Free Radical Biology and Medicine 207 (2023) 194–199

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Do the oxidative stress biomarkers predict COVID-19 outcome? An in-hospital cohort study

Fabio Fernandes Neves [a,*], Henrique Pott-Junior [a], Kaori Maria Carolina Yamashita [a], Sigrid de Sousa Santos [a], Marcia Regina Cominetti [b], Caio Cesar de Melo Freire [c], Anderson Ferreira da Cunha [c], Alceu Afonso Jordão Júnior [d]

[a] Department of Medicine, Federal University of São Carlos - UFSCar, 13565-905, São, Carlos, Brazil
[b] Department of Gerontology, Federal University of São Carlos - UFSCar, 13565-905, São, Carlos, Brazil
[c] Department of Genetics and Evolution, Federal University of São Carlos - UFSCar, 13565-905, São Carlos, Brazil
[d] Department of Health Sciences, Ribeirão Preto Medical School, University of São Paulo - USP, 14049-900, Ribeirão Preto, Brazil

ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
Clinical outcomes
Oxidative stress
ROS
Reactive oxygen species
SOD
Superoxide dismutase
GSH
Glutathione
Vitamin E
AOPP
Advanced oxidation protein products
8OHDG
8-Hydroxy-2′-deoxyguanosine

ABSTRACT

In SARSCoV-2 infections, excessive activation of the immune system dramatically elevates reactive oxygen species levels, harms cell structures, and directly increases disease severity and mortality. We aimed to evaluate whether plasma oxidative stress biomarker levels could predict mortality in adults admitted with Coronavirus Disease 2019 (COVID-19), considering potential confounders. We conducted a cohort study of 115 adults (62.1 ± 17.6 years, 65 males) admitted to a Brazilian public hospital for severely symptomatic COVID-19. Serum levels of α-tocopherol, glutathione, superoxide dismutase, 8-hydroxy-2′-deoxyguanosine, malondialdehyde, and advanced oxidation protein products were quantified at COVID-19 diagnosis using real-time polymerase chain reaction. Serum levels of α-tocopherol, glutathione, superoxide dismutase, and advanced oxidation protein products differed significantly between survivors and non-survivors. Serum glutathione levels below 327.2 μmol/mL were associated with a significant risk of death in COVID-19 patients, even after accounting for other factors (adjusted hazard ratio = 3.12 [95% CI: 1.83–5.33]).

1. Introduction

Approximately 5% of non-immunized patients with Coronavirus Disease 2019 (COVID-19) develop a critical illness, and up to 50% of these critical patients die [1]. The vast literature shows that excessive inflammatory response and hypercoagulation are the primary pathophysiological phenomena associated with poor outcomes [2,3].

Under normal circumstances, aerobic metabolism produces reactive oxygen species (ROS) essential for cell signaling. However, in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, the immune system is excessively activated with an exaggerated increase in ROS levels [2,4]. The imbalance between the rate of ROS production and the antioxidant mechanism leads to oxidative stress (OS). This clinical condition encompasses three distinct stages: a) increased production of ROS, b) mobilization of antioxidant defenses, and c) oxidative damage to the primary molecular targets such as lipids, proteins, and DNA [5].

ROS interfere with the main cellular metabolic pathways by inducing lipid peroxidation, protein degradation, and DNA strand breaks [6]. Some first-line antioxidant substances include glutathione (GSH), vitamin E, and superoxide dismutase (SOD). GSH is a functional molecule with direct antioxidant activity; it prevents the generation of free radicals via hydroperoxide formation. A study showed that patients with severe COVID-19 have low serum GSH levels [7]. Vitamin E is a fat-soluble antioxidant that removes peroxyl radicals and inhibits the oxidation of polyunsaturated fatty acids (PUFAs), mainly due to an interaction between peroxyl radicals and α-tocopherol rather than lipid hydroperoxide [8,9]. SOD is a metal-containing antioxidant enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen

* Corresponding author.
E-mail address: fabioneves@ufscar.br (F.F. Neves).

https://doi.org/10.1016/j.freeradbiomed.2023.06.026
Received 2 March 2023; Received in revised form 7 June 2023; Accepted 24 June 2023
Available online 15 July 2023

peroxide (H_2O_2). Several studies have shown that SOD is essential to defend the organism against excessive ROS generation during viral infections [10]. When ROS overwhelms antioxidants, oxidative stress occurs and can be assessed by quantifying the serum levels of malondialdehyde (MDA), advanced oxidation protein products (AOPP), and 8-hydroxy-2′-deoxyguanosine (8-OHdG), respectively [5].

Despite the rapid development of multiple COVID-19 vaccines, new SARS-CoV-2 variants may be highly infectious, and existing vaccines may be less protective against severe illness and death [11]. In this context, identifying more accurate prognostic markers and deepening the pathophysiological knowledge of COVID-19 may result in new therapeutic approaches that minimize damage caused by future pandemic waves.

According to the current evidence, OS is crucial for the pathogenesis of SARS-CoV-2 infection [12]. However, the role of biomarkers related to OS remains unclear, and further research is required to investigate biomarkers of the redox state in patients with COVID-19. This study aimed to evaluate OS biomarkers and assess their potential application in the mortality prediction of COVID-19 patients.

2. Materials and methods

2.1. Study design and patients

This cohort study was conducted at the University Hospital of the Federal University of São Carlos, São Carlos, Brazil. We gathered the data by conducting face-to-face interviews in the initial months of the pandemic, which took place from May to October 2020. The study sample was a convenience sample of 115 hospitalized adults with confirmed COVID-19 infection. The exclusion criteria were pregnancy, breastfeeding, or current use of vitamin supplements. For each subject, we collected the following data upon hospital admission: sociodemographic characteristics, chronic comorbidities (Charlson comorbidity index [CCI]), and clinical and laboratory data.

We used the Sequential Organ Failure Assessment [SOFA] to estimate an individual's disease severity. Subjects were assessed daily from hospital admission to discharge or 30 days of hospitalization. Patients were treated using a standard protocol, which included antibiotics for concurrent bacterial pneumonia, enoxaparin for thromboembolism prevention, and dexamethasone in selected cases. At the time of the study, no specific treatment for COVID-19 had been approved in Brazil.

2.2. Ethics

The study followed the guidelines of the Declaration of Helsinki and the Brazilian National Health Council (Federal Resolution 466/2012). The study protocol was approved by the Institutional Research Ethics Committee (30184220.8.0000.5504). All patients who fulfilled the eligibility criteria and agreed to participate in the study provided written informed consent.

2.3. Sample collection and processing

Blood samples were collected immediately after COVID-19 diagnosis, which coincided with the day of hospital admission in 40% of the cases. Venous blood samples (5 mL) were collected in sterile vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) and centrifuged at 3000 rpm for 10 min. The serum was split into three aliquots and stored at −80 °C. All OS markers were measured in duplicate at the same time by the same technician who had no access to the clinical data of the patients.

2.4. COVID-19 diagnosis

SARS-CoV-2 infection was diagnosed using real-time polymerase chain reaction (qPCR) in nasopharyngeal samples, according to the guidelines set forth by the US Centers for Disease Control and Prevention [13].

2.5. Oxidative stress biomarkers

2.5.1. Antioxidants

Serum GSH was determined as described previously by Hu [14], in which thiol groups react with dithionitrobenzoic acid (DTNB) to form a deeply colored anion with a maximum peak at 412 nm (e412 = 13,600 M−1 cm−1). The concentration of sulfhydryl groups was calculated using GSH, and the results were reported as micromoles per milliliter.

To assess vitamin E status, α-tocopherol concentration was determined by isocratic high-performance liquid chromatography, as described previously by Arnaud et al. [15], and was reported as micromoles per milliliter.

For SOD determination, a commercial kit (#19160, Sigma-Aldrich, St.Louis, MO, USA) was used according to the manufacturer's instructions and read at 450 nm microplate reader (SpectraMax M5, Molecular Devices, USA). The results were reported as units per milliliter.

2.5.2. Pro-oxidative biomarkers

MDA was measured based on a previously described spectrophotometric method using TCA-TBA-HCl (15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N hydrochloric acid) [5]. The results were presented as micromoles per milliliter.

The levels of 8-OHdG were measured using the Stressgen DNA Damage Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Ann Arbor, MI, USA) according to the manufacturer's instructions and expressed as ng/mL.

Finally, the biochemical analysis of AOPP was performed by chromatography as described by Witko-Sarsat et al. [16].

2.6. Statistical analysis

The primary outcome was the all-cause 30-day mortality rate. Continuous data are presented as mean ± standard deviation or median [first, third quartile], whereas categorical variables are presented as counts (percentage). Comparisons between groups were performed using Student's t-test or Wilcoxon–Mann–Whitney test for continuous variables and Pearson's chi-squared test for categorical variables. The probability of survival was estimated using Kaplan–Meier analysis. The groups were compared using the log-rank test. The hazard ratio (HR) and 95% confidence interval (CI) of mortality were estimated using Cox proportional hazards regression models. Statistical significance was assessed using a two-sided p-value <0.05. All analyses were conducted using TIBCO Statistica® version 14.0.0 (TIBCO Software Inc., CA, USA).

3. Results

3.1. Baseline characteristics of the patients

The study included one hundred fifteen individuals, and the overall all-cause 30-day mortality was 17.4%. As shown in Table 1, non-survivors were older (mean age 74.9 ± 15.9 years vs. 59.2 ± 16.8 years, respectively), had more comorbidities (CCI ≥5 in 50.0% vs. 21.0%, respectively), and had a higher prevalence of organ dysfunction (SOFA ≥3 in 75.0% vs. 24.2%, respectively). Similarly, non-survivors also had worse laboratory test results, characterized by lymphopenia and higher serum levels of D-dimer, lactate dehydrogenase (LDH), and C-reactive protein (CRP). The frequency of therapeutic anticoagulation with heparin was found to be similar in both groups (p = 0.655). However, it was observed that non-survivors were prescribed corticosteroids more frequently than survivors (p = 0.030).

Table 1
Baseline demographic and clinical variables among COVID-19 inpatients.

Variable	Participants		p-value
	Survivors (n = 95)	Non-survivors (n = 20)	
Demographic and clinical features			
Age, years	59.2 ± 16.8	74.9 ± 15.9	<0.001
Male sex	51 (53.7)	13 (65.0)	0.355
White	64 (67.4)	16 (80.0)	0.264
CCI ≥5	20 (21.1)	10 (50.0)	0.007
SOFA ≥3	23 (24.2)	15 (75.0)	<0.001
ICU admission	20 (21.1)	13 (65.0)	<0.001
Length of symptoms*, days	7 [4–10]	7 [4–15]	0.411
Laboratory tests (units)			
Lymphocyte count (× 10^9/L)	1156 [769–1531]	864 [575–1220]	0.002
Leukocyte count (× 10^9/L)	6900 [5400–8515]	6390 [4940–12203]	0.845
Hemoglobin (g/dL)	14.0 [12.4–15.1]	13.8 [12.7–14.9]	0.941
D-dimer (μg/mL)	0.70 [0.40–1.69]	2.51 [1.85–4.71]	<0.001
LDH (U/L)	276 [216–392]	473 [293–629]	0.005
CRP (mg/dL)	6.52 [1.2–13.4]	13.6 [5.1–20.4]	<0.020
Albumin (g/dL)	3.50 [3.15–3.85]	3.30 [3.08–3.42]	0.246
Therapy n (%)			
Corticosteroids	51 (53.7%)	16 (80%)	0.030
Anticoagulants	33 (34.7%)	8 (40%)	0.655

CCI: Charlson coorbidity index; SOFA: sequential organ failure assessment; ICU: intensive care unit; LDH: lactate dehydrogenase; CRP: C-reactive protein. * Time from clinical onset to hospital admission. Bold corresponds to a group of variables.

3.2. Univariate analysis

Comparing survivors and non-survivors, significant differences were observed in the serum levels of α-tocopherol, GSH, SOD, and AOPP. However, the groups had slightly similar levels for MAD and 8-OhdG. Table 2 lists both groups' serum levels of antioxidants and pro-oxidative biomarkers.

3.3. Accuracy of oxidative stress biomarkers in the prediction of mortality

Receiver operating characteristic (ROC) analysis of the biomarkers with statistical significance between groups showed that SOD was the best predictor of mortality [area under the ROC curve (AUC) = 0.834, p < 0.001], and α-tocopherol was the least accurate predictor (Fig. 1).

A SOD concentration of >0.15 U/mL was predictive of death with 90.0% sensitivity, 66.3% specificity, 2.67 positive likelihood ratio, and 0.15 negative likelihood ratio. The ROC analysis of the four biomarkers for predicting death is shown in Table 3.

Fig. 2 shows the survival probability curves according to the serum levels of the OS biomarkers. Serum levels of GSH, SOD, and AOPP were used to assess the survival probability. However, we observed borderline statistical significance in all cases (p = 0.055, 0.054, and 0.066, respectively).

Table 2
Serum levels of oxidative stress biomarkers among COVID-19 inpatients.

Parameters (units)	Participants		p-value
	Survivors (n = 95)	Non-survivors (n = 20)	
Antioxidants			
Vitamin E (μg/mL)	7.09 (0.42–23.8)	5.56 (1.82–11.6)	0.041
GSH (μmol/mL)	409 ± 140	299 ± 140	0.002
SOD (U/mL)	0.150 (0.141–0.159)	0.175 (0.156–0.201)	<0.001
Pro-oxidative biomarkers			
MDA (μmol/mL)	12.7 ± 3.02	12.4 ± 3.38	0.767
8-OhdG (ng/mL)	6.30 (3.4–105.0)	5.86 (2.68–21.0)	0.562
AOPP (μmol/mL)	0.430 (0.09–1.03)	0.274 (0.118–0.501)	<0.001

Vitamin E: α-tocopherol; GSH: glutathione; SOD: superoxide dismutase; MDA: malondialdehyde; 8-OhdG: 8-hydroxy-2′-deoxyguanosine; AOPP: advanced oxidation protein products. Bold corresponds to a group of biomarkers.

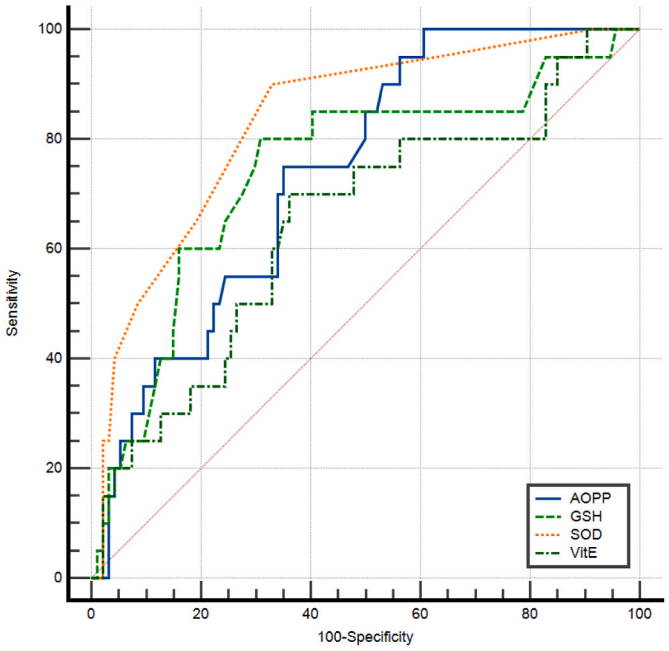

Fig. 1. Receiver operating characteristics (ROC) curves of advanced oxidation protein products (AOPP), glutathione (GSH), superoxide dismutase (SOD), and α-tocopherol (Vitamin E) for prediction of mortality among COVID-19 patients.

3.4. Multivariate analysis

In the multivariate analysis, GSH showed the best results. The Cox regression model showed that the serum levels of GSH ≤327.2 μmol/mL predict 30-day mortality among adults with COVID-19, even after adjusting for potential confounders such as age, sex, race, CCI, length of symptoms, SOFA score at hospital admission, and need for intensive care unit (ICU) admission (adjusted HR 3.12, [95% CI: 1.83–5.33]; p < 0.001).

4. Discussion

This study showed that oxidative stress plays a significant role in the severity of COVID-19. This aspect is consistent with other studies showing an increased imbalance between ROS and antioxidant markers in COVID-19 patients compared to healthy volunteers [10], mostly reduced serum thiol levels [25–28] and increased SOD activity and MDA concentrations [29–31].

As expected, we observed decreased serum levels of two antioxidant biomarkers (vitamin E and GSH) in non-survivors. Surprisingly, SOD concentrations were lower in survivors than in non-survivors. Also, we found no significant differences between the groups for the serum levels of the pro-oxidative biomarkers 8-OhdG and MDA; however, we observed increased levels of AOPP in survivors.

Vitamin E is highly liposoluble and exists universally in human cell membranes and lipoproteins. It exerts significant antioxidant activity mainly by inhibiting the peroxidation of cell membrane lipids [17]. In contrast to our results, two previous studies failed to demonstrate an association between serum vitamin E levels and the severity of COVID-19. A study in Saudi Arabia with 155 patients did not observe a significant difference in the concentration of vitamin E in asymptomatic, mild, moderate, or severe cases of COVID-19 [18]. Another report that evaluated 88 patients and 34 healthy donors in Croatia also showed no difference in vitamin E concentrations between survivors and non-survivors. However, these patients had lower serum levels than the

Table 3
Accuracy of oxidative stress biomarkers for prediction of mortality among COVID-19 inpatients.

Biomarkers (units)<	AUC [95% CI]	p-value	Optimal threshold	Sensibility [95% CI]	Specificity [95% CI]	+LR [95% CI]	-LR [95% CI]
Vitamin E (μg/mL)	0.646 [0.551–0.733]	0.042	≤6.06	70.00% [45.7–88.1]	63.83% [53.3–73.5]	1.94 [1.31–2.87]	0.47 [0.24–0.93]
GSH (μmol/mL)	0.741 [0.651–0.819]	<0.001	≤327.27	80.00% [56.3–94.3]	69.47%	2.62	0.29
					59.2–78.5	1.80–3.81	0.12–0.70
SOD (U/mL)	0.834 [0.753–0.897]	<0.001	>0.15	90.0% [68.3–98.8]	66.32% [55.9–75.7]	2.67 [1.94–3.67]	0.15 [0.04–0.57]
AOPP (μmol/mL)	0.740 [0.649–0.817]	<0.001	≤0.35	75.00% [50.9–91.3]	65.26% [54.8–74.7]	2.16 [1.49–3.14]	0.38 [0.18–0.83]

AUC: area under the ROC curve; CI: confidence interval; +LR: positive likelihood ratio; -LR: negative likelihood ratio; Vitamin E: α-tocopherol; GSH: glutathione; SOD: superoxide dismutase; AOPP: advanced oxidation protein products.

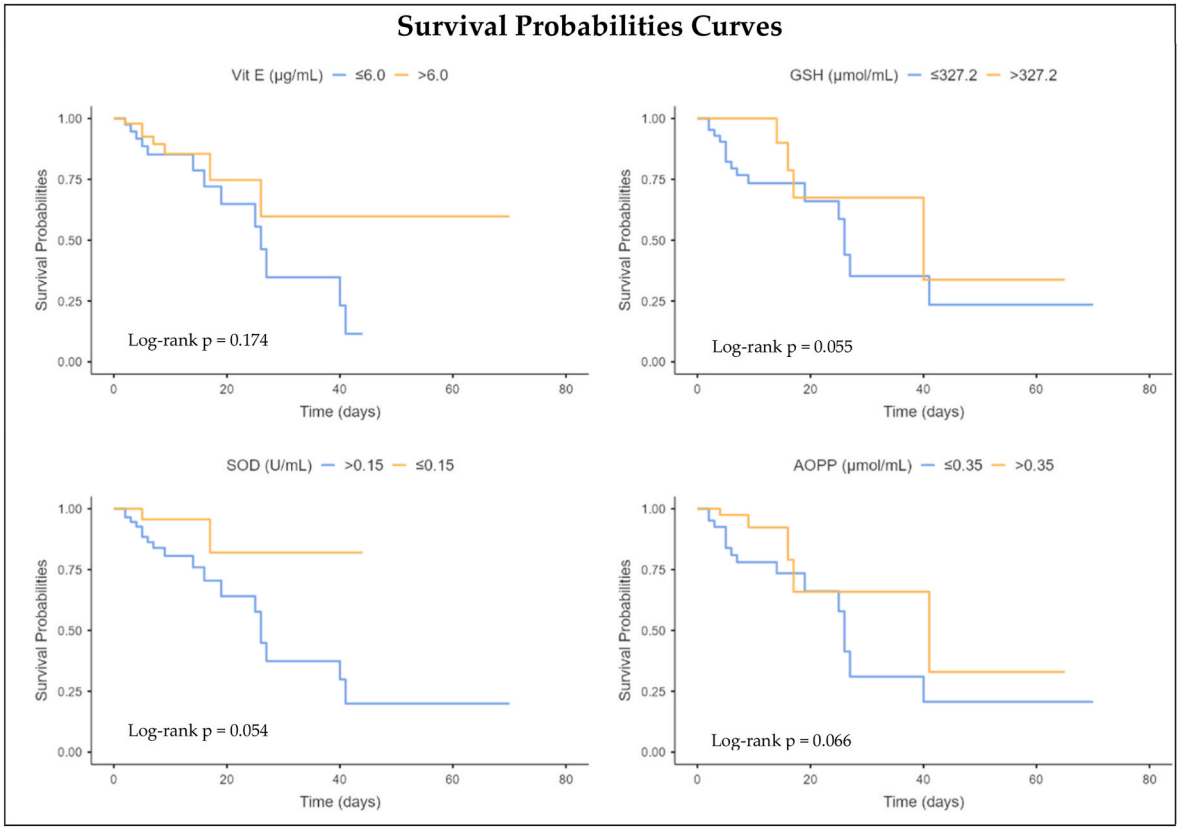

Fig. 2. Kaplan–Meier curves according to α-tocopherol (Vitamin E), glutathione (GSH), superoxide dismutase (SOD), and advanced oxidation protein products (AOPP) serum levels in COVID-19 patients.

controls [19].

GSH is the most abundant endogenous intracellular antioxidant in multiple cellular components, including the cytosol, mitochondria, nucleus, and endoplasmic reticulum [20]. Although the causes of GSH deficiency in hospitalized COVID-19 patients are unknown, several factors are possible, such as decreased synthesis and increased use [21]. Low serum levels of this thiol can predict the severity of COVID-19, such as the need for ventilatory support, ICU admission, and death [22–27]. The findings of Karkhanei and colleagues' study on oxidative stress biomarkers in 78 hospitalized COVID-19 patients indicate a significant difference in GSH levels between the non-ICU and the ICU groups, with the former exhibiting higher levels of GSH in their serum (227.03 vs. 134.54 μmol/mL). Notably, the ICU patients who required endotracheal intubation had the lowest levels of GSH (102.11 ± 36.86 μmol/mL), suggesting a potential correlation between GSH levels and disease severity [22].

Our study has revealed a noteworthy finding that serum GSH levels can predict mortality within 30 days among adults diagnosed with COVID-19. The predictive ability was determined to be an AUC of 0.741 and an HR of 3.12 for serum concentrations below 327.2 μmol/mL. A study in France involving 160 confirmed COVID-19 patients also identified low serum thiol concentrations as predictors of ICU admission and death, with an AUC of 0.762 and 0.750, respectively [27]. Another study found that serum GSH levels can predict mortality rates in hospitalized COVID-19 patients with an AUC of 0.820 [22].

However, several studies have not observed significant differences in thiol or GSH concentrations between groups of COVID-19 patients with different severity [28–33].

It was expected that the activity of essential antioxidant enzymes, such as SOD, would be significantly reduced in the serum of patients with severe COVID-19 [35,36]. However, our study revealed that an increase in SOD activity might be synergistically associated with a higher risk of death (AUC = 0.834). This result is supported by a study that observed significantly higher SOD serum levels in patients placed in the ICU compared to the non-ICU group [34].

Based on immunohistochemical analysis, a study showed that individuals who died due to COVID-19 had elevated levels of SOD-2 in both their pneumocytes and alveolar macrophages [35]. As the SARS-CoV-2 virus primarily targets pneumocytes, it triggers a state of hyperinflammation in the cells, leading to extensive lung damage [36].

We hypothesized that as the severity of the condition increases, the alveolar damage would also become more severe, ultimately leading to the release of intracellular superoxide dismutase, specifically SOD-1 or SOD-2, and an increase in serum levels of the enzyme. However, other studies have not found significant differences in SOD levels among patients with varying levels of severity [29,31,32,37].

Few studies have evaluated the composition of pro-oxidative biomarkers in different groups of patients with COVID-19. A study in Serbia found no difference in serum levels of thiobarbituric acid-reactive substances between groups of patients with different degrees of severity [29]. A Brazilian study that compared patients with moderate and severe COVID-19 did not observe differences in the serum levels of H_2O_2, MDA, carbonyl, or sulfhydryl groups [30]. Similarly, Ducastel et al. did not observe a significant difference in serum concentrations of AOPP between survivors and non-survivors of COVID-19. In contrast, Tantry et al. observed greater urinary excretion of 8-OhdG in COVID-19 patients diagnosed with thrombotic events such as venous thrombosis, pulmonary thromboembolism, myocardial infarction, and ischemic stroke [38].

We also observed lymphopenia and higher serum D-dimer, LDH, and CRP levels in the non-survivor group. These findings were consistent with those described in the scientific literature. The vast literature shows the relevance of D-dimer in predicting adverse clinical outcomes in patients with COVID-19; D-dimer can predict severity, mortality, and venous thromboembolism in COVID-19 patients with high sensitivities of 77%, 75%, and 90%, respectively [39]. Moreover, patients with severe COVID-19 frequently have reduced lymphocyte counts, which can be attributed to direct lymphocyte infections, inflammation-induced apoptosis, and lactic acidosis, which inhibit lymphocyte and lymph tissue degradation [40]. Similarly, high serum LDH levels may predict disease progression, respiratory failure, and in-hospital death [41]. Our study's median serum LDH level was 473 mg/dL in non-survivors; a Chinese study reported that an LDH $\geq$353.5 U/L predicted mortality with a sensitivity of 94.4% and a specificity of 89.2% [42]. Moreover, a meta-analysis that included 69,762 COVID-19 patients observed a positive correlation between high CRP levels, ICU admission, and mortality [43]. CRP levels greater than 1.5 mg/dL provided a marker of disease severity, and levels greater than 20.0 mg/dL on admission were associated with five times the odds of death [29]. In parallel with these findings, our study observed that the median serum CRP levels were lower in survivors than non-survivors.

It is important to note that our study had some limitations. Firstly, selection bias may have occurred due to the study being conducted at a single center. Secondly, the patients recruited had multiple comorbidities, which may have affected the changes in oxidative stress indicators. Additionally, serum lipids were not measured, which could have impacted the vitamin E serum concentration. Lastly, it should be noted that only hospitalized patients were recruited, and therefore the findings cannot be generalized to all ambulatory patients. Despite these limitations, the study was conducted during the epidemic's peak, providing a unique opportunity to collect homogeneous data from the same outbreak.

5. Conclusions

Our study showed significantly lower serum GSH levels in non-survivors of COVID-19, and this biomarker was proven to help predict the risk of death in hospitalized patients. Other recent observational studies have supported these findings. Unfortunately, other markers of OS did not show good accuracy in predicting mortality in COVID-19 patients.

Author contributions

Conceptualization, F.F.N. and H.P-J.; data curation, F.F.N., H.P-J., , K.M.C.Y. and S.S.S; formal analysis, F.F.N. and H.P-J.; funding acquisition, F.F.N.; investigation, F.F.N., H.P-J. and S.S.S; methodology, F.F.N., H.PJ., C.C.M.F, A.F.C. and A.A.J-J; resources, M.R.C., C.C.M.F, A. F.C. and A.A.J-J; software, H.P-J.; supervision, F.F.N. and H.P-J.; writing original draft, F.F.N. and H.P-J.; final approval: all authors.

Funding

This research was funded by São Paulo Research Foundation (FAPESP) grant number #2020/06725-0.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Research Ethics Committee of the Federal University of São Carlos (protocol 30184220.8.0000.5504).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

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.

Acknowledgments

We are indebted to the staff and coworkers at University Hospital of the Federal University of São Carlos (UFSCar) and Brazilian Hospital Services Company (EBSERH).

References

[1] Z. Wu, J.M. McGoogan, Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention, JAMA 323 (2020) 1239–1242, https://doi.org/10.1001/jama.2020.2648.
[2] M. Laforge, C. Elbim, C. Frère, M. Hémadi, C. Massaad, P. Nuss, J.-J. Benoliel, C. Becker, Tissue damage from neutrophil-induced oxidative stress in COVID-19, Nat. Rev. Immunol. 20 (2020) 515–516, https://doi.org/10.1038/s41577-020-0407-1.
[3] N. Tang, D. Li, X. Wang, Z. Sun, Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia, J. Thromb. Haemost. JTH 18 (2020) 844–847, https://doi.org/10.1111/jth.14768.
[4] L. Delgado-Roche, F. Mesta, Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARS-CoV) infection, Arch. Med. Res. 51 (2020) 384–387, https://doi.org/10.1016/j.arcmed.2020.04.019.
[5] M.G. Da Broi, F.O. de Albuquerque, A.Z. de Andrade, R.L. Cardoso, A.A. Jordão Junior, P.A. Navarro, Increased concentration of 8-hydroxy-2'-deoxyguanosine in follicular fluid of infertile women with endometriosis, Cell Tissue Res. 366 (2016) 231–242, https://doi.org/10.1007/s00441-016-2428-4.
[6] E.E. Tsermpini, U. Glamočlija, F. Ulucan-Karnak, S. Redenšek Trampuž, V. Dolžan, Molecular mechanisms related to responses to oxidative stress and antioxidative therapies in COVID-19: a systematic review, Antioxidants 11 (2022) 1609, https://doi.org/10.3390/antiox11081609.
[7] C.A. Labarrere, G.S. Kassab, Glutathione deficiency in the pathogenesis of SARS-CoV-2 infection and its effects upon the host immune response in severe COVID-19 disease, Front. Microbiol. 13 (2022), 979719, https://doi.org/10.3389/fmicb.2022.979719.
[8] G.Y. Lee, S.N. Han, The role of vitamin E in immunity, Nutrients 10 (2018) 1614, https://doi.org/10.3390/nu10111614.
[9] A.H. Alghadir, S.A. Gabr, S. Anwer, H. Li, Associations between vitamin E, oxidative stress markers, total homocysteine levels, and physical activity or cognitive capacity in older adults, Sci. Rep. 11 (2021), 12867, https://doi.org/10.1038/s41598-021-92076-4.
[10] N.V. Semenova, L.V. Rychkova, M.A. Darenskaya, S.I. Kolesnikov, O.A. Nikitina, A. G. Petrova, E.V. Vyrupaeva, L.I. Kolesnikova, Superoxide dismutase activity in male and female patients of different age with moderate COVID-19, Bull. Exp. Biol. Med. 173 (2022) 51–53, https://doi.org/10.1007/s10517-022-05491-6.
[11] J.D. Sachs, S.S.A. Karim, L. Aknin, J. Allen, K. Brosbøl, F. Colombo, G.C. Barron, M. F. Espinosa, V. Gaspar, A. Gaviria, et al., The lancet commission on lessons for the future from the COVID-19 pandemic, Lancet Lond. Engl. 400 (2022) 1224–1280, https://doi.org/10.1016/S0140-6736(22)01585-9.

[12] M.S. Alam, D.M. Czajkowsky, SARS-CoV-2 infection and oxidative stress: pathophysiological insight into thrombosis and therapeutic opportunities, Cytokine Growth Factor Rev. 63 (2022) 44–57, https://doi.org/10.1016/j.cytogfr.2021.11.001.
[13] Centers for disease control and prevention 2019-novel coronavirus (2019-NCoV) real-time RRT-PCR panel primers and probes. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html. (Accessed 10 April 2020).
[14] M.L. Hu, Measurement of protein thiol groups and glutathione in plasma, Methods Enzymol. 233 (1994) 380–385, https://doi.org/10.1016/s0076-6879(94)33044-1.
[15] J. Arnaud, I. Fortis, S. Blachier, D. Kia, A. Favier, Simultaneous determination of retinol, alpha-tocopherol and beta-carotene in serum by isocratic high-performance liquid chromatography, J. Chromatogr. 572 (1991) 103–116, https://doi.org/10.1016/0378-4347(91)80476-s.
[16] V. Witko-Sarsat, M. Friedlander, C. Capeillère-Blandin, T. Nguyen-Khoa, A. T. Nguyen, J. Zingraff, P. Jungers, B. Descamps-Latscha, Advanced oxidation protein products as a novel marker of oxidative stress in uremia, Kidney Int. 49 (1996) 1304–1313, https://doi.org/10.1038/ki.1996.186.
[17] T. Miyazawa, G.C. Burdeos, M. Itaya, K. Nakagawa, T. Miyazawa, Vitamin E: regulatory redox interactions, IUBMB Life 71 (2019) 430–441, https://doi.org/10.1002/iub.2008.
[18] I. Al-Saleh, N. Alrushud, H. Alnuwaysir, R. Elkhatib, M. Shoukri, F. Aldayel, R. Bakheet, M. Almozaini, Essential metals, vitamins and antioxidant enzyme activities in COVID-19 patients and their potential associations with the disease severity, Biometals Int. J. Role Met. Ions Biol. Biochem. Med. 35 (2022) 125–145, https://doi.org/10.1007/s10534-021-00355-4.
[19] N. Žarković, A. Jastrząb, I. Jarocka-Karpowicz, B. Orehovec, B. Baršić, M. Tarle, M. Kmet, I. Lukšić, W. Łuczaj, E. Skrzydlewska, The impact of severe COVID-19 on plasma antioxidants, Mol. Basel Switz. 27 (2022) 5323, https://doi.org/10.3390/molecules27165323.
[20] G. Teskey, R. Abrahem, R. Cao, K. Gyurjian, H. Islamoglu, M. Lucero, A. Martinez, E. Paredes, O. Salaiz, B. Robinson, et al., Glutathione as a marker for human disease, Adv. Clin. Chem. 87 (2018) 141–159, https://doi.org/10.1016/bs.acc.2018.07.004.
[21] P. Kumar, O. Osahon, D.B. Vides, N. Hanania, C.G. Minard, R.V. Sekhar, Severe glutathione deficiency, oxidative stress and oxidant damage in adults hospitalized with COVID-19: implications for GlyNAC (Glycine and N-acetylcysteine) supplementation, Antioxid. Basel Switz. 11 (2021) 50, https://doi.org/10.3390/antiox11010050.
[22] B. Karkhanei, E. Talebi Ghane, F. Mehri, Evaluation of oxidative stress level: total antioxidant capacity, total oxidant status and glutathione activity in patients with COVID-19, New Microbes New Infect 42 (2021), 100897, https://doi.org/10.1016/j.nmni.2021.100897.
[23] A.K. Kalem, B. Kayaaslan, S. Neselioglu, F. Eser, İ. Hasanoglu, A. Aypak, E. Akinci, H.N. Akca, O. Erel, R. Guner, A useful and sensitive marker in the prediction of COVID-19 and disease severity: thiol, Free Radic. Biol. Med. 166 (2021) 11–17, https://doi.org/10.1016/j.freeradbiomed.2021.02.009.
[24] G. Çakırca, T. Damar Çakırca, M. Üstünel, A. Torun, İ. Koyuncu, Thiol level and total oxidant/antioxidant status in patients with COVID-19 infection, Ir. J. Med. Sci. 191 (2022) 1925–1930, https://doi.org/10.1007/s11845-021-02743-8.
[25] A. Martinez Mesa, E. Cabrera César, E. Martín-Montañez, E. Sanchez Alvarez, P. M. Lopez, Y. Romero-Zerbo, M. Garcia-Fernandez, J.L. Velasco Garrido, Acute lung injury biomarkers in the prediction of COVID-19 severity: total thiol, ferritin and lactate dehydrogenase, Antioxid. Basel Switz. 10 (2021) 1221, https://doi.org/10.3390/antiox10081221.
[26] S.L. Lage, E.P. Amaral, K.L. Hilligan, E. Laidlaw, A. Rupert, S. Namasivayan, J. Rocco, F. Galindo, A. Kellogg, P. Kumar, et al., Persistent oxidative stress and inflammasome activation in CD14highCD16- monocytes from COVID-19 patients, Front. Immunol. 12 (2021), 799558, https://doi.org/10.3389/fimmu.2021.799558.
[27] M. Ducastel, C. Chenevier-Gobeaux, Y. Ballaa, J.-F. Meritet, M. Brack, N. Chapuis, F. Pene, N. Carlier, T.-A. Szwebel, N. Roche, et al., Oxidative stress and inflammatory biomarkers for the prediction of severity and ICU admission in unselected patients hospitalized with COVID-19, Int. J. Mol. Sci. 22 (2021) 7462, https://doi.org/10.3390/ijms22147462.
[28] K. Aykac, Y. Ozsurekci, B.C.C. Yayla, S.L. Gurlevik, P.D. Oygar, N.B. Bolu, M. A. Tasar, F.S. Erdinc, G.T. Ertem, S. Neselioglu, et al., Oxidant and antioxidant balance in patients with COVID-19, Pediatr. Pulmonol. 56 (2021) 2803–2810, https://doi.org/10.1002/ppul.25549.
[29] I. Cekerevac, T.N. Turnic, N. Draginic, M. Andjic, V. Zivkovic, S. Simovic, R. Susa, L. Novkovic, Z. Mijailovic, M. Andjelkovic, et al., Predicting severity and intrahospital mortality in COVID-19: the place and role of oxidative stress, Oxid. Med. Cell. Longev. (2021), 6615787, https://doi.org/10.1155/2021/6615787, 2021.
[30] A.C. Gadotti, A.L. Lipinski, F.T. Vasconcellos, L.F. Marqueze, E.B. Cunha, A. C. Campos, C.F. Oliveira, A.N. Amaral, C.P. Baena, J.P. Telles, 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. 165 (2021) 184–190, https://doi.org/10.1016/j.freeradbiomed.2021.01.044.
[31] S. Golabi, S. Ghasemi, M. Adelipour, R. Bagheri, K. Suzuki, A. Wong, M. Seyedtabib, M. Naghashpour, Oxidative stress and inflammatory status in COVID-19 outpatients: a Health center-based analytical cross-sectional study, Antioxid. Basel Switz. 11 (2022) 606, https://doi.org/10.3390/antiox11040606.
[32] E. Grossini, D. Concina, C. Rinaldi, S. Russotto, D. Garhwal, P. Zeppegno, C. Gramaglia, S. Kul, M. Panella, Association between plasma redox state/mitochondria function and a flu-like syndrome/COVID-19 in the elderly admitted to a long-term care unit, Front. Physiol. 12 (2021), 707587, https://doi.org/10.3389/fphys.2021.707587.
[33] A. Orea-Tejada, C. Sánchez-Moreno, O.G. Aztatzi-Aguilar, M.P. Sierra-Vargas, D. González-Islas, Y. Debray-García, M.S. Ortega-Romero, C. Keirns-Davis, L. Cornejo-Cornejo, J. Aguilar-Meza, Plasma endothelial and oxidative stress biomarkers associated with late mortality in hospitalized COVID-19 patients, J. Clin. Med. 11 (2022) 3950, https://doi.org/10.3390/jcm11143950.
[34] F. Mehri, A.H. Rahbar, E.T. Ghane, B. Souri, M. Esfahani, Changes in oxidative markers in COVID-19 patients, Arch. Med. Res. 52 (2021) 843–849, https://doi.org/10.1016/j.arcmed.2021.06.004.
[35] N. Zarkovic, A. Jakovcevic, A. Mataic, M. Jaganjac, T. Vukovic, G. Waeg, K. Zarkovic, Post-mortem findings of inflammatory cells and the association of 4-hydroxynonenal with systemic vascular and oxidative stress in lethal COVID-19, Cells 11 (2022) 444, https://doi.org/10.3390/cells11030444.
[36] A.F. Rendeiro, H. Ravichandran, Y. Bram, V. Chandar, J. Kim, C. Meydan, J. Park, J. Foox, T. Hether, S. Warren, et al., The spatial landscape of lung pathology during COVID-19 progression, Nature 593 (2021) 564–569, https://doi.org/10.1038/s41586-021-03475-6.
[37] N. Yaghoubi, M. Youssefi, F. Jabbari Azad, F. Farzad, Z. Yavari, F. Zahedi Avval, Total antioxidant capacity as a marker of severity of COVID-19 infection: possible prognostic and therapeutic clinical application, J. Med. Virol. 94 (2022) 1558–1565, https://doi.org/10.1002/jmv.27500.
[38] U.S. Tantry, K.P. Bliden, A. Cho, N. Walia, J.R. Dahlen, G. Ens, M. Traianova, C. Jerjian, A. Usman, P.A. Gurbel, First experience addressing the prognostic utility of novel urinary biomarkers in patients with COVID-19, Open Forum Infect. Dis. 8 (2021), ofab274, https://doi.org/10.1093/ofid/ofab274.
[39] H. Zhan, H. Chen, C. Liu, L. Cheng, S. Yan, H. Li, Y. Li, Diagnostic value of D-dimer in COVID-19: a meta-analysis and meta-regression, Clin. Appl. Thromb. Off. J. Int. Acad. Clin. Appl. Thromb. 27 (2021), 10760296211010976, https://doi.org/10.1177/10760296211010976.
[40] L. Tan, Q. Wang, D. Zhang, J. Ding, Q. Huang, Y.-Q. Tang, Q. Wang, H. Miao, Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study, Signal Transduct. Targeted Ther. 5 (2020) 33, https://doi.org/10.1038/s41392-020-0148-4.
[41] B. Fialek, M. Pruc, J. Smereka, R. Jas, M. Rahnama-Hezavah, A. Denegri, A. Szarpak, M.J. Jaguszewski, F.W. Peacock, L. Szarpak, Diagnostic value of lactate dehydrogenase in COVID-19: a systematic review and meta-analysis, Cardiol. J. 29 (2022) 751–758, https://doi.org/10.5603/CJ.a2022.0056.
[42] X. Dong, L. Sun, Y. Li, Prognostic value of lactate dehydrogenase for in-hospital mortality in severe and critically ill patients with COVID-19, Int. J. Med. Sci. 17 (2020) 2225–2231, https://doi.org/10.7150/ijms.47604.
[43] S. Katzenschlager, A.J. Zimmer, C. Gottschalk, J. Grafeneder, S. Schmitz, S. Kraker, M. Ganslmeier, A. Muth, A. Seitel, L. Maier-Hein, et al., Can we predict the severe course of COVID-19 - a systematic review and meta-analysis of indicators of clinical outcome? PLoS One 16 (2021), e0255154 https://doi.org/10.1371/journal.pone.0255154.