 and T3 compared to T1 ($p < .01$, Figure 2E). Non-ICU group showed higher ROS content at T3 compared to T1, and the ICU group had higher ROS content at T2 and T3 compared to T1 of the same group, and higher ROS concentration in T3 of ICU patients compared to T3 of non-ICU ($p < .05$, Figure 3F). The protein oxidation concentration showed higher levels at T3 in ICU patients compared to T1 of the same group and to T3 of non-ICU group ($p < .01$, Figure 2G). TBARS concentration did not differ among groups ($p < .05$, Figure 2H). Non-ICU COVID-19 patients demonstrated higher IL-6 concentration at T3 compared to T1, and, in the ICU patients, the IL-6 was higher at T2 and T3 compared to T1, and T3 was higher compared to the T3 of non-ICU patients ($p < .05$, Figure 2I).

3.3 | Phagocytes generates higher amounts of ROS through changes in MMP

Granulocytes and monocytes produce higher ROS levels during infectious episodes such as viral infection. We firstly accessed the MMP and the ROS generation in granulocytes and monocytes in the peripheral blood of COVID-19 patients by flow cytometry. Both non-ICU and ICU COVID-19 patients presented a state of mitochondrial membrane depolarization in monocytes and granulocytes, identified by the decreases in Rhd-123 fluorescence intensity, compared to the controls ($p < .01$ for all comparisons). Furthermore, increased ROS production was identified in both immune cell types of COVID-19 patients compared to controls ($p < .05$). However, ICU COVID-19 patients presented higher ROS production by granulocytes and monocytes than non-ICU COVID-19 patients ($p < .01$ for both) (Figure 3).

3.4 | Plasma from severe COVID-19 patients induces decreases in cell viability and higher apoptosis rate in THP-1 monocytic cell line

Increased ROS generation are linked to cell death events in several conditions, such as acute infectious diseases. In this line, we incubated THP-1 monocytic cell line with the plasma obtained from non-ICU and ICU COVID-19 patients at T1 and T3 hospitalization periods. Plasma from both non-ICU and ICU COVID-19 groups collected at T3 reduced the cell viability of THP-1 cells ($p < .05$). On the other hand, increased annexin-V+ cells, indicating apoptosis, was found in THP-1 cells incubated with T3 plasma of non-ICU compared to T1 plasma from the same group ($p < .05$). Interestingly, T3 plasma of ICU COVID-19 group had the higher effects on cell viability ($p < .05$) and apoptosis rate ($p < .05$) than those observed in T3 plasma of non-ICU COVID-19 trial in THP-1 cells (Figure 4).

4 | DISCUSSION

Our study performed a follow-up of the redox status along the hospitalization of patients with different degrees of COVID-19 severity. This study showed the increase of antioxidant capacity, oxidation of protein, and TBARS levels in non-ICU COVID-19 and ICU COVID-19 patients compared to healthy individuals, as well as, higher nitrite concentration in non-ICU COVID-19 compared to ICU COVID-19 patients. Considering the progression of the hospitalization (T1, T2, and T3), it was observed an increase of protein oxidation at T3 of ICU patients, higher antioxidant status and lower TAC, reduced nitrite in T3 of ICU COVID-19, and higher ROS and IL-6 in both T2 and T3 of ICU COVID-19 patients. Furthermore, we showed that peripheral leukocytes produce a higher amount of ROS during severe SARS-CoV-2, with ICU COVID-19 patients presenting higher levels of ROS generation and decreased MMP in granulocytes and monocytes. Finally, the incubation of THP-1 monocytic cell line with plasma obtained from ICU-COVID-19 at T3 of hospitalization resulted in lower cell viability and higher apoptosis rate.

The symptoms and underlying conditions described in the patients enrolled in our study were observed either in mild or severe infection of SARS-CoV-2. The most frequent underlying conditions observed in the groups with COVID-19 were hypertension (28.57%), diabetes mellitus (21.42%), and neurological diseases (21.42%), which consists in group more susceptible to severe forms of SARS-CoV-2 infection.[3] The symptoms more frequently reported from the patients included fever (76.19%), cough (57.14%), and dyspnea (33.33%), while the patients with severe infection also reported respiratory failure, a common symptom from patients in this condition. Same symptoms were observed in studies analyzing SARS-CoV-2 infection.[2,18] Patients from infected groups were older and with higher body mass than noninfected group, these characteristics with the underlying condition are associated with oxidative stress, chronic inflammation, and metabolic alterations that could lead to low antioxidant

capacity, oxidative damage and a pro-inflammatory condition, contributing to the exacerbation of the pro-inflammatory cytokines release and a poor viral clearance increasing the severity of the infection.[19]

Patients with SARS-CoV-2 infection demonstrated high C-reactive protein levels especially in the beginning of the diseases. In the non-ICU groups, the levels of C-reactive protein decreased with the progression of the hospitalization until reach the reference value. The ICU group had higher levels than non-ICU, indicating a more severe infection. The C-reactive protein is a nonspecific acute phase reactant elevated in infection or inflammation and it is used as indicator of COVID-19 disease severity. A study of Stringer and colleagues identified that a simple threshold ≥40 mg/dl should be used within clinical practice to guide disease severity and likely disease progression, and recommended that CRP ≥ 40 mg/dl on admission may indicate an increased risk of disease progression and death, and warrants an enhanced level of discussion and clinical support.[20] In our study, the levels of C-reactive protein were 3.5-fold and 2-fold higher in the ICU patients and non-ICU patients, respectively, compared to the threshold.[20]

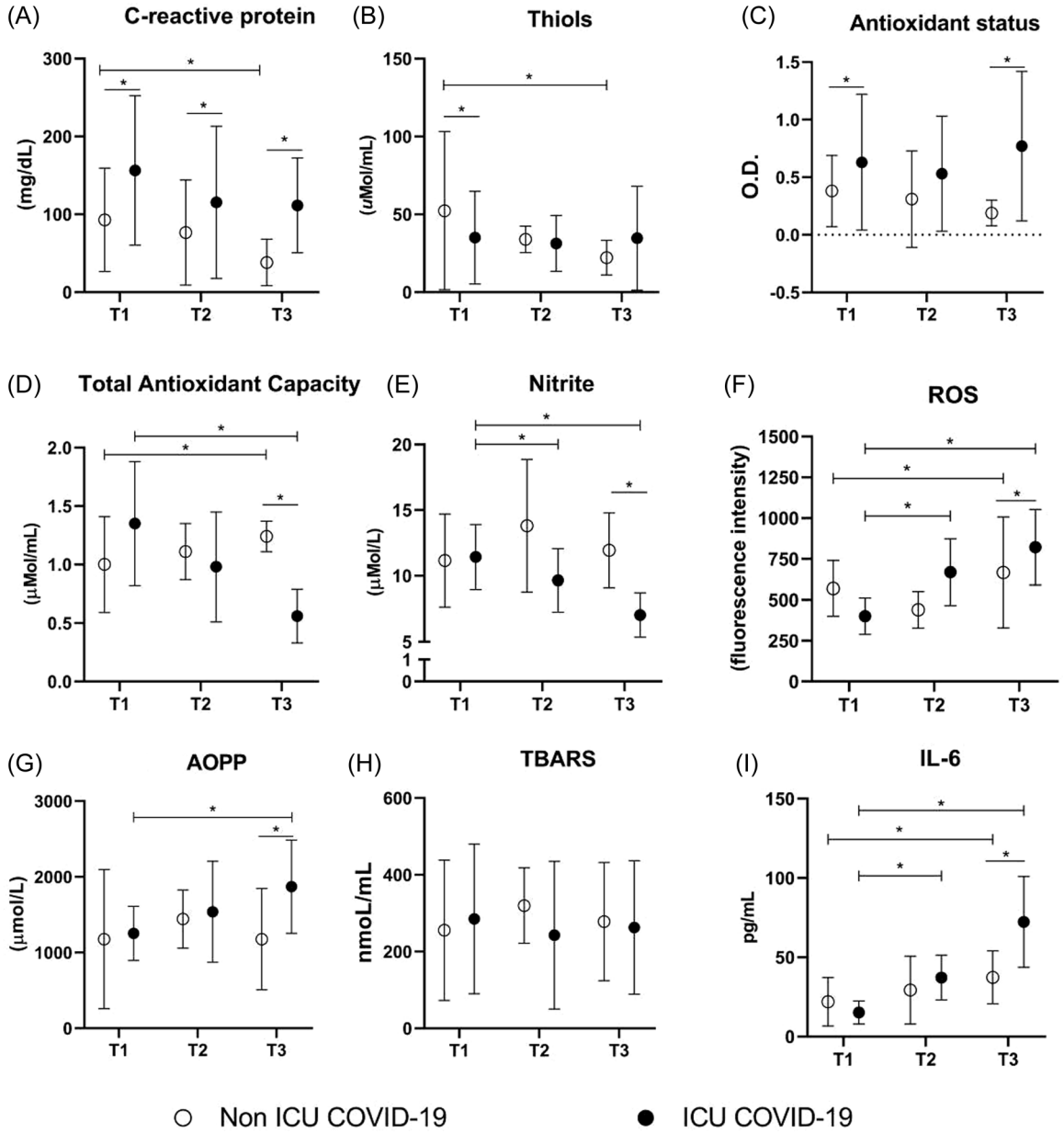

FIGURE 2 Biological markers of oxidative status and IL-6 in non-ICU COVID-19 patients (open spheres) and ICU COVID-19 patients (solid spheres) along the hospitalization (T1, T2, and T3). (A) C reactive protein. (B) Thiol concentration. (C) Antioxidant status. (D) Total Antioxidant Capacity. (E) Nitrite concentration. (F) ROS concentration. (G) Protein oxidation (AOPP). (H) TBARS. (I) Interleukin-6. Data are presented as mean ± SD. Difference among the groups were verified one way ANOVA followed by Bonferroni post hoc test. *$p < .05$. ANOVA, analysis of variance; ICU, intensive care unit; ROS, reactive oxygen species; TAC, total antioxidant capacity.

Our study showed an increase of ROS content at the end of the hospitalization in both groups, but with higher concentration in the ICU patients. Additionally, nitrite concentration and antioxidant capacity decreased along the hospitalization, while protein oxidation and IL-6 increased in ICU groups. ROS formation could be a physiological response of the organism once it had a role in signaling transduction, however, under pathological conditions ROS plays an essential function in the diseases processes.[21,22] The nitrite concentrations were higher in non-ICU patients compared to ICU, and during the hospitalization the levels in the ICU patients decreased. The nitrite can act as a precursor to form peroxinitrite, a free radical that induce oxidative damage.[23,24] Here, our results could indicate an increase in the oxidation processes that is related with reduced antioxidant capacity and nitrite concentration in the ICU group.[23,24]

Viral infections, including SARS-CoV-2, induce in the organism a range of oxidative and inflammatory response. The viral infection could trigger oxidative stress through the increase of reactive oxygen and nitrogen species (RONS), activation of immune response, and production of cytokines.[6,25] Alterations in enzyme function, as XO and NADPH oxidase, and mitochondrial dysfunction results in the increased production of superoxide and hydrogen peroxide, and reduced antioxidant defense.[26] Additionally, the bound between angiotensin-converting enzyme 2 and SARS-CoV-2 during viral invasion increases the binding of Ang II to angiotensin type 1 receptor, activating NADPH oxidase and induces the production of ROS mitochondrial that reacts with NO and reduce its bioavailability forming peroxynitrite.[23,27] The augment formation of RONS mediate signaling pathways responsible for the increased production of inflammatory cytokines.

Regarding antioxidant response, we measured thiol concentration, antioxidant status, and antioxidant capacity. Studies investigating oxidative stress markers in COVID-19 patients observed a depletion in antioxidant response together with increased production of ROS, pro-inflammatory cytokines, and oxidative damage.[6,8,11] Here, we showed higher antioxidant capacity in the non-ICU and ICU patients compared to healthy individuals, which might indicate the antioxidant response to the increased formation of ROS due to the infection. Additionally, when observed the hospitalization progression during T1, T2, and T3, the TAC increased in non-ICU and lowered in ICU groups on T3, indicating a recovery of the non-ICU patients and the progression of the infection and, possibly a consequent poor outcome of the ICU patients.

The results found corroborates to the oxidation markers evaluated, protein oxidation and lipid peroxidation (TBARS). TBARS concentration in non-ICU and ICU patients were higher than healthy individuals, showing that the SARS-CoV-2 infection cause an oxidative stress and inflammation capable of damage the membrane of the cells.[25] Furthermore, protein oxidation was higher in the infected patients compared to healthy individuals, and during the hospitalization it was observed an ascending concentration of protein oxidation in ICU patients, demonstrating protein damage, which might include DNA damage. So, the oxygen reactive species

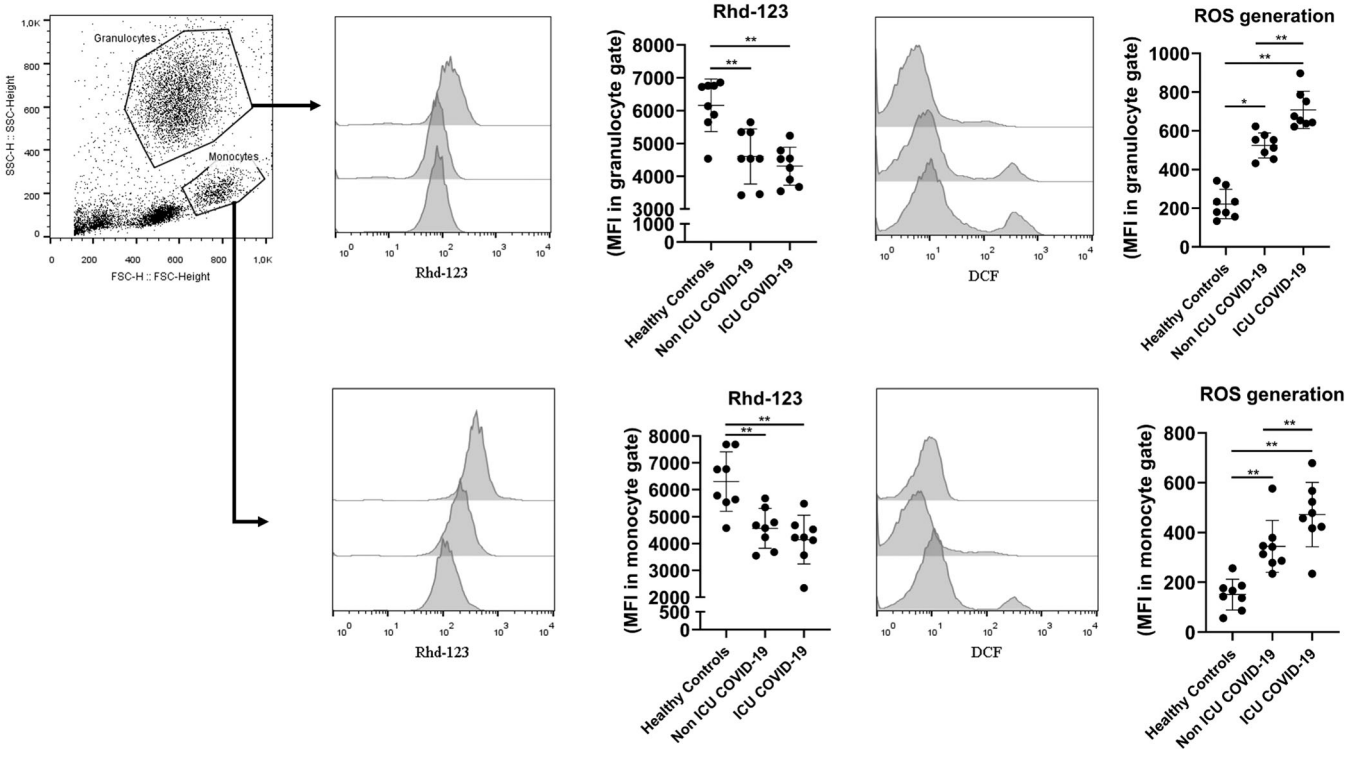

FIGURE 3 Mitochondrial membrane potential and ROS generation in granulocytes and monocytes in the peripheral blood of non-ICU and ICU COVID-19 patients. Data are presented as mean ± SD. Difference among the groups were verified one way ANOVA followed by Bonferroni post hoc test. *$p < .05$; **$p < .01$; ***$p < .001$. ANOVA, analysis of variance; ICU, intensive care unit; ROS, reactive oxygen species.

generated through the SARS-CoV-2 infection might result in the primary damage of membrane cells followed by the oxidation of proteins, DNA damage, and induce apoptosis.

Phagocytes are a greater source of ROS during viral and bacterial infections.[28] Here, we observed that non-ICU and ICU patients showed lower mitochondrial depolarization and higher ROS production, with higher alterations observed in severe cases of SARS-CoV-2 infection. In severe infections, the uncontrolled ROS production generated by innate immune cells leads to extensive cell damage and tissue injury which is associated with worse clinical outcomes. In this sense, excessive levels of ROS from neutrophils have been implicated in a cascade of biological events that drive pathological host response in COVID-19, including tissue damage, thrombosis and red blood cell dysfunction.[29] Furthermore, oxidative stress induced by neutrophils predict COVID-19-associated mortality,[30] and increased mitochondrial ROS production and impaired MMP in CD14+ monocytes were observed in severe COVID-19 patients who presented higher SARS-CoV-2 viral load.[9] The imbalance in redox state of innate immune cells leads to the activation of intracellular inflammatory pathways, mainly inflammasome, contributing to the COVID-19 hyperinflammation in severe cases.[31] In fact, several pro-oxidant genes were upregulated in mononuclear cells of severe COVID-19,[32] which confirms the redox state imbalance evoked in leukocytes during SARS-CoV-2 infection. Interestingly, the incubation of a THP-1 monocytic cell line with plasma obtained from non-ICU and ICU patients at T3 hospitalization period resulted in lower cell viability and only severe cases of COVID-19 induced higher apoptosis. Collectively, these results indicate that the redox imbalance induced during the SARS-CoV-2 infection impacts on cellular process, inducing cell damage and death.

The first phase of the SARS-CoV-2 infection is the viral replication and the innate immune response, followed by symptoms manifestation and higher levels of cytokines. Then, it is observed a severe systemic inflammatory syndrome with the progression of the disease.[33,34] So, the exacerbated response of the immune system against SARS-CoV-2 infection is characterized by a hypercytokinaemia state, an increase in cytokines release leading to a hyperinflammatory condition to contain the viral infection. The increase in cytokines release might be an indicator of the diseases severity. Here, we observed the augment of the IL-6 concentration along the hospitalization progression in non-ICU and ICU patients, however, higher levels were observed in the ICU group. IL-6 is mainly produce in response to pathogens by macrophages and T lymphocytes to control viral infection, and it had a great correlation with disease severity.[35] Previous studies showed that patients with severe form of the disease had higher IL-6 concentration compared to patients with mild or moderate forms, and IL-6 values was used to predict the risk of mortality in COVID-19 patients hospitalized, which is observed in our study that observed 46% of mortality in the ICU group.[36,37]

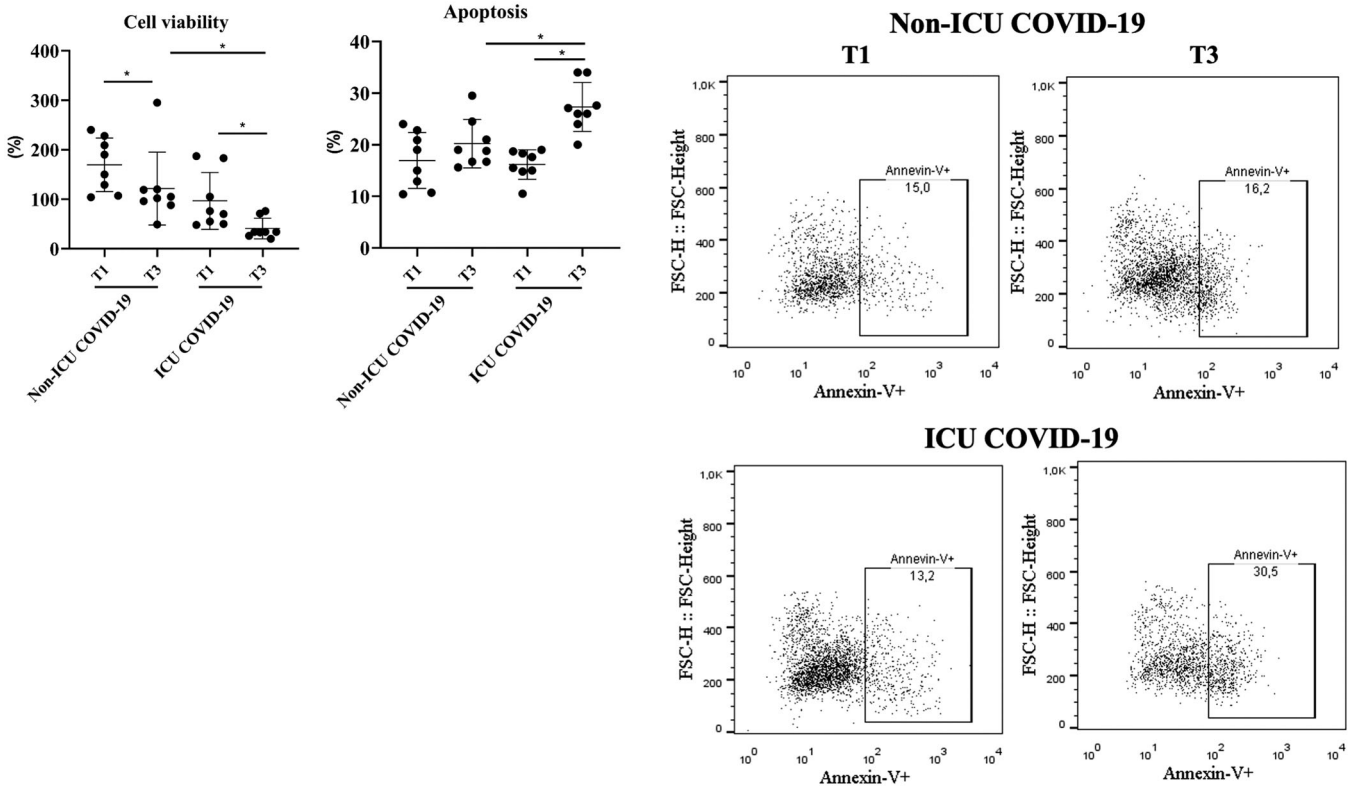

FIGURE 4 Cell viability and apoptosis rate in THP-1 monocytic cell line incubated with plasma from non-ICU and ICU COVID-19 patients. Data are presented as mean ± SD. Difference among the groups were verified one way ANOVA followed by Bonferroni post hoc test. *$p < .05$. ANOVA, analysis of variance; ICU, intensive care unit.

Oxidative stress had an important role in the pathogenesis of COVID-19, in the present study, we monitored the modification in redox mechanism caused by SARS-CoV-2 infection in the beginning of the disease and its progression in the hospitalization period. It would be critically important to use the oxidative and inflammatory parameters evaluated to COVID-19 monitoring. Here, we showed that there is an interesting dynamic in the biomarkers with a potential use in clinical management of the disease.

ACKNOWLEDGMENTS

We thank the Secretaria Municipal de Saúde de Esteio and São Camilo Hospital (Esteio, RS, Brazil) for supporting our work. This project was funded, in part, with support from the *Fundação de Amparo à Pesquisa do Estado de Rio Grande do Sul* (FAPERGS), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil* (CAPES) Finance Code 001, *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), and Federal University of Health Sciences of Porto Alegre. G. P. D. is recipient of a PNPD/CAPES fellowship. P. R. T. R. is of the CNPq.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be available upon reasonable request.

ORCID

Paula C. Teixeira https://orcid.org/0000-0001-7702-3313
Carla Andretta Moreira Neves https://orcid.org/0000-0002-1338-9399
Paulo Santana Filho https://orcid.org/0000-0002-1420-7841
Bruna Marmett https://orcid.org/0000-0002-2982-0323
Roseana Carvalho https://orcid.org/0000-0002-8677-7763
Alessandra Peres https://orcid.org/0000-0002-3587-2832
Liane Rotta https://orcid.org/0000-0003-1043-5203
Claudia Elizabeth Thompson https://orcid.org/0000-0003-3639-1851
Gilson P. Dorneles https://orcid.org/0000-0001-6524-3204
Pedro R. T. Romão https://orcid.org/0000-0002-1039-2509

REFERENCES

1. WHO. *Transmission of SARS-CoV-2: Implications for Infection Prevention Precautions*. COVID-19: Infection Prevention and Control/World Health Organization; 2020.
2. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *JAMA*. 2020;324(8):782-793.
3. Yang J, Zheng Y, Gou X, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Infect Dis*. 2020;94:91-95.
4. Codo AC, Davanzo GG, Monteiro LB, et al. Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1α/glycolysis-dependent axis. *Cell Metab*. 2020;32(3):437-446.e435.
5. Siska PJ, Decking SM, Babl N, et al. Metabolic imbalance of T cells in COVID-19 is hallmarked by basigin and mitigated by dexamethasone. *J Clin Invest*. 2021;131(22):e148225.
6. Delgado-Roche L, Mesta F. Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARS-CoV) infection. *Arch Med Res*. 2020;51(5):384-387.
7. Mcelvaney OJ, Mcevoy NL, Mcelvaney OF, et al. Characterization of the inflammatory response to severe COVID-19 illness. *Am J Respir Crit Care Med*. 2020;202(6):812-821.
8. Cecchini R, Cecchini AL. SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. *Med Hypotheses*. 2020;143:110102.
9. Romão PR, Teixeira PC, Schipper L, et al. Viral load is associated with mitochondrial dysfunction and altered monocyte phenotype in acute severe SARS-CoV-2 infection. *Int Immunopharmacol*. 2022;108:108697.
10. Teixeira PC, Dorneles GP, Santana Filho PC, et al. Increased LPS levels coexist with systemic inflammation and result in monocyte activation in severe COVID-19 patients. *Int Immunopharmacol*. 2021;100:108125.
11. Karkhanei B, Talebi Ghane E, Mehri F. Evaluation of oxidative stress level: total antioxidant capacity, total oxidant status and glutathione activity in patients with COVID-19. *New Microbes New Infect*. 2021;42:100897.
12. Ellman G. Tissue sulfhydryl groups. *Arch Biochem Biophys*. 1959;82(1):70-77.
13. Medina LO, Veloso CA, De Abreu Borges É, et al. Determination of the antioxidant status of plasma from type 2 diabetic patients. *Diabetes Res Clin Pract*. 2007;77(2):193-197.
14. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci (Lond)*. 1993;84(4):407-412.
15. Guevara I, Iwanejko J, Dembińska-Kieć A, et al. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. *Clin Chim Acta*. 1998;274(2):177-188.
16. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int*. 1996;49(5):1304-1313.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351-358.
18. Gadotti AC, Lipinski AL, Vasconcellos FT, et al. Susceptibility of the patients infected with Sars-Cov2 to oxidative stress and possible interplay with severity of the disease. *Free Radic Biol Med*. 2021;165:184-190.
19. Trump S, Lukassen S, Anker MS, et al. Hypertension delays viral clearance and exacerbates airway hyperinflammation in patients with COVID-19. *Nat Biotechnol*. 2021;39(6):705-716.
20. Stringer D, Braude P, Myint PK, et al. The role of C-reactive protein as a prognostic marker in COVID-19. *Int J Epidemiol*. 2021;50(2):420-429.
21. Cadenas S. ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection. *Free Radic Biol Med*. 2018;117:76-89.
22. Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt PIH. Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochem J*. 2016;473(24):4527-4550.
23. Wang J, Mei F, Bai L, et al. Serum nitrite and nitrate: a potential biomarker for post-covid-19 complications? *Free Radic Biol Med*. 2021;175:216-225.
24. Yamasaki H. Blood nitrate and nitrite modulating nitric oxide bioavailability: potential therapeutic functions in COVID-19. *Nitric oxide*. 2020;103:29-30.

25. Chang R, Mamun A, Dominic A, Le NT. SARS-CoV-2 mediated endothelial dysfunction: the potential role of chronic oxidative stress. *Front Physiol*. 2020;11:605908.
26. Li X, Fang P, Mai J, Choi ET, Wang H, Yang XF. Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. *J Hematol Oncol*. 2013;6:19.
27. Mete AÖ, Koçak K, Saracaloglu A, Demiryürek S, Altınbaş Ö, Demiryürek AT. Effects of antiviral drug therapy on dynamic thiol/disulphide homeostasis and nitric oxide levels in COVID-19 patients. *Eur J Pharmacol*. 2021;907:174306.
28. Herb M, Schramm M. Functions of ROS in macrophages and antimicrobial immunity. *Antioxidants (Basel)*. 2021;10:313.
29. Laforge M, Elbim C, Frère C, et al. Tissue damage from neutrophil-induced oxidative stress in COVID-19. *Nat Rev Immunol*. 2020;20:515-516.
30. Badawy MA, Yasseen BA, El-Messiery RM, et al. Neutrophil-mediated oxidative stress and albumin structural damage predict COVID-19-associated mortality. *eLife*. 2021;10:e69417.
31. Lage SL, Amaral EP, Hilligan KL, et al. Persistent oxidative stress and inflammasome activation in CD14 high CD16—monocytes from COVID-19 patients. *Front Immunol*. 2022;12:799558.
32. Saheb sharif-Askari N, Saheb Sharif-Askari F, Mdkhana B, et al. Upregulation of oxidative stress gene markers during SARS-COV-2 viral infection. *Free Radic Biol Med*. 2021;172:688-698.
33. Santa Cruz A, Mendes-Frias A, Oliveira AI, et al. Interleukin-6 is a biomarker for the development of fatal severe acute respiratory syndrome coronavirus 2 pneumonia. *Front Immunol*. 2021;12:613422.
34. Siddiqi HK, Mehra MR. COVID-19 illness in native and immunosuppressed states: a clinical-therapeutic staging proposal. *J Heart Lung Transplant*. 2020;39(5):405-407.
35. Velazquez-Salinas L, Verdugo-Rodriguez A, Rodriguez LL, Borca MV. The role of interleukin 6 during viral infections. *Front Microbiol*. 2019;10:1057.
36. Laguna-Goya R, Utrero-Rico A, Talayero P, et al. IL-6-based mortality risk model for hospitalized patients with COVID-19. *J Allergy Clin Immunol*. 2020;146(4):799-807.e799.
37. Mojtabavi H, Saghazadeh A, Rezaei N. Interleukin-6 and severe COVID-19: a systematic review and meta-analysis. *Eur Cytokine Netw*. 2020;31(2):44-49.

How to cite this article: Postiga IAL, Teixeira PC, Neves CAM, et al. Systemic redox imbalance in severe COVID-19 patients. *Cell Biochem Funct*. 2022;40:694-705. doi:10.1002/cbf.3735