Open Clinical Trial of the Use of Antioxidants and Pentoxifylline as Coadjuvant Measures to Standard Therapy to Improve Prognosis of Patients with Pneumonia and Septic Shock due to Covid-19

Adrián Chavarria
Critical Care Unit of the Temporal COVID-19 Unit, Citibanamex Center.
Rafael Ricardo Vázquez
Critical Care Unit of the Temporal COVID-19 Unit, Citibanamex Center.
José Guillermo Cherit
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
Héctor Bello
Critical Care Unit of the Temporal COVID-19 Unit, Citibanamex Center.
Humberto Suastegui
Critical Care Unit of the Temporal COVID-19 Unit, Citibanamex Center.
Lidia Castañeda
Critical Care Unit of the Temporal COVID-19 Unit, Citibanamex Center.
Gabriela Estrada
Critical Care Unit of the Temporal COVID-19 Unit, Citibanamex Center.
Fabián Valdez
Critical Care Unit of the Temporal COVID-19 Unit, Citibanamex Center.
Omar Marcos
Critical Care Unit of the Temporal COVID-19 Unit, Citibanamex Center.
Huitzilihuitl Orozco
Instituto Nacional de Cardiología
Linaloe Pech
Instituto Nacional de Cardiología
Ricardo Velasco
Instituto Nacional de Cardiología
Verónica Lans
Instituto Nacional de Cardiología
Israel Torres (pertorisr@yahoo.com.mx)
Instituto Nacional de Cardiología
Maria Elena López
Instituto Nacional de Cardiología

Research Article

Keywords: SARS-CoV-2, Antioxidants, Sepsis, COVID-19, Pneumonia

Posted Date: December 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-122123/v1

Version of Record: A version of this preprint was published on February 27th, 2021. See the published version at https://doi.org/10.1016/j.csbj.2021.02.009.

OPEN CLINICAL TRIAL OF THE USE OF ANTIOXIDANTS AND PENTOXIFYLLINE AS COADJUVANT MEASURES TO STANDARD THERAPY TO IMPROVE PROGNOSIS OF PATIENTS WITH PNEUMONIA AND SEPTIC SHOCK DUE TO COVID-19

Adrián Palacios Chavarria[1,3†], Rafael Ricardo Valdez Vázquez[1†], José Guillermo Domínguez Cherit[2], Héctor Herrera Bello[1], Humberto Castillejos Suastegui[1], Lidia Moreno-Castañeda[1], Gabriela Alanís Estrada[1], Fabián Hernández[1], Omar González-Marcos[3], Huitzilihuitl Saucedo-Orozco[4], Linaloe Manzano Pech[6], Ricardo Márquez[4], Verónica Guarner-Lans[5], Israel Pérez Torres[6*] and Maria Elena Soto[1,4,3*].

[1,†]Critical Care Unit of the Temporal COVID-19 Unit, Citibanamex Center Av. del Conscripto 311, Lomas de Sotelo, Hipódromo de las Américas, Miguel Hidalgo, 11200 Ciudad de México, CDMX and American British Cowdray Medical Center.

[2]Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" Vasco de Quiroga 15, Sección XVI, Tlalpan, México City 14000, México and Tecnológico de Monterrey EMCS.

[3]Pharmacist American British Cowdray (ABC) Medical Center, I.A.P. ABC I.A.P. ABC Sur 136 No. 116 Col. Las Américas, México City 01120.

[4]Departament of Immunology, Instituto Nacional de Cardiología Ignacio Chávez. Juan Badiano 1, Sección XVI, Tlalpan, México City 14080, México.

[5]Departament of Physiology, Instituto Nacional de Cardiología Ignacio Chávez. Badiano 1, Sección XVI, Tlalpan, México City 14080, México.

[6]Departament of Cardiovascular Biomedicine, Instituto Nacional de Cardiología Ignacio Chávez. Badiano 1, Sección XVI, Tlalpan, México City 14080, México.

*Correspondence: The authors Maria Elena Soto and Israel Pérez-Torres share author correspondence. Mails; mesoto50@hotmail.com Tel.: +52-5573-2911 (25707) and pertorisr@yahoo.com.mx; Tel.: +52-5573-2911 (25203); Fax: +52-5573-0926 respectively.

[†]The authors Adrian Palacios Chavarria and Rafael Valdez Vázquez share first authorship of this study.

Abstract

Background: SARS-CoV-2 infection produces pneumonia with pulmonary alveolar collapse and in some cases sepsis and septic shock. There is no specific treatment for COVID-19. Vitamin C (Vit C), Vitamin E (Vit E), N-acetylcysteine (NAC) and Melatonin (MT) increase the intracellular content of GSH, kidnap free radicals and protect DNA, proteins in the cytosol and lipids of cell membranes. Pentoxifylline (Px) has anti-inflammatory activities.

Methods: Here we evaluate the effect of Vit C, Vit E, NAC, and MT plus Px in COVID-19 patients with moderate and severe pneumonia. 110 patients of either sex were included. They were divided into five groups with 22 patients each. Group 1 received Vit C+Px, group 2 Vit E+Px, group 3 NAC+Px, group 4 MT+Px, and group 5 only Px. Oxidative stress markers as Lipid peroxidation levels, evaluation of total antioxidant capacity and nitrites were evaluate by spectrophotometry, and by ELISA assay IL-6 levels.

Results: The antioxidant therapy improved the survival scores including SOFA, Apache II, SAPS II, COVIDGRAM and GCS. OS markers in plasma such as LPO ($p \leq 0.04$) and TAC ($p \leq 0.03$) decreased in COVID-19 patients administered with antioxidants. There was an increase of IL-6 ($p \leq 0.01$) and decreases of CRP ($p \leq 0.01$) and PCT ($p \leq 0.05$) in COVID-19 patients when entering the hospital and the different antioxidants reversed this alteration at the end of the hospital stay.

Conclusions: This study confirms the presence of OS in COVID-19 patients. The results suggest that the treatment with antioxidant supplements such as Vit C, E, NAC, and MT plus Px could contribute to the deceleration of the aggressive and

lethal development COVID-19. There is evidence that antioxidants in moderate doses decrease inflammation and control of OS; therefore, their use as an adjuvant therapy to improve prognosis is confirmed. The antioxidant therapy can be effective in this pandemia since it improves all of the survival scores including SOFA, Apache II, SAPS II, COVIDGRAM, GCS by lowering the LPO, IL-6, CRP, PCT and increasing systemic TAC.

Keywords: SARS-CoV-2, Antioxidants, Sepsis, COVID-19, Pneumonia

Background

COVID-19 is caused by the SARS-CoV-2 virus and results in pneumonia with different presentations ranging from mild to severe. Pneumonia is defined as a lung inflammation caused by bacterial or viral infections in which the air sacs fill with pus and may solidify. In patients with the severe form of the disease pneumonia leads to pulmonary alveolar collapse with cessation of oxygen exchange. Patients also show dyspnea, (respiratory rate ≥30/min), decreased blood oxygen saturation (≤93%), reduced ratio of partial pressure of arterial oxygen to fraction of inspired oxygen (<300) and/or presence of pulmonary infiltrates (>50%) within 24 to 48 hours [1]. There may also be respiratory distress. Lymphopenia, lactate, creatinine and kinase dehydrogenase levels are elevated, and there are increased concentrations of interleukins such as IL-1β, IL-5, IL-7, IL-8, IL-9, IL-10, IL-15, IL-12p70, FGF, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1A, MIP1B, PDGF, TNF-α and VEGF. Alterations in the redox homeostasis in respiratory tract infected cells are one of the key events that is linked to infection and to inflammation [2].

Patients with moderate and severe pneumonia by COVID-19 may develop sepsis which is defined as life-threatening organ dysfunction caused by the host's inadequate response to infection. Septic shock is a consequence of sepsis and is associated with multiple organ failure (MOF) and high mortality [3]. Sepsis is the leading cause of mortality in intensive care units (ICU) worldwide, reaching up to 80% in patients with MOF [4]. In experimental models and in humans with septic shock, there is a high production of reactive species oxygen (ROS), nitric oxide (NO) and reactive nitrogen species (RNS) that can cause multiple organ failure (pulmonary, cardiac, neurological, and hepatic). During septic shock, there is an increase in cardiac output accompanied by a decrease in systemic vascular resistance caused by arterial dilation. Excessive or prolonged reduction in vascular resistance produces vasopressor-resistant hypotension, which contributes to severe heart failure [5].

There is no specific treatment for COVID-19. Most treatments are accompanied by measures that decrease the inflammatory condition known as the cytokine storm [6]. However, much less attention has been placed in the use of antioxidant therapy. ROS are produced by phagocytic leukocytes such as neutrophils, monocytes, macrophages, and eosinophils during invasion of the tissues by microorganisms, and may lead to cytotoxicity and cellular injury. ROS also participate the mechanism of inflammation by activating NFkB resulting in the transcription of cytokine producing genes that may be involved in the cytokine storm present in COVID-19 patients [1].

The use of antioxidant therapy for septic shock was proposed since Hippocrates, who used myrrh (Commiphora mukul, Commiphora myrrha) for therapeutic and anti-inflammatory medicinal purposes. The supplementation with antioxidants improves oxygenation rates, leads to higher levels of GSH and strengthens the immune response [3]. In addition, with its use, the hospital stay is reduced as well as the time of mechanical ventilation, the length of the ICU stay, multiple organ dysfunction rates, and the mortality rate in patients with acute lung injury (ALI) and reduce the sequential organ failure assessment score (SOFA). Among antioxidants Vitamin C (Vit C), Vitamin E (Vit E), N-acetylcysteine (NAC), melatonin (MT) and pentoxifylline (Px) have been proposed [3]. Therapies using these agents might reduce the effects of COVID-19 on several organs.

Vit C is a cofactor of multiple enzymes. It inhibits the production of superoxide (O_2^-) and peroxynitrite ($ONOO^-$) by inhibiting the O_2^- producing NADPH oxidase and the expression of the mRNA of the inducible nitric oxide synthase (iNOS) [7]. Furthermore, patients with sepsis that receiving Vit C, have a greater decrease in the SOFA score compared with placebo groups [8]. Vit E is considered as the most important lipophilic antioxidant in cell membranes, lipids, plasma, and red blood cells. [9]. It protects cell membranes from lipid peroxidation (LPO) by ending the lipid chain reaction. It also functions as an O_2^- and hydroxyl radical (OH) scavenger. A study ex vivo that evaluated the effect of Vit E on the production of O_2^- in septic and non-septic patients in the ICU, showed that in the septic patients, the administration of Vit E significantly decreased the O_2^- overproduction through inactivation of the NADPH oxidase [10]. A study in humans with 7469 elderly men

with community acquired pneumonia reported that this condition was lowered by treatment with vitamin E [11]. Supplementation with nutrients that are a source of vitamin E, controls nutritional deficiencies, overweight and promotes an adequate nutritional status in COVID-19 patients, improving the immune response and the antioxidant status during the infective phase.

NAC is a glutathione (GSH) precursor. It increases the activities of redox enzymes that employ GSH such as glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), thioredoxin reductase (TrxR) [12]. It also has a direct action on ROS, thus limiting oxidative lung injury [13], and may reduce the levels of IL-8, IL-6 and ICAM. The use of NAC in patients with septic shock was associated with a shorter time of mechanical ventilation and a shorter stay in the ICU [14].

MT sequesters ROS, thus protecting lipids in cell membranes, proteins in the cytosol, DNA, and mitochondrias [15]. In vitro and in vivo studies have shown that it can prevent increases in LPO [16], preserve cellular membrane permeability by increasing its fluidity [17], and reduce levels of H_2O_2 in mitochondria and cytosol by restoring GSH homeostasis [16], through the stimulation of the activity of the ɣ-glutamyl cysteine synthase and glutathione synthase. These actions increase the intracellular synthesis of GSH [18]. MT may also stimulate antioxidant enzymes, such as CAT, SOD isoforms, GPx and GR [19]. SARS-CoV2 infection leads to inflammation in the lungs by pyroptosis [20]. COVID-19 patients suffer from anxiety and lack of sleep and MT improves sleep habits, reduces anxiety and stimulates immunity. It has therefore been proposed as an adjuvant therapy [21].

Pentoxifylline (Px), exerts several antioxidant and anti-inflammatory activities, such as the maintenance of GSH levels and mitochondrial viability, the inhibition of the production of TNF-α and the preservation of vascular endothelial functions [22].

In addition, a recent work by our group showed that the antioxidant therapy with Vit C, Vit E, NAC and MT in patients with septic shock reduced MOF, the SOFA score, the time in ICU, oxidative stress (OS) and inflammation [3]. Therefore, the aim of this study was to evaluate the effect of the use of Vit C, Vit E, NAC, MT and Px on the outcome of patients with moderate and severe COVID-19 with and without sepsis.

Materials and Methods

Description of the study population

This was an open, quasi-experimental, analytical, prospective, and longitudinal (before-after) study run between August 20 and September 20, 2020. The study population consisted of patients over 18 years of age who were admitted to the ICU of the CITIBANAMEX Center and who developed or not septic shock, secondary to moderate or severe pneumonia due to COVID-19. The diagnostic criteria for septic shock were based on the Sepsis-3 consensus [23]. Patients considered to have septic shock had to have an acute increase of at least 2 points in the SOFA score [24], lactate levels greater than 2 mmol/L and they had to be dependent on a vasopressor for at least 2 hours before the time of enrollment. Exclusion from this study occurred when patients were younger than 18 years, were not able to grant an informed consent, refused to be included, if pregnant or

breastfeeding or if they were under chronic use (last 6th months) or recent use of steroids, statins or antioxidants. Patients were also excluded if there was any contraindication for the use of Vit C, Vit E, NAC, MT and Px.

The hospitalized patients included were considered as moderate or severe according to the ventilatory status. Patients with the severe condition required invasive mechanical intubation according to the criteria of Berlin for Acute respiratory distress syndrome (ARDS). The Berlin definition proposes 3 categories of ARDS based on the severity of hypoxemia: mild (200 mm Hg<Pao2/Fio2≤300 mm Hg), moderate (100 mm Hg<Pao2/Fio2≤200 mm Hg), and severe (Pao2/Fio2 ≤100 mm Hg), along with explicit criteria related to timing of the syndrome's onset, origin of edema, and the chest radiograph findings. The ARDS definition task force was considered [25].

Ethical approval was obtained from the local ethics committee on August 19th, 2020 (Control-9867/2020, register REG. CONBIOETICA-09-CEI-011-20160627). A written informed consent for enrollment or consent to use patient data was obtained from each patient or their legal surrogate. The protocol was registered (TRIAL REGISTRATION: ClinicalTrials.gov Identifier: NCT 04570254).

Study Measurements and Procedures

Collection samples to verify infection by SARS-CoV-2

In the study design, samples from patients and from personnel engaged in clinical and laboratory activities were collected. Paired saliva and nasopharyngeal swab samples were collected from 110 patients who were suspected to be infected by

SARS-CoV-2. Samples were classified as positive for SARS-CoV-2 when both the N1 and N2 primer-probe sets were detected. The presence of the SARS-CoV-2 virus was evaluated for (COVID-19) using specific probes for the detection of the virus in conjunction with the real-time reverse transcriptase polymerase chain reaction technique (qRT-PCR).

To evaluate organ dysfunction, the SOFA score (neurologic, respiratory, hemodynamic, hepatic, and hematologic) was calculated at admission and during all of the days of treatment [26].

Standard therapeutic management

The treatment applied during hospitalization was chosen according to standard maneuvers considering the requirements of each individual patient, the hemodynamic and electrolyte demands, and the ventilator demands. Treatment was initiated during the first hour after admission and before the recognition of the presence or absence of septic shock. Cultures were performed on admission before starting the administration of a broad-spectrum intravenous antibiotic. In this study, only one patient required management with antibiotics since the coexistence with pseudomonas infection was found and the patient was treated with Meropenem. In all patients, the use of hydroxychloroquine or antivirals was not considered, since they received individualized management according to the algorithm proposed [1].

Management with crystalloid solutions and/or albumin was considered depending on the hemodynamic status, by means of dynamic indicators. Use of vasopressors to maintain a MAP ≥65 mmHg was given if necessary. Norepinephrine was the first

option and/or vasopressin was used when there was a need to increase the MAP or reduce the dose of norepinephrine. Inotropics were administered in cases of myocardial dysfunction (dobutamine). Transfusion of blood packs were applied in case of a decrease in hemoglobin (<7.0 g/dl) in the absence of myocardial ischemia, severe hypoxemia or severe bleeding. Mechanical ventilation with tidal volumes of 6 ml/kg was used in patients with ARDS. Plateau pressure was maintained at less than 30 cmH_2O, and alveolar conduction pressure of less than 13 cmH_2O. PEEP (Positive end expiration pressure) titration was managed by the use of the FiO_2/PEEP (Fraction inspired of oxygen/Positive end expiration pressure) according to the table published by Ancukiewicz [27]. The treatment with anticoagulants was based on the Thachil guidelines [28]. The management with the prone position was necessary in patients with PaO_2/FiO_2 of less than 150 [29].

In all patients, the standard therapeutic management with dexamethasone 8 mg i.v. each 24 hours for 7 days was applied between day 1 and 21 of the onset of symptoms when not contraindicated. It was contraindicated when there was a requirement of 0_2 greater than 3 L, progressive requirement of = 2, $PAO_2/FiO_2 \leq$ 250 mmHg, use of O_2 plus bilateral infiltrates in the radiography, use of O_2 plus DHL ≤ 250U/L or ferritin ≥ 300 or DD ≥ 1000ng/mL, CPK ≥ 2 times the upper normal value. The following conditions were not considered as contraindications or relative contraindications: glucose greater than 250 mg/dL with hypoglycemic, hypokalemia <3.3 meq, blood pressure > 155/95 mmHg with antihypertensive treatment, glaucoma, triglycerides > 500 mg/dL (start treatment), history of known peptic ulcer or bleeding from recent gastrointestinal tract, untreated or

decompensated dementia or psychiatric illness, use of non-potassium sparing diuretics or use of inhaled B2 agonists.

The next conditions were monitored at follow-up: pre-prandial capillary glucometer (7-13-19 hrs.) for 10 days, even in fasting patients, blood pressure per shift and basal potassium every 72 hours.

Antioxidant therapy

To administer the antioxidant therapy, a medical management algorithm was used considering the presence of comorbidities in each patient; this algorithm was previously reported by our group [1]. The antioxidant was adjusted to each comorbid condition or to the presence of potential allergies or heart rhythm disorders due to each individual history. All antioxidants were orally administered or administered by a nasogastric tube, depending on the clinical status of the patient during the 5 days of the treatment. All data entry was monitored at the coordinating center, with site visits for source data verification.

Doses of the antioxidant therapy

Five treatments were used each in an independent group of twenty-two patients. Group 1; Vit C tablets of 1 g were administered every 12 hours by oral route or naso-enteral tube for 5 days. Group 2; Vit E (α-tocopheryl acetate) capsules of 400 IU equivalent to 800 mg were administered every 12 hours for 5 days. Group 3; effervescent tablets of NAC 600 mg were used every 12 hours by the oral route or naso-enteral tube for 5 days. Group 4; MT of 5 mg (50 mg) (10 prolonged-release capsules) were given every 12 hours by oral route or naso-enteral tube for 5 days.

Group 5; Px tablets of 400 mg a dose every 12 hours by oral route or naso-enteral tube for 5 days. Additionally, groups 1-4 received Px at the same concentration as in group 5.

Blood Sample obtainment and storage

Blood samples were obtained from each patient that entered the draw, before initiation of the treatment and 48 hours after the administration of the antioxidant. The blood samples were centrifuged for 20 min at 936 g and 4°C. The plasma of the samples were placed in 3 or 4 aliquots and stored at -30 °C.

Laboratory tests were made in patients with COVID-19 to determine acute-phase reactants, hemoglobin, leukocytes, lymphocytes, platelets, creatinine, urea nitrogen, glucose, C-reactive protein (CRP), albumin, D-dimer, ferritin, fibrinogen, procalcitonin (PCT), interleukin-6 (IL-6) and oxygen saturation. Data from the patient's medical history including demographic, prior illnesses to infection by SARS-CoV-2, test result for COVID-19, whether mechanical ventilation was used, and type of treatment given were used for the analysis of the results. Additionally, other biochemical variables were determined in plasma such as OS markers, lipid peroxidation (LPO), total antioxidant capacity (TAC), and nitrite (NO_2^-).

Oxidative stress markers

Nitrites

The NO_2^- levels in plasma were determined by the Griess reaction. 100 µl of plasma previously deproteinated with 0.5 N, NaOH and 10%, $ZnSO_4$ were

centrifuged at 5000 rpm for10 min. The supernatant was recovered and 200 µl of sulfanilamide1% and 200 µl of N-naphthyl-ethyl diamine 0.1% were added. The total volume was adjusted to 1 ml. The calibration curve was obtained with solution KNO_2 of 5-0.156 nM and the absorbance was measured at 540 nm.

Lipid peroxidation levels

50 µl CH_3-OH with 4% BHT plus phosphate buffer pH 7.4 were added to 100 µl of plasma. The reaction tube was incubated to 100°C for 1 hour and centrifuged at 4000 rpm at room temperature for 2 min. Then, the n-butanol phase was extracted, and the absorbance was measured at 532 nm [30].

Evaluation of total antioxidant capacity

100 µl of plasma were suspended in 1.5mL of a reaction mixture prepared as follows: 300 mM acetate buffer pH 3.6, 20 mM hexahydrate of ferric chloride, and 10mM of 2,4,6-Tris-2- pyridyl-s-triazine dissolved in 40mM chlorhydric acid. These reactants were added in a relation of 10:1:1 v/v, respectively. After mixing, the samples were incubated at 37°C for 15 min in the dark. The absorbance was measured at 593 nm [30].

Interleukin-6 concentration

IL-6 levels were measured in plasma samples by enzyme-linked immunosorbent assay (ELISA) using a commercial kit according to the manufacturer's instructions (BioLegend, San Diego, CA, USA).

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation or median with minimum and maximum, depending on their distribution. Categorical variables are expressed as frequencies and percentages. The normality of the variables was evaluated using the Shapiro-Wilk or Shapiro-France test, depending on the size of the sample. A graphical analysis of the distribution of the variables was also performed with histograms and/ or stem and leaf graphs. We used non-parametric tests (Mann-Whitney test, Kruskal-Wallis t, depending on the particular case) to contrast variables without Gaussian distribution. The analysis of paired samples (before-after) was carried out with Friedman or Wilcoxon signed rank test depending on the distribution of the data. The Pearson correlation was used for the estimation of biomarkers. Statistical analyses were performed with SPSS version 26. The Sigma Plot 14 program (Jendel Corporation, 1986-2017) was used to generate the analysis and graphs of the OS markers and statistical significance was determined by the Mann-Whitney rank sum test followed by the normality test (Shapiro-Wilk). Differences were considered statistically significant when $p \leq 0.05$.

Results

Demographic characteristics

A total of 110 patients were examined, of which 78 (71%) were men and 32 (29%) were women. Patients had an age range of 57.9 ± 12.8 years. In all patients, infection by SARS-CoV-2 was diagnosed through a CRP-test. The demographic characteristics of the patients are shown in Table1.

The average body mass index was 29.1 ± 4.1 kg/m^2 with normal weight in 13 (12%) subjects, overweight in 52 (47%) and obesity in 45 (41%). Comorbid conditions prior to SARS-CoV-2 infection were, dyslipidemia 52 (47.3%), systemic arterial hypertension 44 (40%) diabetes mellitus 41 (37%), chronic obstructive lung disease 5 (4.5%), chronic kidney disease 4 (4%) and coronary heart disease 4 (3.6%). There were no patients with cancer, liver disease or previous autoimmune diseases or infection associated with bacteria and fungi.

The main symptoms at the time of hospital admission were cough 74 (67%), fever 73 (66%) headache 51 (46.4%), anosmia 26 (24%), dysgeusia 23 (21%), conjunctivitis 10 (9%), diarrhea 14 (13%), arthralgia 39 (35.5%), myalgia 43 (39%), pharyngodynia 23 (21%), rhinorrhea 16 (14.5%).

Interventional treatment was warranted. 35 patients required invasive mechanical ventilation and in 75 required non-invasive techniques at the time of admission. The days required of invasive mechanical ventilation and length of stay in the ICU in patients with severe condition had a median of 13 days with a minimum of 2 and a maximum of 30 days. The total time of hospital stay had a median of 17 days with a minimum of 6 and a maximum of 36 days.

Thirty-one patients developed septic shock and were kept in the ICU. Of them, 23 were admitted with this condition and 8 were admitted without data of septic shock and developed this condition during their stay in the ICU. Three patients died out of which one developed an infection by pseudomonas. Of the other two patients who did not develop septic shock and died, one had end-stage chronic kidney failure

and the other an acute myocardial infarction. Regarding adverse effects, only one patient had an allergic reaction to the antioxidant that was treated without presenting consequences. The indexes for organic and physiological severity, risk, thrombo-prophylaxis, and state of consciousness were evaluated in the experimental groups at the time of admission and throughout the in-hospital period and are shown in Table 2 according to the severity of the condition. The survival rate to 30 days and 90 days was of 90% as shown in Table 3.

Oxidative stress markers in plasma

The LPO index in the general group (moderate and severe) of the patients in the basal condition (entrance to the hospital) showed a significant increase in all groups. After of the antioxidant therapy there was a decrease in the Vit E+Px, NAC+Px and MT+Px groups in comparison with the basal conditions ($p=0.002$, $p=0.004$ and $p<0.001$ respectively, Figure 1A). However, when the patients were separated into moderate and severe, the LPO index in moderate patients showed a significant decrease in the Vit C+Px, NAC+Px and MT+Px groups in comparison with the basal conditions ($p=0.01$, $p=0.04$ and $p=0.01$ respectively, Figure 1B). In the severe patients the LPO index was decrease in the Vit E+Px and NAC+Px groups in comparison with basal conditions ($p=0.02$ and $p=0.05$ respectively, Figure 1C).

The TAC in the general patient group (moderate and severe) under basal conditions (entrance to hospital) showed a significant decrease in the Vit C+Px and NAC+Px groups. After of the antioxidant therapy there were increases ($p=0.02$,

and p=0.03 respectively) in comparison to the basal conditions, Figure 2A. There was only a tendency to a decrease in the patients treated with Vit E+Px (p=0.06, Figure 2A). However, when the patients were separated into moderate and severe, the TAC in the moderate patients showed a significant increase in the Vit C+Px group (p=0.01, Figure 2B). In the severe patients the TAC was increase in the group treated with NAC+Px (p=0.03, Figure 2C).

The NO_2^- concentration in the general patient group (moderate and severe) in basal conditions (entrance to hospital) showed a significant decrease in all groups. After the antioxidant therapy there was an increase in the Vit C+Px, Vit E+Px, NAC+Px and MT+Px groups ($p<0.001$). However, in the Px group there was only a p=0.02 significance against the basal conditions, Figure 3A. However, when the patients were separated into the moderate and severe presentations, the NO_2^- in the moderate patients showed a significant increase in the Vit C+Px, Vit E+Px, NAC+Px and MT+Px groups ($p<0.001$ and p=0.04 respectively, Figure 3B). In the severe patients the NO_2^- was increased in the Vit C+Px, Vit E+Px, NAC+Px and MT+Px groups (p=0.002, $p<0.001$, p=0.004 and p=0.04 respectively, Figure 3C).

Table 3 shows that the IL-6 levels in the general patient group (moderate and severe) in basal conditions (entrance to hospital) showed a significant increase in all groups. After of the antioxidant treatment, there was a decrease in the Vit C+Px, Vit E+Px, and NAC+Px groups ($p\leq0.01$), but in the MT+Px group there was only a tendency to decrease (p=0.07). However, when the patients were separated into moderate and severe, the IL-6 levels in moderate patients, showed a significant decrease in all of the groups except the Px only group (p=0.02, p=0.04, p=0.005 and p=0.001 respectively). In the severe patients the IL-6 levels were decreased

only in the Vit E+Px group (p=0.02), and there was a tendency to a decrease in the MT+Px group (p=0.08). Furthermore, the same table shows that the CRP levels in the general patient group under basal conditions (entrance to hospital) showed a significant increase in all groups. After the antioxidant treatment there was a decrease in all groups (p≤0.01). In addition, when the patients were separated into moderate and severe, the CRP levels in the moderate patients, showed a significant decrease in all groups except in the NAC+Px group (only with tendency (p=0.06) in comparison with basal conditions (p=0.04, p=0.02, p=0.005 and p=0.001 respectively). In the severe patients, the CRP levels were decreased only in the NAC+Px and Px groups (p=0.01 and p=0.04 respectively). In addition, the PCT levels in the general patient group under basal conditions (entry hospital) showed a significant increase in the Vit C+Px, Vit E+Px and NAC+Px groups. After of the antioxidant treatment it was decreased (p=0.04, p=0.004 and 0.01 respectively). However, when the patients were separated into moderate and severe, the CPR levels in the moderate patients, showed a significant decrease in the Vit C+Px, Vit E+Px and MT+Px groups (p=0.05, p=0.04 and p=0.03 respectively). In the severe patients the CRP levels was decreased only in the Vit E+Px and NAC+Px groups (p=0.04 and p=0.01 respectively, Table 4).

Discussion

There is still no vaccine against SARS-CoV-2, and despite the overwhelming information in the literature, the effects and interactions of multiple therapies that may be used against COVID-19 are still unknown, and there is not yet an effective therapy. A two-phase combined therapy strategy has proven to be the most effective measure for the treatment of COVID-19. The first phase aims to lower the

viral load that is the source and origin of the chronic inflammatory condition leading to severe sepsis and MOF. The second phase reduces the septic condition, thereby reducing MOF, and the uncontrolled cytokine storm. The use of antioxidants might decrease the uncontrolled inflammatory response without collateral effects. Since the two phases occur simultaneously, the therapeutic strategy should be selected avoiding subsequent collateral damage in patient with COVID-19. The aim of this study was to evaluate the use of antioxidants such as Vit C, Vit E, NAC MT and Px (anti-inflammatory) in patients with moderate and severe infection by SARS-CoV-2. There is evidence that most people infected with SARS-CoV-2 (81%) have mild or uncomplicated forms of the disease [31]. However, some patients develop the serious form of the illness requiring oxygen therapy (14%) and approximately 5% need treatment in the ICU. Most critically ill patients require mechanical ventilation [32].

The most common condition in patients with severe COVID-19 is pneumonia, and the death rate by severe pneumonia in China was from 15% [33]. In this COVID-19 pandemic, severe pneumonia and septic shock are the leading causes of morbidity and mortality in ICU around the world.

Regarding the pathophysiological mechanisms responsible for this disease, OS seems to play and important role and the use of antioxidant therapies could be effective. Our group has found that antioxidant therapy is useful in other conditions such as sepsis [3] In chronic obstructive pulmonary disease (COPD), ALI, and ARDS, there is an increase in ROS [34], which is associated with a high release of pro-inflammatory mediators including IL-6, IL-8, and TNFα by bronchial epithelial cells and alveolar macrophages [35]. ROS can activate neutrophils and

macrophages, resulting in destruction of the alveolar wall and collapse of small airways [36]. These changes can induce endothelial damage, pulmonary capillary hyperpermeability, and pulmonary edema, resulting in impaired pulmonary gas exchange [37]. Furthermore, during severe sepsis in ALI/ARDS, where there is life-threatening organ dysfunction caused by an inadequate host response to infection, the cardiovascular system increases cardiac output and decreases peripheral resistance, adopting a hemodynamic profile that leads to arterial dilation. An excessive drop in peripheral resistance or its prolonged time lead to progressive hypotension that is refractory to catecholamines can contribute to severe cardiovascular failure [38].

In different experimental animal models and in humans with severe septic shock, there is high production and release of O_2^- and $ONOO^-$ by different pathways that contributes to the failure of the lungs, heart, brain, and liver [39]. Although clinical data are limited, many viral diseases such as SARS-CoV can lead to moderate and severe septic shock and increase ROS. This condition is associated with overexpression of iNOS, NADP oxidases, cyclooxygenase 2 and xanthine oxidase which activate the transcription of factors such as NF-κB resulting in an exacerbated pro-inflammatory host response [1]. In addition, O_2^- and $ONOO^-$ participate as important mediators of a pro-inflammatory interleukin storm, which may stimulate the production and release of more ROS. This can interfere with mitochondrial respiration and ATP depletion, since mitochondrial dysfunction is commonly induced in an environment of septic shock [40].

Our results show that the OS markers in plasma such as LPO and TAC and the IL-6, CRP and PCT were increase and decrease respectively in COVID-19 patients

when they entered the hospital. The use of the different antioxidants including Vit C +Px, Vit E+Px, NAC+Px and MT+Px, reversed this alteration at the end of the hospital stay. However, in the group that received only Px, there were no significant changes in OS markers. However, IL-6 and CRP were modified in this group. This suggests an additive effect between Px which has anti-inflammatory properties which is associated with the antioxidant capacity that is shown by each of the antioxidants used in this study.

Vit C is a potent natural antioxidant that primarily removes ROS which are over-produced in inflammation. Alveolar epithelial type II cells in the lung require high concentrations of intercellular Vit C (mM) to sustain their pivotal functions in innate immunity [41]. Low levels of Vit C are present in patients with septic shock [42]. This decrease may be caused by an inadequate intake, acute or chronic consumption secondary to an OS increase and/or an increase in the loss of this vitamin. Levels of <10 µmol/L have been reported in critically ill patients despite the administration of the recommended daily requirements of Vit C, in the CITRIS-ALI trial, was recommended intravenous administration of 200 mg/kg/day of vitamin C for 4 days to reduce mortality of 30% [43] Also, there is a decreased level of Vit C related to the severity of MOF. However, in a phase I study the Vit C administration at two different doses (50 mg/kg/24 hours and 200 mg/kg/24 hours) versus placebo in patients with severe sepsis; decrease the SOFA score when compared to the placebo group. No adverse effects were reported. However, in the VITAMINS trial study with ARDS although there was not a significant difference in the SOFA score, there were decreased CRP levels and reduced mortality. [44] In another study with ARDS patients, there were decreased levels of CRP after the Vit C

treatment [45]. In an in vivo mice model of sepsis, the administration of Vit C (100 mg/kg bw i.v.) attenuated the elevation of the serum aminotransferase, TNFα, cyclooxygenase 2 mRNAs and of LPO [46]. In another study, Vit C (100 mg/kg bw i.p.) or vitamin E (15 mg/kg b.w. per day) administered for 3 days, attenuated the increase in the CYP1A1 and CYP2E1 mRNA expression in the liver and decreased aminotransferase and the LPO levels in serum [47]. Vit C in plasma is incorporated into cells by a sodium dependent Vit C transporter. In parallel, dehydroascorbic acid, an oxidized form of Vit C, is taken up through glucose transporters. Since glucose competes with dehydroascorbic acid for the transporters, Vit C availability may be limited in cells when high blood sugar conditions are present. This might be a potential reason for the pathological severity of COVID-19 in diabetic patients. [47].

Few studies have evaluated the antioxidant effect of Vit E as monotherapy and most have been carried out with the administration in conjunction with other antioxidants. An ex vivo study showed the effect of Vit E on the O_2^- production in septic and non-septic patients in the ICU and concluded that septic patients have significantly decreased levels of Vit E and O_2^- overproduction. However, the administration of the combination of Vit E and simvastatin reduces this phenomenon through inactivation of NADPH oxidase [48]. In addition, there is a decrease in the incidence of pneumonia in old men who quit smoking after administration of the Vit E [49]. Also, a recent study by our group demonstrated that Vit E treatment (400 UI every 8 h) reduces the PCT levels in serum in patients with severe sepsis [3]. The results of this study suggest that the combination of Vit E with Px is capable of reducing the LPO and TAC increase present in COVID-19

patients and that the treatment has a synergic effect decreasing markers of inflammation such as IL-6, CRP and PCT. This treatment can be used as an adjuvant without showing collateral effects.

MT is not an antiviral drug, but it may have indirect anti-viral actions due to its anti-inflammatory, antioxidant, and immune enhancing properties. MT levels are decrease in the pineal gland and in mitochondrias and this decrease can contribute to elevate the replication of viruses and the severity of many viral infections [50]. In mice whose central nervous system is infected by some virus, MT decreases the viral load, and reduces paralysis and death [51]. In models of respiratory syncytial virus infections, MT reduces down regulation of acute lung oxidative injury, pro-inflammatory cytokine release and inflammatory cell recruitment [52]. Also, MT down-regulates NF-κB activation in T cells and lung tissue [53]. The anti-oxidative effect of MT may be through its anti-inflammatory and antioxidant actions by up-regulating anti-oxidative enzymes such as superoxide dismutase, and down-regulating pro-oxidative enzymes as iNOS. MT may also interact directly with ROS as a free radical scavenger, and induce up regulation of Nrf2 which is depleted in COVID-19 [54]. Furthermore, MT can exert regulatory actions on the immune system and enhance responses by improving proliferation and maturation of natural killer cells, T and B lymphocytes, granulocytes and monocytes in the bone marrow and other tissues [55]. In a clinical trial of patients suffering of severe multiple sclerosis, MT administration was associates with significant reduction in serum concentrations of TNF-α, IL-6, IL-1β and LPO [55]. A recent meta-analysis of a total of 22 randomized controlled trials suggested that supplementary MT use significantly reduced TNF-α and IL-6 levels [56]. The results from the present study

suggest that MT use as an adjuvant may effectively reduce the circulating cytokine levels and lower the pro-inflammatory cytokine storm and the OS present in COVID-19 patients. Published reports also indicate that MT application may ameliorate septic shock via the NLRP3 pathway. Specifically, MT has a preventive effect against sepsis-induced renal injury, septic cardiomyopathy and liver injury [57].

Moreover, MT exerts neurological protection by reducing the cerebral inflammatory response, cerebral edema and brain-blood barrier permeability under a number of experimental conditions [58]. In addition, the deep sedation of the patients in the ICU is associated with increased long-term mortality, and MT application can reduce the use of sedatives and the frequency of anxiety, agitation and pain [58]. It has also been reported that administration the MT up to a dose of 1 g/day for a month, does not present adverse effects [59].

Another antioxidant that has protective actions in sepsis is NAC which is a precursor of the GSH. Low levels of GSH are found in COVID-19 patients [60]. In animal models, in in vitro studies and in clinical trials, the use of NAC has beneficial effects. NAC administration reverses the enteropathogenic effects of the epidemic caused by the porcine coronavirus and by diarrhea viruses. It decreases levels of H_2O_2 in plasma and mucosas. NAC is also able to inhibit the H5N1 infection in lung epithelial cells in vitro and the production of pro-inflammatory mediators [61]. NAC administration 20 min after sepsis induction by injecting LPS in rats, prevented the decrease in the mean arterial pressure, and diminished markers of organic injury such as BUN, creatinine, lactate dehydrogenase, creatine phosphokinase, ALT, AST TNF-α, IL-6 [62]. Similarly, NAC administration 1 hour

before injecting endotoxin to rats showed decreased lung NF-kB activation and diminished cytokine-induced neutrophil chemo attractant mRNA expression in lung tissue. This was associated with a diminished inflammatory response in the lungs [63]. Treatment with NAC increased the TAC in patients with sepsis [3]. NAC has also been used as a treatment in numerous pulmonary ailments including COPD and chronic bronchitis. It prevents COPD exacerbations and chronic bronchitis flare-ups and this was also shown in a meta-analysis. NAC also exhibited promising results d when used as an adjuvant treatment for idiopathic pulmonary fibrosis [64]. A recent review discussed the potential use of NAC for the treatment of COVID-19 [64]. NAC may bind to Cys-145, the active site of M protein, and thus inhibit the protease activity and viral replication [65]. This structural characteristics might render NAC as a potentially specific first-line drug for SARS-Cov-2.

NAC reduces the incidence of pneumonia at an oral doses of (600 mg, bid), as well as the frequency and severity of influenza [66]. With this dose it also reduces levels of TNF and malondialdehyde and improves OS [67], and modulates inflammation. In patients with mild to moderate acute lung injury, intravenous treatment with NAC (i.v.) at doses of (40 mg/kg/day) significantly improves systemic oxygenation, reduces the need for ventilatory support, and also slightly decreased the mortality rate [68].

The effect of NAC in COVID-19 in a double-blind, randomized, placebo-controlled and unicentric trial, conducted in 135 patients with the severe disease (confirmed or suspected), having as a primary endpoint the need for mechanical ventilation was recently published. Time of mechanical ventilation, admission to ICU, time in ICU, and mortality were secondary endpoints of this paper and it was

found that administration of NAC in high doses did not affect the evolution of severe form of COVID-19 [69]. The difference with our study resides in the studied populations, the doses of NAC and in the fact that the authors did not explain whether the therapy was an adjuvant to standard comprehensive management. Here we evaluated the clinical anti-inflammatory status and the OS deregulation, and our results are promising. Therefore, our results and others shown in the literature suggest that NAC could contribute to the deceleration of the aggressive and lethal development of COVID-19 with the use of moderate doses [1, 70].

ARDS patients have decreased plasma concentrations of GSH in alveolar epithelial tissue and erythrocytes when compared to normal subjects. In a randomized crossover study, patients who were given intravenous NAC, showed an increase in GSH levels. These individuals also exhibited a clinical response to the treatment with increased oxygen delivery, improved lung compliance, and resolution of pulmonary edema. In another randomized controlled trial in patients with community acquired pneumonia, NAC treatment in conjunction with conventional therapy decreased the inflammatory response in comparison to conventional therapy alone [67].

ROS production can increase IL-6 production and LPO resulting in cell damage. Early treatment with NAC+Px during COVID-19 may bypass the excessive inflammation and cell damage that leads to the severe form of the infection, since SARS-CoV-2 influences intracellular GSH levels by decreasing the function of intracellular Nrf2 [71].

On the other hand, the combination of Px with any of the antioxidants used in this study, decreased IL-6 and CRP concentrations. This suggests a synergic effect by

decreasing inflammation markers. Px is a xanthine drug indicated in some severe cases of alcoholic hepatitis. It may act on the plasma membrane of red blood cells and render it more malleable, thus improving blood perfusion. Px exerts anti-inflammatory activities, decreasing the production of TNF-α and CRP. In a randomized controlled study that included 120 newborns with a mean gestational age of 30 weeks, the administration of Px 5 mg/kg/hour i.v. for 6 h/6 days was associated with reduced levels of TNF-α and CRP. It decreased the need for vasopressors, the duration of respiratory support, the antibiotic treatment, it shortened hospitalization, reduced the incidence of disseminated intravascular coagulopathy, of metabolic acidosis, and thrombocytopenia [72]. However, no difference in short-term morbidity was found between Px-treated and untreated septic infants [73]. In a meta-analysis that included 6 studies, the administration of Px in septic newborns showed it to be effective in reducing all-cause mortality and length of hospital stay. A subgroup analysis demonstrated significantly reduced mortality in premature newborns and infants, newborns with proven sepsis, and infants with gram-negative sepsis [74]. This led to the conclusion that Px may represent a beneficial adjuvant therapy in COVID-19 sepsis. However, it is contraindicated in patients with recent or active cerebral or retinal hemorrhage, in coronary artery disease and in patients with impaired renal or hepatic function.

In addition, production and release from extra thyroidal sources of PCT into the circulation is enormously amplified during bacterial infections. It is actively sustained by enhanced concentrations of TNF-α and IL-6. Nevertheless, the synthesis of this biomarker is inhibited by INF-γ, whose concentration increases during viral infections. Therefore, PCT value would be expected to stay within the

reference range in patients with non-complicated SARS-CoV-2 infection. Therefore, its substantial increase would reflect bacterial co-infection in patients developing the severe form of COVID-19 and could contribute to complicate the clinical picture [75]. However the results of this study suggest that the combination therapy with antioxidants is able to elevate the decreased levels of PCT.

An increase in circulating neutrophils enhances ROS release, LPO and reduces nitric oxide (NO) during obstructive sleep apnea and ARDS. This may cause an increase in platelet aggregation, elevated levels of adhesion molecules, endothelin and vascular endothelial growth that contributes to sepsis [76]. In ARDS there is a low local pH in the damaged capillaries which might help produce NO from NO_2^-. Even at a near neutral pH, NO_2^- can be reduced to NO in hypoxic conditions by multiple enzymes, such as deoxy hemoglobin from erythrocytes, blood xanthine oxidoreductase, or cytochrome oxidase from the mitochondrial respiratory chain. Lower or impaired NO metabolism is associated with the pathological severity of COVID-19. The NO synthesis by nitric oxide synthases requires O_2, and in ARDS, this is due to hypoxia that is decreased. As a consequence, the NO oxidation products NO_3^- and/or NO_2^- in plasma would be expected to be low [77]. The results in this study show that NO_2^- was decreased in all groups from the entrance to the hospital and that the antioxidant therapy increased it. In the absence of blood circulation in the lungs there is LPO and OS due to the hypoxic condition. Furthermore, NADP oxidase and iNOS in the endothelium are one of the main causes of oxidation in pulmonary ischemia. Immune cells such as macrophages and neutrophils can also contribute to oxidative damage in the lungs by the same enzymatic mechanism [78].

However, NO overproduction could be expected in sepsis by infection SARS-CoV-2 since it results from the activation of iNOS which contributes to the inflammation process. Nevertheless, our results show a decrease in the plasmatic levels of NO_2^-. The possible explanation to this may be through the hypoxic condition, in which the O_2 concentration decreased and optimal concentrations of O_2 are required for NO synthesis by the oxide nitric synthases. In addition the NO which is synthetized might be oxidized by ROS to $ONOO^-$ and this nitrogen specie may contribute to the inflammatory process in COVID-19 patients [37].

In this study, when patients with severe pneumonia were only treated with Px there was a decrease in CPR, which confirms the participation of this drug in the control of inflammation. There was not a decrease in LPO, but an increase the TAC was found. There was also a lower average of days of stay in ventilation, of days in the ICU and of days of hospitalization (date not shown).

We observed that Px had a better effect in patients with moderate pneumonia. It improved levels of inflammatory biomarkers in patients with moderate and severe pneumonia. The combination of Vit E+Px reduced IL-6 levels more than Px alone. There were no statistically relevant data in severe patients. However, CRP levels decreased with the use of NAC combined with Px and Px alone. On the other hand, the use of NAC and Vit E combined with Px produced better effects up-TPC levels. This suggests that the inflammatory state could have a better performance with the combination of two antioxidants and the use of Px, NAC and MT were the ones that showed the best performance. Therefore, we can propose that this could be a further improvement to the therapy for patients with severe pneumonia due to COVID-19.

On the other hand, the decrease observed in the inflammatory state in this study through the decrease in IL-6, CRP and PCT in most patients who did not develop septic shock; suggest that the use of antioxidants or Px should be started early.

Finally, it is also relevant to point out that we obtained only a 2.7% of mortality in this series and therefore survival was high. Deaths were not related to complications of COVID and this study generates the hypothesis that the treatment should be applied using the combination of Px with the antioxidants since it shows a better clinical performance in the regulation of biomarkers of inflammation and OS. The antioxidants proposed are NAC, MT, Vit E, Vit C and Px and their preventive use should also be analyzed in the context of clinical trials. This therapy should also be evaluated as a means to stop the use of invasive intubation. The figure 4 resume the results the antioxidant therapy on the SARS-CoV-2 infection in COVID-19 patients.

Conclusion

This study confirms the presence of OS in COVID-19 patients. The results suggest that the treatment with antioxidant supplements such as Vit C, E, NAC, and MT plus Px could contribute to the deceleration of the aggressive and lethal development COVID-19. There is evidence that antioxidants in moderate doses decrease inflammation and control of OS; therefore, their use as an adjuvant therapy to improve prognosis is confirmed. The antioxidant therapy can be effective in this pandemia since it improves all of the survival scores including SOFA, Apache II, SAPS II, COVIDGRAM, GCS by lowering the LPO, IL-6, CRP, PCT and increasing systemic TAC. The survival prognosis is greater than 90%, in

patients with pneumonia by COVID-19 which supports its use. There is still needed to evaluate the reproducibility of our findings in a similar treatment and context.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions A.P.C., R.R.V.V., J.G.D.C., H.H.B., H.C.S., L.M.-C., G.A.E., F.H., O.G.-M., H.S.-O., treated and recruited the patients in the intensive care unit and collected all of the results, including the pretreatment and post treatment dates; **:** M.E.S., V.G.-L., and I.P.-T., and designed the study and wrote the manuscript. V.G.-L. revised and structured the manuscript; I.P.-T., and M.E.S. made the laboratory determination, designed the tables, figures and performed and planned the statistical analysis. R. M. made the IL-6 determination; L.M.P. designed and made the graphical abstract. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Conacyt # 312167.

Conflicts of Interest: The authors declare no conflict of interest.

Reference

1. Soto ME, Guarner-Lans V, Soria-Castro E, Manzano-Pech L, Pérez-Torres I. Is antioxidant therapy a useful complementary measure for covid-19 treatment? An algorithm for its application. Medicina (Kaunas). 2020;56:386.
2. Khomich OA, Kochetkov SN, Bartosch B, Ivanov AV. Redox Bbology of respiratory viral infections. Viruses. 2018;10: 392.
3. Aisa-Alvarez A, Soto ME, Guarner-Lans V, Camarena-Alejo G, Franco-Granillo J, Martinez-Rodríguez EA, et al. Usefulness of antioxidants as

adjuvant therapy for septic shock: A randomized clinical trial. Medicina (Kaunas). 2020;56:619.

4. Timsit JF, Perner A, Bakker J, Bassetti M, Benoit D, Cecconi M, et al. Year in review in intensive care Medicine 2014: III. Severe infections, septic shock, health care associated infections, highly resistant bacteria, invasive fungal infections, severe viral infections, Ebola virus disease and paediatrics. Intensive Care Med. 2015;41:575–588.
5. Marshall JC, Vincent JL, Guyatt G, Angus DC, Abraham E, Bernard G, et al. Outcome measures for clinical research in sepsis: A report of the 2nd Cambridge Colloquium of the International Sepsis Forum. Crit Care Med. 2005;33:708–1716.
6. Feng H, Yu D, Weina L. Coronavirus disease 2019 (COVID-19): What we know? J Med Virol 2020;92:719–725.
7. Pérez-Torres I, Manzano-Pech L, Rubio-Ruíz ME, Soto ME, Guarner-Lans V. Nitrosative stress and its association with cardiometabolic disorders. Molecules. 2020;25:2555.
8. Berger MM, Oudemans-van SHM. Vitamin C supplementation in the critically ill patient. Curr Opin Clin Nutr Metab Care 2015;18:193–201.
9. Pérez-Torres I, Guarner-Lans V, Rubio-Ruiz ME. Reductive stress in inflammation-associated diseases and the pro- effect of antioxidant agents. Int J Mol Sci. 2017;18:2098.
10. Lassnigg A, Punz A, Barker R, Keznickl P, Manhart N, Roth E, et al.. Influence of intravenous vitamin E supplementation in cardiac surgery on

oxidative stress: A double-blinded, randomized, controlled study. Br J Anaesth. 2003;90:148–154.

11. Harri H. Vitamin E administration may decrease the incidence of pneumonia in elderly males. Clin Interv Aging 2016;11:1379–1385.
12. Zúñiga-Muñoz AM, Pérez-Torres I, Guarner-Lans V, Núñez-Garrido E, Velázquez ER, Huesca-Gómez C, et al. Glutathione system participation in thoracic aneurysms from patients with Marfan syndrome. Vasa. 2017;46: 177–186.
13. Rank N, Michel C, Haertel C, Lenhart A, Welte M, Meier-Hellmann A, et al. N acetylcysteine increases liver blood flow and improves liver function in septic shock patients: Results of a prospective, randomized, double-blind study. Crit Care Med. 2000;28:3799–3807.
14. Paterson RL, Galley HF, Webster NR. The effect of N-acetylcysteine on nuclear factor-kappa B activation, interleukin-6, interleukin-8, and intercellular adhesion molecule-1 expression in patients with sepsis. Crit Care Med. 2003;31:2574–2578.
15. Sánchez-Barceló, EJ, Mediavilla MD, Tan DX, Reiter RJ. Clinical uses of melatonin: Evaluation of human trials. Curr Med Chem. 2010;17:2070–2095.
16. D'Amato LA, Mistraletti G, Longhi D, Piva IR, Marrazzo F, Villa C, et al. Melatonin blood values and total antioxidant capacity in critically ill patients. Crit Care. 2014;18:P436.
17. Lowes DA, Almawash AM, Webster NR, Reid VL, Galley HF. Melatonin and structurally similar compounds have differing effects on inflammation and

mitochondrial function in endothelial cells under conditions mimicking sepsis. Br J Anaesth. 2011;107:193–201.

18. Urata Y, Honma S, Goto S, Todoroki S, Iida T, Cho S, et al. Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. Free Radic Biol Med 1999;27:838–847.

19. Galley HF, Lowes DA, Allen L, Cameron G, Aucott LS, Webster NR. Melatonin as a potential therapy for sepsis: A phase I dose escalation study and an ex vivo whole blood model under conditions of sepsis. J Pineal Res. 2014;56:427–438.

20. Yang M. Cell pyroptosis, a potential pathogenic mechanism of 2019-nCoV infection. SSRN. 2020;1–7:doi:10.2139/ssrn.3527420

21. Shneider A, Kudriavtsev A, Vakhrusheva A. Can melatonin reduce the severity of COVID-19 pandemic? Int Rev Immunol. 2020;39:153–162.

22. Yung-Ming C, Chao-Jung T, Kung-Yu H, Kwan-Dun W, Tun-Jun Tsai. Inhibition by pentoxifylline of TNF-alpha-stimulated fractalkine production in vascular smooth muscle cells: evidence for mediation by NF-kappa B down-regulation. Br J Pharmacol. 2003;138:950–958.

23. Olson G, Andrew MD. Diagnosis and Treatment of Adults with Community-Acquired Pneumonia. JAMA. 2020;323:885–886.

24. Lambden S, Laterre PF, Levy MM, Francois B. The SOFA score-development, utility, and challenges of accurate assessment in clinical trials. Crit Care. 2019;23:374.

25. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012;307:2526–2533.

26. Lambden S, Laterre PF, Levy MM, Francois B. The SOFA score-development, utility, and challenges of accurate assessment in clinical trials. Crit Care. 2019;23:374.

27. Brower RG, Lanken PN, MacIntyre N, Matthay MA, Morris A, Marek A, et al. National heart, lung, and blood institute ARDS clinical trials network higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. N Engl J Med. 2004;351:327–336.

28. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. J Thromb Haemost. 2020;18:1023–1026.

29. Guimarães HP, Timerman S, Rodrigues RDR, Corrêa TD, Schubert DUC, Freitas AP, et al. Position statement: Cardiopulmonary resuscitation of patients with confirmed or suspected COVID-19-2020. Arq Bras Cardiol. 2020;114:1078–1087.

30. Manzano-Pech LG, Caballero-Chacón SC, Guarner-Lans V, Díaz-Díaz E, Moreno GA, Pérez-Torres I. Effect of oophorosalpingo-hysterectomy on serum antioxidant enzymes in female dogs. Sci Rep. 2019;9:9674.

31. Narayanasamy K, Natarajan M, Ramachandran A, Wesley J, Thangaraj V, Etherajan T, et al. Clinical outcomes among asymptomatic or mildly symptomatic COVID-19 patients in an isolation facility in Chennai, India. Am J Trop Med Hyg. 2020;doi: 10.4269/ajtmh.20-1096.

32. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al., Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020;8:475–481.

33. Chen N, Zhou M, Dong X, Qu J, Gong F, Han T, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395:507–513.

34. Howe KP, Clochesy JM, Goldstein LS, Owen H. Mechanical ventilation antioxidant trial. Am J Crit Care. 2015;24:440–445.

35. Cazzola M, Calzetta L, Facciolo F, Rogliani P, Matera MG. Pharmacological investigation on the antioxidant and anti-inflammatory activity of N-acetylcysteine in an ex vivo model of COPD exacerbation. Respir Res. 2017;18:26.

36. Singh VP, Aggarwal R, Singh S, Banik A, Ahmad T, Patnaik BR, et al. Metabolic syndrome is associated with increased oxo-nitrative stress and asthma-like changes in lungs. PLoS One. 2015;10:e0129850.

37. Delgado-Roche L, Mesta F. Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARS-CoV) Infection. Arch Med Res. 2020;51:384–387.

38. Rivers EP, McIntyre L, Morro DC, Rivers KK. Early and innovative interventions for severe sepsis and septic shock: taking advantage of a window of opportunity. CMAJ. 2005;173:1054–1065.

39. Clapp BR, Hingorani AD, Kharbanda RK, Mohamed-Ali V, Stephens JW, Vallance P, et al. Inflammation-induced endothelial dysfunction involves

reduced nitric oxide bioavailability and increased oxidant stress. Cardiovasc Res. 2004;64:172–178.

40. Pérez-Torres I, Soto ME, Castrejón-Tellez V, Rubio-Ruiz ME, Manzano-Pech L, Guarner-Lans V. Oxidative, reductive, and nitrosative stress effects on epigenetics and on posttranslational modification of enzymes in cardiometabolic diseases. Oxid Med Cell Longev 2020;2020:8819719.

41. Ang A, Pullar JM, Currie MJ Vissers MCM. Vitamin C and immune cell function in inflammation and cancer. Biochem Soc Trans. 2018;46:1147–1159.

42. Fowler AA, Syed AA, Knowlson S, Sculthorpe R, Farthing D, DeWilde C, et al. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. J Transl Med. 2014;12:32.

43. Fowler, AA, Truwit JD, Hite RD, Morris PE, DeWilde C, Priday A, et al. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: The CITRIS-ALI randomized clinical trial. JAMA. 2019;322:1261–1270.

44. Fujii T, Luethi N, Young PJ, Frei DR, Eastwood GM, French CJ, et al. Effect of vitamin C, hydrocortisone, and thiamine vs hydrocortisone alone on time alive and free of vasopressor support among patients with septic shock: The Vitamins Randomized Clinical Trial. JAMA. 2020;323:423–431.

45. Boretti A, Banik, BK. Intravenous vitamin C for reduction of cytokines storm in acute respiratory distress syndrome. Pharma Nutrition. 2020;12:100190.

46. Kim J-Y, Lee S-M. Effect of ascorbic acid on hepatic vasoregulatory gene expression during polymicrobial sepsis. Life Sci. 2004;75:2015–2026.

47. Kim J-Y, Lee S-M. Vitamins C and E protect hepatic cytochrome P450 dysfunction induced by polymicrobial sepsis. Eur J Pharmacol. 2006;534:202–209.

48. Maret G, Traber J, Stevens F. Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Radic Biol Med. 2011;51:1000–1013.

49. Hemilä, H. Vitamin E administration may decrease the incidence of pneumonia in elderly males. Clin Interv Aging. 2016;11:1379–1385.

50. Boga JA, Coto-Montes A, Rosales-Corral SA, Tan D-X, Reiter RJ. Beneficial actions of melatonin in the management of viral infections: a new use for this “molecular handyman”? Rev Med Virol. 2012;22:323–338.

51. Ben-Nathan D, Maestroni GJ, Lustig S, Conti A. Protective effects of melatonin in mice infected with encephalitis viruses, Arch Virol. 1995;140: 223–230.

52. Hardeland, R. Melatonin and inflammation-story of a double-edged blade. J Pineal Res. 2018;65:e12525.

53. Shang Y, Xu SP, Wu Y, Jiang YX, Wu ZY, Yuan SY, et al. Melatonin reduces acute lung injury in endotoxemic rats, Chin Med J. 2009;122: 1388–1393.

54. Ahmadi Z, Ashrafizadeh M. Melatonin as a potential modulator of Nrf2, Fund. Clin Pharmacol. 2020;34:11–19.

55. Miller SC, Pandi-Perumal SR, Esquifino AI, Cardinali DP, Maestroni GJM. The role of melatonin in immuno-enhancement: potential application in cancer, Int J Exp Pathol. 2006;87:81–87.

56. Zarezadeh M, Khorshidi M, Emami M, Janmohammadi P, Kord-Varkaneh H, Mousavi SM, et al. Melatonin supplementation and pro-inflammatory mediators: a systematic review and meta-analysis of clinical trials. Eur J Nutr. 2019;59:1803–1813.

57. Chen J, Xia H, Zhang L, Zhang H, Wang D, Tao X. Protective effects of melatonin on sepsis-induced liver injury and dysregulation of gluconeogenesis in rats through activating SIRT1/STAT3 pathway. Biomed Pharmacother. 2019;117:109150.

58. Tordjman S, Chokron S, Delorme R, Charrier A, Bellissant E, Jaafari N, et al. Melatonin: pharmacology, functions and therapeutic benefits. Curr Neuropharmacol. 2017;15:434–443.

59. Andersen LPH, Gogenur I, Rosenberg J, Reiter RJ. The safety of melatonin in humans. Clin Drug Investig. 2016;36:169–175.

60. Silvagno F, Vernone A, Pescarmona GP. The Role of glutathione in protecting against the severe inflammatory response triggered by COVID-19. Antioxidants (Basel). 2020;9:624.

61. Geiler J. N-acetyl-L-cysteine (NAC) inhibits virus replication and expression of pro-inflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus. Biochem Pharmacol. 2010;79:413–420.

62. Hsu BG, Lee RP, Yang FL, Harn HJ, Chen HI. Posttreatment with N-acetylcysteine ameliorates endotoxin shock-induced organ damage in conscious rats. Life Sci. 2006;79:2010–2016.

63. Prauchner CA. Oxidative stress in sepsis: Pathophysiological implications justifying antioxidant co-therapy. Burns. 2017;43:471–485.

64. Nasi A, McArdle S, Gaudernack G, Westman G, Melief C, Rockberg J, et al. Reactive oxygen species as an initiator of toxic innate immune responses in retort to SARS-CoV-2 in an ageing population, consider N-acetylcysteine as early therapeutic intervention. Toxicol Rep. 2020;7:768–771.

65. Guthappa R. Molecular docking studies of N-acetylcysteine, zinc acetylcysteine and niclosamide on SARS Cov 2 protease and its comparison with hydroxychloroquine. Chemarxiv. 2020;doi:10.26434/chemrxiv.12161493.v1.

66. Sharafkhah M, Abdolrazaghnejad A, Zarinfar N, Mohammadbeigi A, Ali MA. Abaszadeh S. Safety and efficacy of N-acetylcysteine for prophylaxis of ventilator-associated pneumonia: a randomized, double blind, placebo-controlled clinical trial. Med Gas Res. 2018;8:19–23.

67. Zhang Q, Ju Y, Ma Y, Wang T. N-acetylcysteine improves oxidative stress and inflammatory response in patients with community acquired pneumonia A randomized controlled trial. Medicine. 2018;97:e13087.

68. Suter PM, Domenighetti G, Schaller MD, Laverrière MC, Ritz R, Perret C. N-Acetylcysteine enhances recovery from acute lung injury in man: a randomized, double-blind, placebo-controlled clinical study. Chest. 1994;105:190–194.

69. Garcia de Alencar JC, Moreira CL, Müller AD, Chaves CE, Fukuhara MA, Aparecida da Silva E, et al. Covid Register Group Double-blind, randomized, placebo-controlled trial with N-acetylcysteine for treatment of severe acute respiratory syndrome caused by COVID-19. Clin Infect Dis. 2020;ciaa1443: doi: 10.1093/cid/ciaa1443.

70. Zhongcheng S, Puyo CA. N-Acetylcysteine to combat COVID-19: an evidence review. Ther Clin Risk Manag. 2020;16:1047–1055.

71. Guloyan V, Oganesian B, Baghdasaryan N, Yeh C, Singh M, Guilford F, et al. Glutathione supplementation as an adjunctive therapy in COVID-19. Antioxidants (Basel). 2020;9:914.

72. Shabaan AE, Nasef N, Shouman B, Nour I, Mesbah A, Abdel-Hady H. Pentoxifylline therapy for late-onset sepsis in preterm infants: a randomized controlled trial. Pediatr Infect Dis J. 2015;34:E143–E148.

73. Speer EM, Dowling DJ, Ozog LS, Xu J, Yang Jie, Kennady G, et al. Pentoxifylline inhibits the production of inflammatory cytokines mediated by TLR and inflammasome in human blood with greater efficacy and potency in neonates, Pediatric Res. 2017;81:806–816.

74. Salman S, Hibbert J, Page-Sharp M, Manning L, Simmer K, Doherty DA, et al. Effects of maturation and size on population pharmacokinetics of pentoxifylline and its metabolites in very preterm infants with suspected late-onset sepsis or necrotizing enterocolitis: a pilot study incorporating clinical outcomes. Br J Clin Pharmacol 2019;85:147–159.

75. Kotula JJ, Moore WS, Chopra A, Cies JJ. Association of procalcitonin value and bacterial coinfections in pediatric patients with viral lower respiratory

tract infections admitted to the pediatric intensive care unit. J Pediatr Pharmacol Ther. 2018;23:466–472.

76. Kaur R, Kaur M, Singh J. Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: Molecular insights and therapeutic strategies. Cardiovasc Diabetol. 2018;17:121.

77. Yamasaki H. Blood nitrate and nitrite modulating nitric oxide bioavailability: Potential therapeutic functions in COVID-19. Nitric Oxide. 2020;103:29–30.

78. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal. 2014;20:1126–1167.

Table 1 Demographic characteristics at admission of patients infected with COVID-19

	All (median, min-max)	**Moderate 59 (54%) (Median, min-max)**	**Severe 51 (46%) (Median, min-max)**	p
Women	32 (29%)	18 (31)	14 (27)	NS
Men	78 (71%)	41 (69)	37 (73)	NS
Age	57.9 ± 12.8	54 ± 12.3	62 ± 12.3	0.002
BMI	29.1 ± 4.1	29.3 ± 4.4	28.9 ± 3.8	NS
Temperature	36.6 ± 0.46	36.6 ± 0.48	36.6 ± 0.45	NS
PaO_2	84.7 ± 44.1	84.4 ± 37.9	85.02 ± 50.7	NS
PCO_2	31.7 ± 6.1	30.65 ± 4.3	33.04 ± 7.5	0.052
PaO_2/FiO2	149.7 ± 60.7	171.3 ± 55.1	125.4 ± 57.9	0.0001
SpO2/FiO2	146.3 ± 54.5	162.5 ± 52.6	125.1 ± 51.3	0.0001
FC	80.8 ± 19.3	84.5 ± 17.6	76.4 ± 20.4	0.03
TAM	80.3 ± 11.7	81 ± 12	79 ± 12	NS
Glucose	147.5 ± 68.1	141.9 ± 64.05	153.9 ± 72.5	NS
Urea	43.9 ± 38.3	33.02 ± 11.5	55.3 ± 51.3	0.005
Ureic nitrogen	22 ± 16.40	18.7 ± 7.8	25.7 ± 21.9	0.04
Total cholesterol	139.4 ± 33.8	141.04 ± 32.2	137.6 ± 35.8	NS
Triglycerides	163.4 ± 88.09	174.7 ± 106	150.4 ± 58.4	NS
HDL	32.3 ± 8.9	32.2 ± 9.9	32.5 ± 8.5	NS
LDL	75.7 ± 25.5	76.8 ± 24	74.4 ± 27.2	NS
DHL	290.4 ± 103	266.5 ± 95.1	317.3 ± 105.9	0.01
BT	0.65 ± 0.30	0.59 ± 0.20	0.71 ± 0.39	0.05
DB	0.19 ± 0.11	0.17 ± 0.10	0.22 ± 0.11	0.03
Leukocytes	10.2 ± 4.0	9.5 ± 3.5	11.1 ± 4.5	0.05
Lymphocytes	0.91 ± 0.87	1.05 ± 1.1	0.74 ± 0.45	0.05
Platelets	254.2 ± 89	260.8 ± 99.9	246.8 ± 87.2	NS
Ferritin	586 (24.7-3373)	579 (24.7-2354)	59 7.7 (146-3373)	NS
IL-6 admission	43.2 (7.8-638.5)	24.6 (7.8-626)	75.4 (7.8-638)	0.001
Index N/L	11 (1-106)	10 (2-46)	12 (1-106)	0.05
D-Dimer	740 (200-35,200)	610 (200-35200)	880 (210-34920)	0.006
DM	41 (37.3)	17 (29)	24 (47)	0.05
SAH	44 (4)	21 (36)	23 (45)	NS
Dyslipidemia	52 (47.3)	32 (54)	20 (39)	NS
COPD	5 (4.5)	2 (4)	3 (6)	NS
CD	2 (1.8)	0	2 (4)	NS
ECKD	4 (3.6)	0	4 (8)	0.04
Norepinephrine	21 (19)	0	21 (41)	0.0001
Enteral nutrition	45 (41)	33 (56)	12 (24)	0.0001
Deaths	3 (2.7)	0	3 (6)	0.09

Abbreviations: BMI= Body mass index, HR= Heart rate, MAP= mean arterial pressure, HDL=high-density lipoproteins, LDL=low-density lipoproteins, TB= Total bilirubin, DB=Direct bilirubin, IL= interleukin, N/L= neutrophil&/ lymphocyte, DM= Diabetes Mellitus, SAH= Systemic arterial hypertension, CD= cardiovascular disease, COPD= Chronic obstructive pulmonary disease, ECKD= End-stage chronic kidney disease. FiO2= inspired fraction of oxygen

Table 2 Characteristics of the scores between initially seriously ill patients and patients who progressed to severity and those who were stable and persisted stable

	All (median, min-max)	**Moderate 51 (46 %)**	**Severe 59 (54 %)**	p
SOFA	1 (0-8)	0 (0-5)	2 (0-8)	0.001
Apache II	5 (3-14)	5 (3-8)	6 (4-14)	0.001
SAPS II	27 (3-32)	26 (3-31)	28 (13-32)	0.001
COVIDGRAM	116 (50-240)	113 (50-162)	120 (81-240)	0.009
Padua	6 (5-7)	5 (5-7)	6 (5-7)	NS
GCS	15 (12-15)	15 (14-15)	15 (12-15)	0.001

Abbreviations: SOFA= Sequential Organ Failure Assessment, Apache= Acute Physiology and chronic Health Evaluation II, SAPS= Simplified Acute Physiology Score II, COVIDGRAM= Name of Critical Illness Risk Score, Launched during COVID-19 crisis, Padua= Name of prediction score for risk of venous thromboembolism, GCS=Glasgow Coma Scale

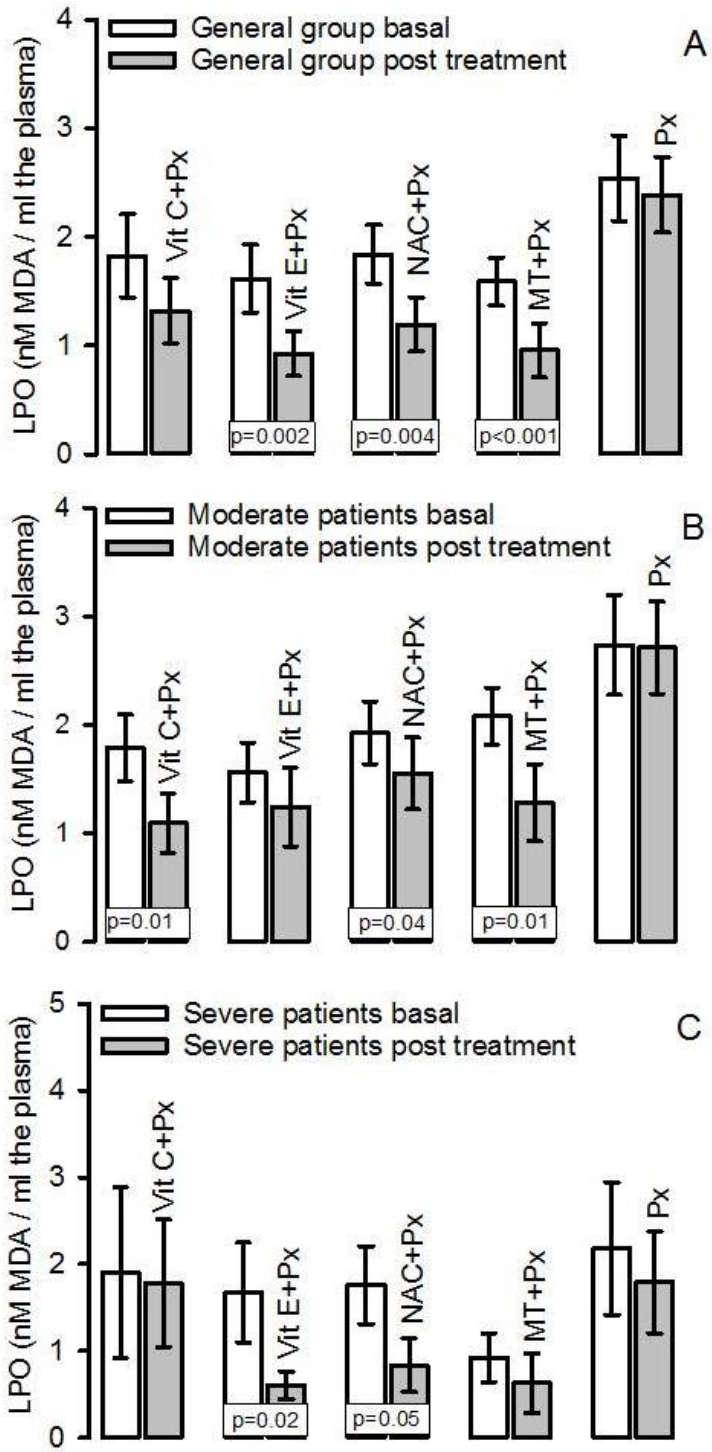

Figure 1 LPO index in the general group both in basal and posttreatment conditions (Panel A), Panel B shows the LPO index in moderate patients both in basal and post treatment conditions, and panel 1C shows the LPO index in severe patients both in basal and post treatment conditions

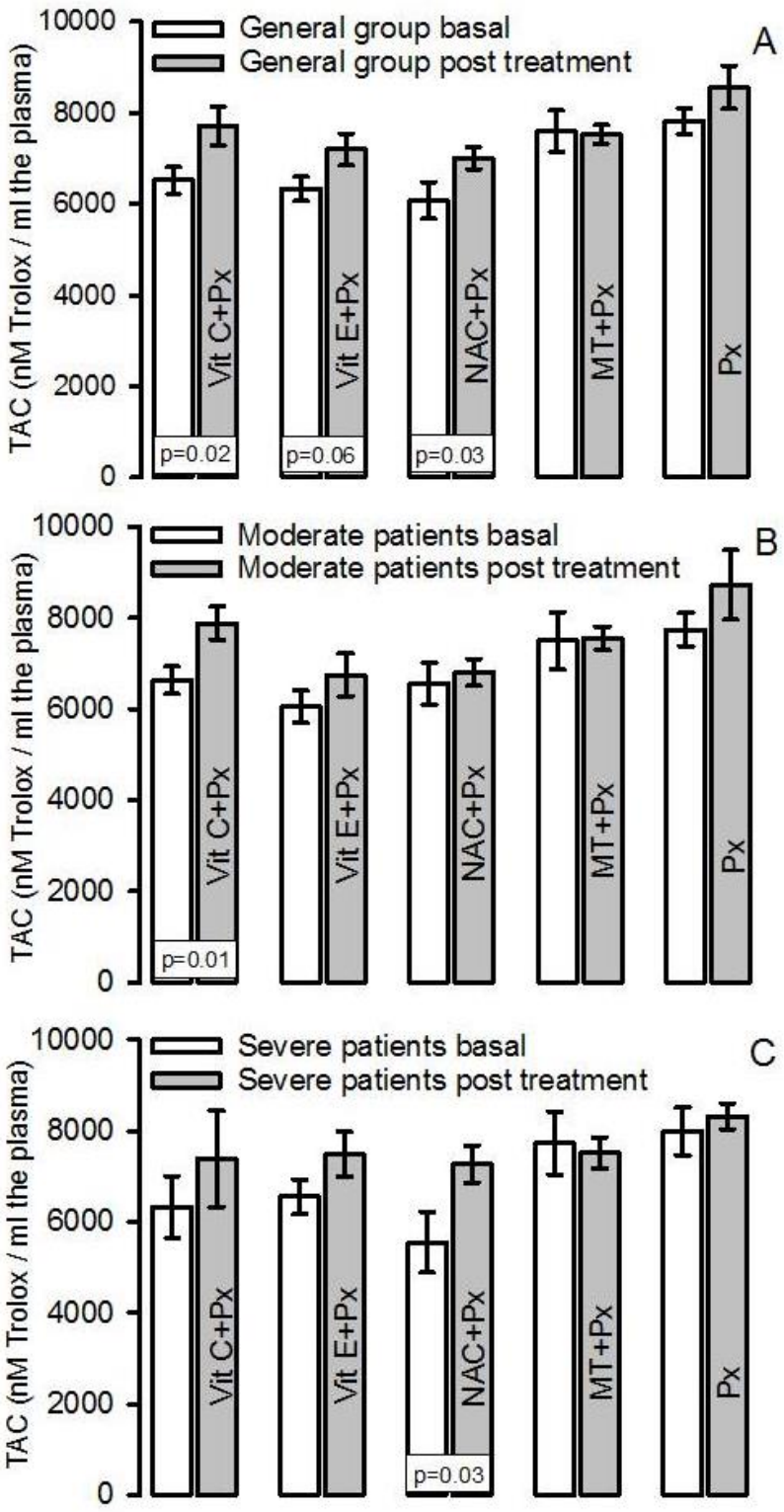

Figure 2 TAC index in the general group both in basal and post treatment conditions (panel A). Panel B shows the TAC index in moderate patients both in basal and post treatment conditions, and panel C shows the TAC index in severe patients both in basal and post treatment conditions

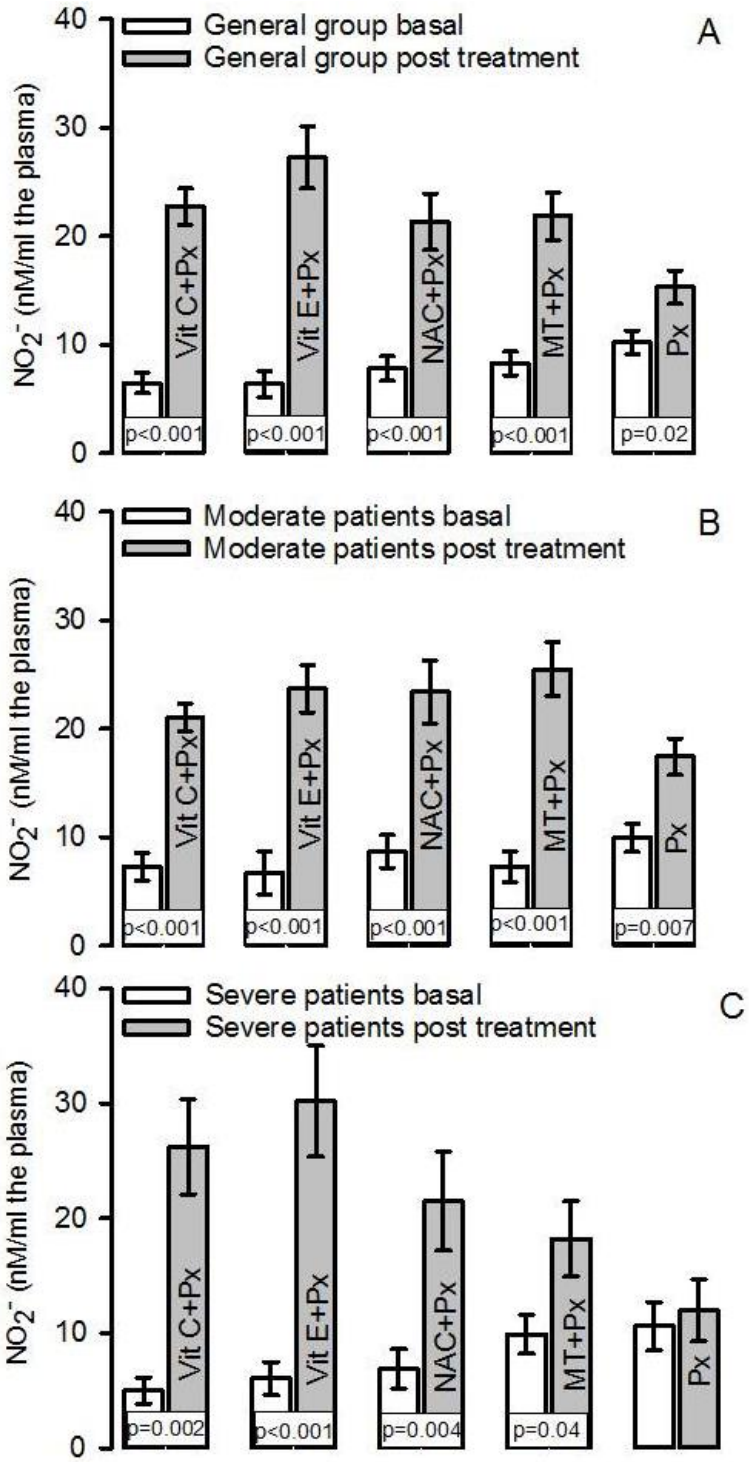

Figure 3 Plasma nitrite concentration in the general group both in basal and post treatment conditions (panel A), panel B shows the NO_2^- concentration in moderate patients both in basal and post treatment conditions. The panel C shows the NO_2^- concentration in severe patients both in basal and post treatment conditions

Table 4 Determination of IL-6, CRP and PCT levels before (basal) and after antioxidant treatment in the group of COVID-19 patients, in the general group and divided into moderate and severe

	General group 110 (100%)			Moderate 59 (54%)			Severe 51 (46%)		
	Basal (Median, min-max)	Post treatment (Median, min-max)	P	Basal (Median, min-max)	Post treatment (Median, min-max)	P	Basal (Median, min-max)	Post treatment (Median, min-max)	P
IL-6									
Vit C+Px	22.4 (7.8–638)	7.8 (7.8–547)	0.01	13.5 (7.8–214.08)	7.8 (7.8–10.6)	0.02	57.89 (7.8–638)	11.8 (7.8–547)	NS
Vit E+Px	51.6 (7.8–395)	7.8 (7.8–284.9)	0.001	29.9 (7.8–177)	7.8 (7.8–45.2)	0.04	217 (7.8–637)	7.8 (7.8–282)	0.02
NAC+Px	77.9 (7.8–637)	7.8 (7.8–282.08)	0.004	20.5 (7.8–466)	7.8 (7.8–108)	0.06	37.6 (9.1–324.11)	7.8 (7.8–406)	NS
MT+Px	68.4 (7.8–626)	7.8 (7.8–406)	0.07	34.2 (7.8–293.2)	7.8 (7.8–7.8)	0.005	74.9 (9.5–395.6)	8 (7.8–284.9)	0.08
Px	68.4 (7.8–626)	7.8 (7.8–640)	NS	56.4 (7.8–626.8)	7.8 (7.8–150)	0.001	190.1 (23–315)	198.6 (7.8–640.1)	NS
CRP									
Vit C+Px	117 (20–494)	44.6 (4.10–201.48)	0.01	120.4 (20.1–494)	37.6 (6.8–91.1)	0.04	57.8 (7.8–638.5)	11.8 (7.8–547.7)	NS
Vit E+Px	165 (28.5–384.7)	43.8 (1.8–263.5)	0.01	143.4 (28.5–259.2)	22.3 (1.8–110)	0.02	217.4 (7.8–637)	7.8 (7.8–282)	NS
NAC+Px	144 (15.1–221)	43.3 (7.3–148)	0.001	146 (15.1–178)	33.4 (7.3–148)	0.06	37.6 (9.1–324.1)	7.8 (7.8–406.9)	0.01
MT+Px	145 (6.8–308)	23.4 (3.3–357.9)	0.004	152 (6.8–308)	14.1 (3.3–57.3)	0.005	74.9 (9,5–395.6)	8 (7.8–284.9)	NS
Px	141.6 (33.7–283)	20.3 (2.09–191.77)	0.0001	90.4 (33.7–252)	12.5 (2.09–191.7)	0.001	190.1 (9.5–395.6)	8 (7.8-284.9)	0.04
PCT									
Vit C+Px	0.16 (0.03–2.04)	0.07 (0.03–0.78)	0.04	0.15 (0.03–0.41)	0.04 (0.03–0.08)	0.05	0.20 (0.09–2.04)	0.12 (0.04–0.78)	NS
Vit E+Px	0.33 (0.06–2.2)	0.16 (0.01–0.50)	0.004	0.40 (0.14–1.7)	0.11 (0.01–0.50)	0.04	0.33 (0.06–2.2)	0.19 (0.04–0.39)	0.04
NAC+Px	0.23 (0.08–11.4)	0.12 (0.02–0.90)	0.01	0.21 (0.10–0.90)	0.07 (0.04–0.90)	NS	0.28 (0.08–11.4)	0.13 (0.02–0.32)	0.01
MT+Px	0.40 (0.06–34.7)	0.15 (0.01–8.3)	NS	0.46 (0.0–1.23)	0.08 (0.01–0.19)	0.03	0.40 (0.09–34.7)	0.29 (0.06–8.3)	NS
Px	0.19 (0.03–1.35)	0.06 (0.01–1.25)	NS	0.12 (0.03–0.68)	0.05 (0.01–0.21)	NS	0.45 (0.12–1.35)	0.80 (0.16–1.25)	NS

Abbreviations: Vit C= Vitamin C, Vit E= Vitamin E, NAC= N-acetylcysteine, MT= Melatonin, Px= Pentoxifylline

Table 3 Determination of IL-6, CRP and PCT levels before (basal) and after antioxidant treatment in the group of COVID-19 patients, in general population and divided into patients with and without septic shock

	Patients with septic shock			Patients without septic shock		
	IL-6 basal (Median, min-max)	**IL-6 post treatment (Median, min-max)**	**P**	**IL-6 basal (Median, min-max)**	**IL-6 post treatment (Median, min-max)**	**P**
Vit C+Px	57.89 (7.8–638)	11.8 (7.8–547)	NS	13.5 (7.8–214.08)	7.8 (7.8–10.6)	0.02
Vit E+Px	217 (7.8–637)	7.8 (7.8–282)	0.02	29.9 (7.8–177)	7.8 (7.8–45.2)	0.04
NAC+Px	37.6 (9.1–324.11)	7.8 (7.8–406)	NS	20.5 (7.8–466)	7.8 (7.8–108)	0.06
MT+Px	74.9 (9.5–395.6)	8 (7.8–284.9)	0.08	34.2 (7.8–293.2)	7.8 (7.8–7.8)	0.005
Px	**190.1 (23–315)**	198.6 (7.8–640.1)	NS	56.4 (7.8–626.8)	7.8 (7.8–150)	0.001
	CRP basal	**CRP post treatment**		**CRP basal**	**CRP post treatment**	
Vit C+Px	57.8 (7.8–638.5)	11.8 (7.8–547.7)	NS	120.4 (20.1–494)	37.6 (6.8–91.1)	0.04
Vit E+Px	217.4 (7.8–637)	7.8 (7.8–282)	NS	143.4 (28.5–259.2)	22.3 (1.8–110)	0.02
NAC+Px	37.6 (9.1–324.1)	7.8 (7.8–406.9)	0.01	146 (15.1–178)	33.4 (7.3–148)	0.06
MT+Px	74.9 (9,5–395.6)	8 (7.8–284.9)	NS	152 (6.8–308)	14.1 (3.3–57.3)	0.005
Px	190.1 (9.5–395.6)	8 (7.8–284.9)	0.04	90.4 (33.7–252)	12.5 (2.09–191.7)	0.001
	PCT basal	**PCT post treatment**		**PCT basal**	**PCT post treatment**	
Vit C+Px	0.20 (0.09–2.04)	0.12 (0.04–0.78)	NS	0.15 (0.03–0.41)	0.04 (0.03–0.08)	0.05
Vit E+Px	0.33 (0.06–2.2)	0.19 (0.04–0.39)	0.04	0.40 (0.14–1.7)	0.11 (0.01–0.50)	0.04
NAC+Px	0.28 (0.08–11.4)	0.13 (0.02–0.32)	0.01	0.21 (0.10–0.90)	0.07 (0.04–0.90)	NS
MT+Px	0.40 (0.09–34.7)	0.29 (0.06–8.3)	NS	0.46 (0.06–1.23)	0.08 (0.01–0.19)	0.03
Px	0.45 (0.12–1.35)	0.80 (0.16–1.25)	NS	0.12 (0.03–0.68)	0.05 (0.01–0.21)	NS

Abbreviations: Vit C= Vitamin C, Vit E= Vitamin E, NAC= N-acetylcysteine, MT= Melatonin, Px=Pentoxifylline

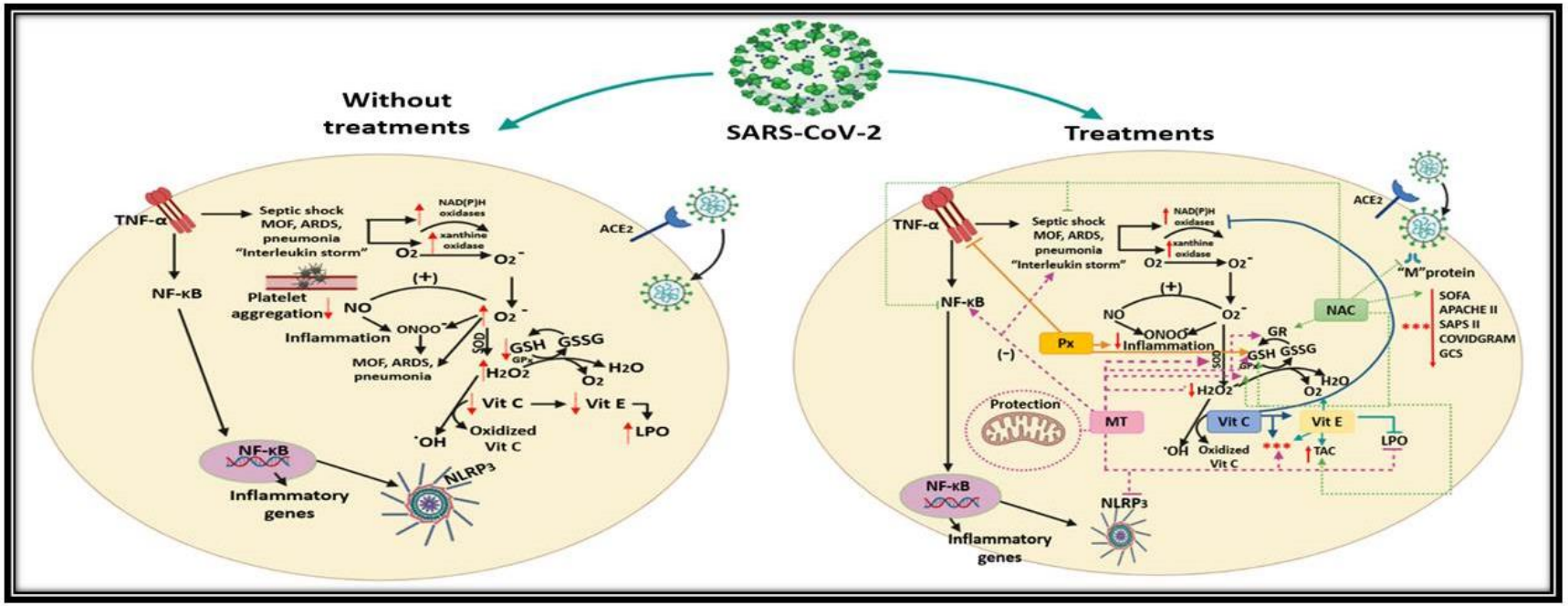

Figure 4. Effect of different treatments for SARS-CoV-2 on antioxidant systems. Abbreviations: **ACE2** = angiotensin converting enzyme 2, **Apache II** = acute physiology and chronic health evaluation II, **ARDS** = acute respiratory distress syndrome, **COVIDGRAM** = name of critical Illness risk score, **H2O2** = hydrogen peroxide, **GCS** = glasgow coma scale, **GPx** =glutathione peroxidase, **GR** = glutathione reductase, **GSH** = glutathione, **GSSG** = oxidized glutathione, **LPO =** lipid peroxidation, **MOF =** multiple organ failure, **MT** =melatonin, **NAC** = N-acetylcysteine, **NF-κB =** nuclear factor κ-light-chain-enhancer of activated B cells, **NLR3 =** NLR family pyrin domain containing 3, **NO** = nitric oxide, **O2-** = superoxide anion, **OH** = hydroxyl radical, **ONOO−** = peroxynitrate, **Px** = pentoxifylline, **SAPS** = Simplified Acute Physiology Score II **SOD** = superoxide dismutase, **SOFA** = sequential organ failure assessment, **TAC** = total antioxidant capacity, **TNF-α** = factor de necrosis tumoral alfa. NLRP3 = NOD-like receptor protein 3 that activation of the inflammasome

Figures

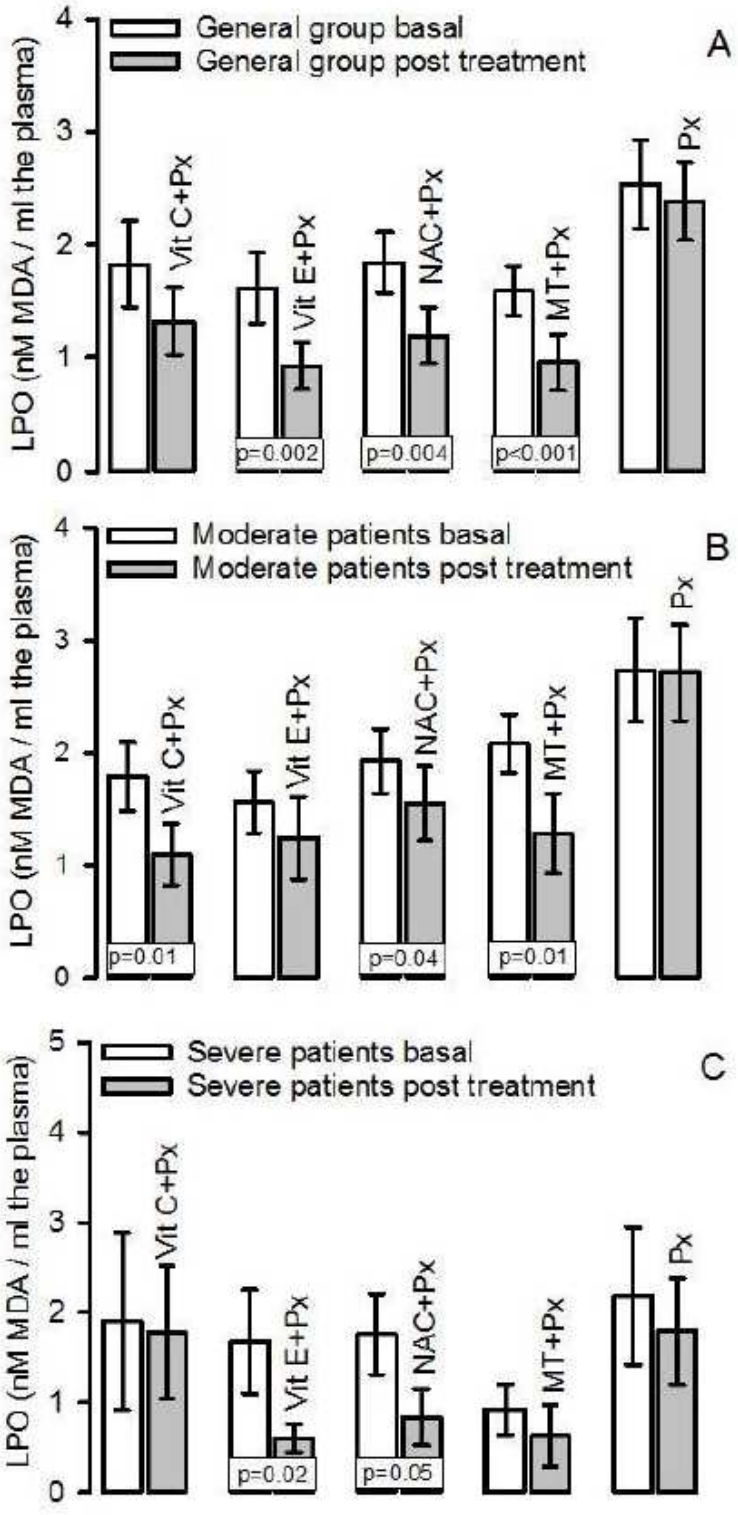

Figure 1

LPO index in the general group both in basal and posttreatment conditions (Panel A), Panel B shows the LPO index in moderate patients both in basal and post treatment conditions, and panel 1C shows the LPO index in severe patients both in basal and post treatment conditions

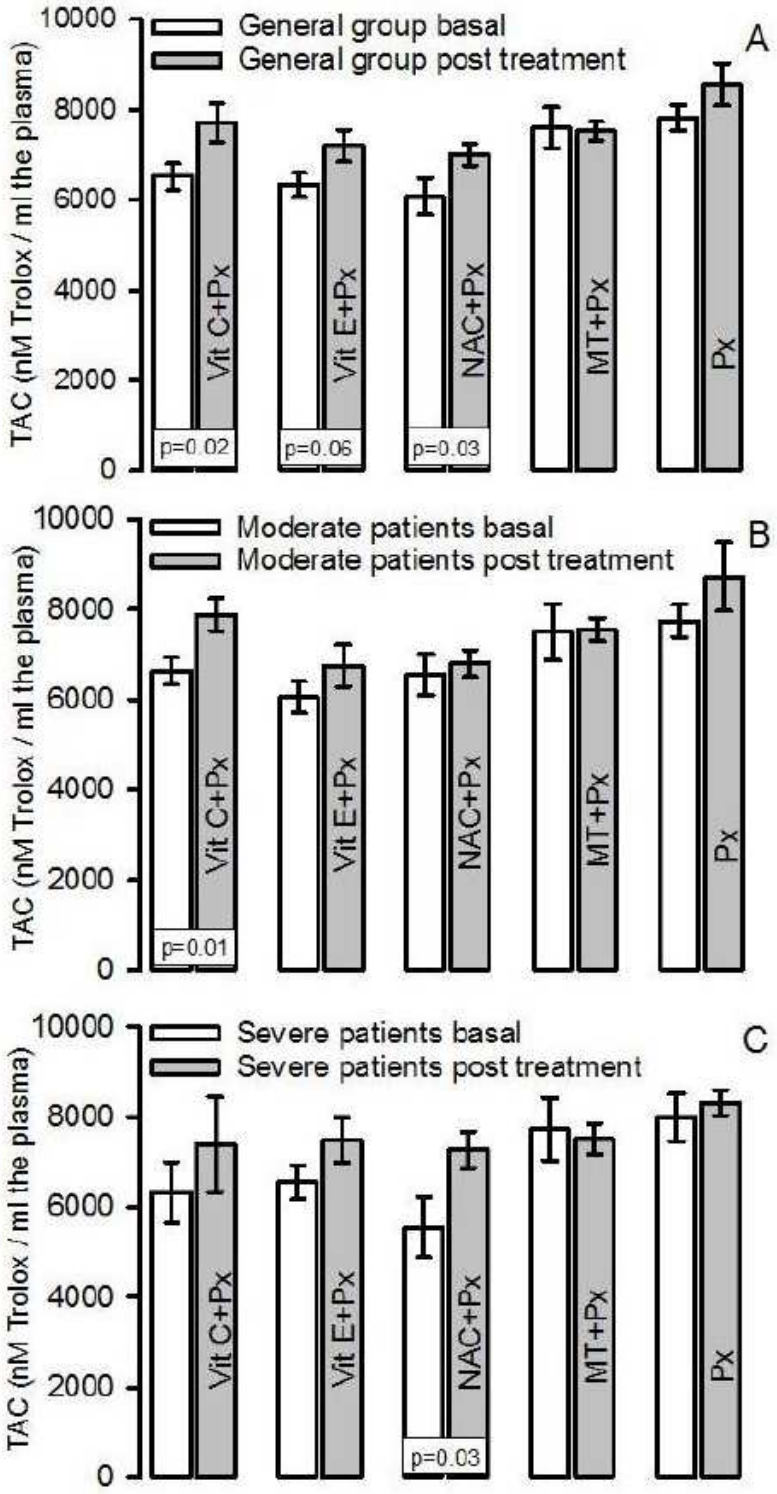

Figure 2

TAC index in the general group both in basal and post treatment conditions (panel A). Panel B shows the TAC index in moderate patients both in basal and post treatment conditions, and panel C shows the TAC index in severe patients both in basal and post treatment conditions

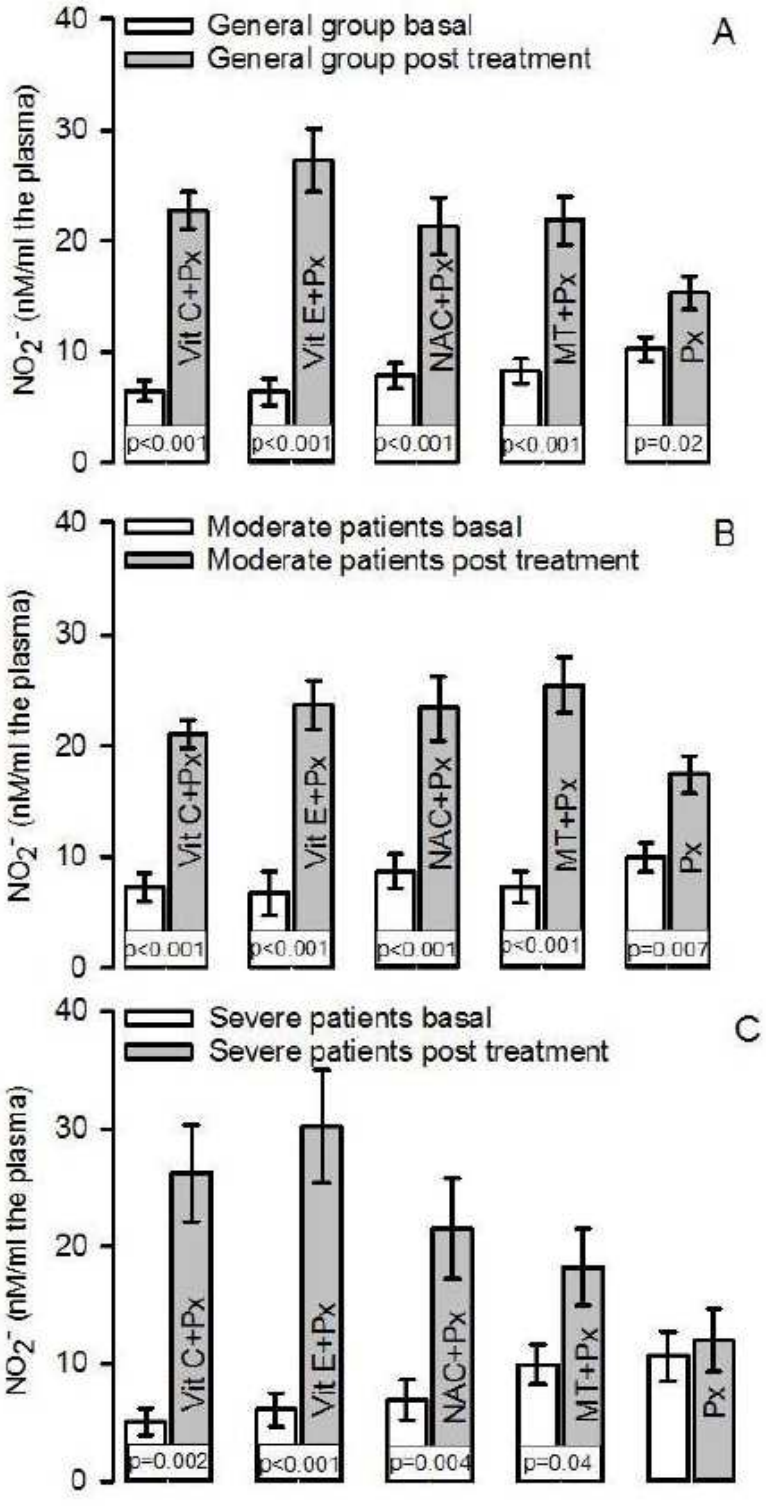

Figure 3

Plasma nitrite concentration in the general group both in basal and post treatment conditions (panel A), panel B shows the NO2– concentration in moderate patients both in basal and post treatment conditions. The panel C shows the NO2– concentration in severe patients both in basal and post treatment conditions

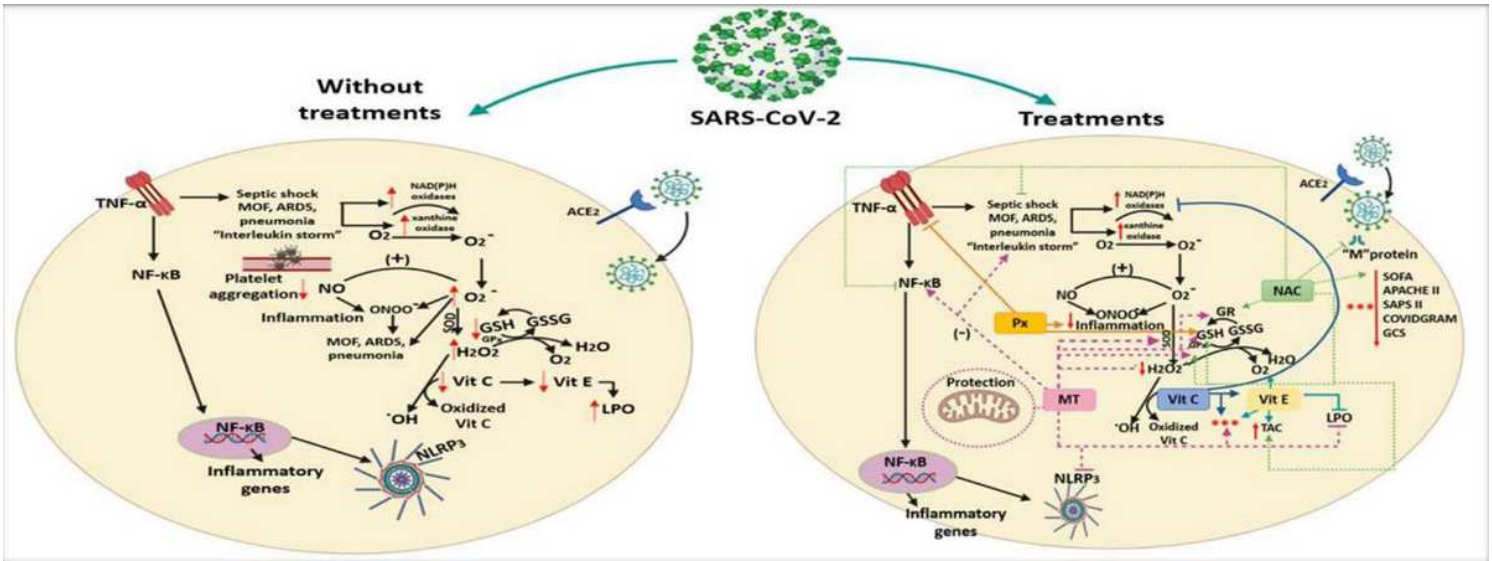

Figure 4

Effect of different treatments for SARS-CoV-2 on antioxidant systems. Abbreviations: ACE2= angiotensin converting enzyme 2, Apache II= acute physiology and chronic health evaluation II, ARDS- acute respiratory distress syndrome, COVIDGRAM= name of critical illness risk score, H2O2= hydrogen peroxide, GCS= glasgow coma scale, GPx= glutathione peroxidase, GR= glutathione reductase, GSH= glutathione, GSSG= oxidized glutathione, LPO= lipid peroxidation, MOF= multiple organ failure, MT= melatonin, NAC= N-acetylcysetine, NF-kB= nuclear factor k-light chain enhancer of activated B cells, NLR3= MLR family pyrin domain containing 3, NO=nitric oxide, O2-= superoxide anion, OH= hydroxl radical, ONOO- = peroxynitrate, Px = pentoxifiyline, SAPA,= Simplified Acute Physiology Score II SOD= Superoxide dismutase, SOFA= sequential organ failure assessment, TACS= total antioxidant capacity, TNF-a= factor de necrosis tumoral alfa. NLRP3= NOD-like receptor protein 3 that activation of the inflammasome.