A Randomized clinical trial of antioxidant therapy in patients with septic shock. Reference study to propose adjuvant therapy in patients with critical organic damage by COVID-19

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Research

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Abstract

Background: Oxidative stress (OS) participates in the pathophysiology of patients with septic shock having multiple organ failure (MOF), ischemia-reperfusion injury and acute respiratory failure syndrome (ARDS). Antioxidants have been proposed in their therapy.

Objective: To evaluate the effect of antioxidant treatment in patients with septic shock with MOF and levels OS after treatment.

Research question: Will the administration of specific antioxidant therapy decrease deregulatory factors of oxidative stress and organ failure in patients with septic shock?

Study design and Methods: Double-blind, placebo-controlled randomized clinical trial run in 2 ICU in Mexico City between May 2018 and January 2020. The random allocation sequence was generated using computer methods. Patients older than 18 years of either sex, with septic shock were included, were excluded when informed consent could not be obtained, they received chronic or recent use of steroids, statins, or antioxidants or if they had contraindications to the use of antioxidants. All antioxidants were administered by mouth or nasogastric tube during 5 days and were added to standard.

Results: 97 patients were included with median age of 66 years. 20 were treated with MT and 18 with Vit C and they showed post-treatment decreased SOFA scores [p=0.007 and p<0.001 respectively]. Also, total antioxidant capacity (TAC) was improved by NAC. All patients had decreased basal levels of Vit C and patients that received Vit C had decreased levels of the NO_3^-/NO_2^- (p=0.02) and RCP levels [p=0.045]. Procalcitonin (PCT) levels were reduced by Vit E, [p=0.047], NAC; [p=0.001] and MT [p=0.045]. LPO was reduced in patients that received MT p=0.042

Conclusion: In septic shock, antioxidant therapy associated with standard intensive care unit therapy reduces MOF, the oxidative and inflammatory state. These results could be a reference to use adjuvant antioxidant therapy in patients with septic shock in COVID19.

Trial Registration: ClinicalTrials.gov Identifier: NCT 03557229

Introduction

Damage caused by OS participates in the pathophysiology of serious diseases like MOF due to sepsis. Bacteria, fungi and viruses or a combination of them cause these diseases (1). Sepsis and septic shock are the largest cause of mortality worldwide in the Intensive Care Units (ICU), MOF (2) constituting a high cost to in health systems (3).

Studies in animal models and in patients with septic shock have shown an imbalance between the production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and antioxidant defenses (4–6).

In sepsis, ROS are generated by phagocytic cells, by the increased activity of enzymes such as NAD(P)H oxidase, xanthine oxidase and inducible nitric oxide (iNOS) and by increased inflammatory mediators through the activation of nuclear factor κB (NFκB) (7). Mitochondrial damage caused by OS is a component of the pathophysiology of MOF secondary to sepsis (8).

Antioxidants such as N-acetylcysteine (NAC), melatonin (MT), vitamins (A, C and E), enzyme cofactors (selenium and zinc) and endogenous compounds (ubiquinone, α lipoic acid, bilirubin, albumin, ferritin, and quercetin) inhibit ROS and RNS, by counteracting their effects (9).

NAC has anti-inflammatory and antioxidant properties (10, 11). Its antioxidant capacity is due to the replenishment of glutathione (GSH) deposits and to sequestration of ROS (12). NAC improves hemodynamic variables, cardiac indexes (13) oxygenation, compliance of lung statics, hepatosplenic flow and liver function in septic shock. Thus, NAC decreases MOF (14) and reduces levels of IL-8, soluble α receptor tumor necrosis factor p55 (15), IL-6 and ICAM-1 (16). NAC reduces mechanical ventilation length, days in ICU stay and mortality (17).

Vit C prevents the excessive production of nitric oxide (NO), decreases vasoconstriction and loss of vascular permeability. (18). Decreased Vit C levels are related to severity of MOF and mortality (19). Vit C therapy decreases SOFA scores, PCT, C-reactive protein (CRP) and thrombomodulin leading to a lower mortality rate (20).

E (Vit E) is an important lipophilic antioxidant in cell membranes. It protects them from lipid peroxidation (LPO), (21). In septic patients with decreased levels of Vit E and O_2^- overproduction, the administration of Vit E and simvastatin inactivates NAD(P)H oxidase (22).

MT lowers OS both at the plasma and intracellular membranes due to its hydrophilic and lipophilic properties. MT possesses ROS sequestration properties, thus protecting cell membrane lipids, cytosol proteins, and nuclear and mitochondrial DNA (23–25).

Recently, there has been an increase in the prevalence and incidence of sepsis and septic shock due to the current pandemic caused by SARS-CoV2 (26, 27).

Although there is a marked increase in ROS and a decrease in endogenous antioxidant defenses in critically ill patients with sepsis (28), the usefulness of different antioxidants has not yet been evaluated through clinical-randomized trials. In this study we evaluated the antioxidant effect of Vit C, Vit E, NAC and MT in patients with septic shock through the Sequential Organ Failure Assessment (SOFA) score and the measurement of antioxidant markers and OS.

Methods

This was a double- blind study in 2 ICU in Mexico City with a parallel randomized group.

Study Population

Patients were admitted to the ICU with a primary diagnosis of septic shock. All diagnostic criteria for septic shock were based on the Sepsis-3 consensus (29) which had to be fulfilled within a maximum of 24 hours prior to enrollment, with an acute increase of at least 2 points in the SOFA score (30), lactate level greater than 2 mmol/L and patients were dependent on a vasopressor for at least 2 hours at the time of enrollment. Exclusion occurred when they were younger than 18 years, not able to grant an informed consent, refused to be included, if they were under chronic use (last 6th months) or recent use of steroids, statins or antioxidants, any contraindication for the use of Vit C, Vit E, NAC o MT of if pregnant or breastfeeding.

Ethical approval was obtained from the local ethics committee (PT 10-0-76; ABC 18–19). Written informed consent for enrollment or consent to continue and use patient data was obtained from each patient or their legal surrogate.

Randomization, Masking, and Drug Administration

The random allocation sequence was generated at the coordinating center using computer-generated random program. (Fig. 1). Blinding was maintained by the investigational pharmacy at each institution. Investigators were blinded from the onset until the analysis outcomes were completed.

Administration of all antioxidants was by mouth or nasogastric tube for 5 days. Tablets of 600 mg every 12 hours of NAC were used. MT capsules of 5 mg were given to patients 50 mg once a day. Vit C 1 gm tablets every 6 hours were given. We used Vit E capsules of 400 UI every 8 hours. Patients of the control group did not receive any type of therapy. All data entry was monitored at the coordinating center, with site visits for source data verification.

Standard therapy at the ICU

Patients were treated according to the recommendation of the International Guidelines for Management of Sepsis and Septic Shock (30).

Outcomes

Primary outcome was SOFA scores for up to 5 days. There were 14 pre-specified secondary outcomes, including plasma OS markers (nitrate/nitrite (NO_3^-/NO_2^-), LPO, GHS levels, TAC, carbonylation and Vit C levels) at 48 hours. Secondary outcomes were measured on day 28 including mortality due to any cause, ventilator-free days, ICU-free days, and hospital-free days. Ventilator-free days were defined as the number of days a patient was extubated from mechanical ventilation, after ICU admission and requiring reintubation were subtracted from the total days. If the patient died in the hospital, a value of zero was

assigned to postextubation. ICU-free days began the moment the patient was transferred out of the ICU to day 28. Hospital- and ICU-free days were calculated similarly.

Study Measurements and Procedures

To evaluate the organ dysfunction, SOFA score (neurologic, respiratory, hemodynamic, hepatic, and hematologic) was calculated at admission and during all days of treatment. The CRP and the PCT determinations were performed on admission before the beginning of the antioxidant therapy and during the next 7 days.

Sampling for the determination of oxidative stress and antioxidant state

The measurement of OS markers was done before the beginning of the antioxidant therapy and 48 hours after its initiation.

Sample obtention and storage

Blood samples were obtained from each patient entered to the draw, before initiation of the treatment and 48 hours after it began. The plasma of the samples was placed in 3 or 4 aliquots and was stored at -70 °C.

Oxidative stress markers

Nitrates and nitrites

The NO_3^- was reduced to NO_2^- by the nitrate reductase enzyme reaction. At the end of the incubation period 200 µl of sulfanilamide1% and 200 µl of N-naphthyl-ethyldiamine 0.1% were added and the total volume was adjusted to 1 ml. The absorbance was measured at 540 nm.

Lipid Peroxidation

50 µl CH3-OH with 4% BHT plus phosphate buffer pH 7.4 was added to 100 µl of plasma. It was incubated, centrifuged at 4000 rpm at room temperature for 2 min. Then the n-butanol phase was extracted, the absorbance was measured at 532 nm.

Glutathione concentration

800 µl of phosphate buffer 50 mM, pH 7.3, plus 100 µl of Ellman reactive (5,5' dithiobis 2-nitrobenzoic) 1M, were added to 100 µg of plasma previously deproteinization with 20% trichloroacetic acid (vol/vol). It was incubated at room temperature and absorbance was read at 412 nm.

Evaluation of total antioxidant capacity

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

Carbonylation

100 µl of plasma were added to 500 µl of HCl 2.5 N in parallel with another sample with 500 µl of 2, 4-dinitrophenylhydrazine (DNPH), and incubated. At the end of the incubation period, they were centrifuged at 15,000 g for 5 min. The supernatant was discarded. Two washings were performed. The mixture was incubated again at 37 °C for 30 min. Absorbance was read in a spectrophotometer at 370 nm, using water bi-distilled as blank and a molar absorption coefficient of 22000 M^{-1} cm^{-1}.

Vitamin C

100 μl of 20% trichloroacetic acid was added to 100 μL of plasma., centrifuged at 5000 rpm for 5 min. 200 μl of Folin-Ciocalteu reagent 0.20 mM was added to the supernatant. The mixture was incubated for 10 min. The absorbance was measured at 760 nm.

Statistical Analysis

Based on a SD of 2.9 of the SOFA score, the study estimated to require 55 (11 per group) patients to have 84% power (2- sided with an $\alpha = 0.05$) and 160 (32 per group). In accordance with these calculations, our study enrolled 97 patients to allow for 10% dropouts, providing a statistical power of 99%, with an $\alpha < .05$. Testing was 2 sided. Effects are reported with a point estimate and 95% CIs in addition to *P* values.

Group comparisons were made using $\chi 2$ tests for equal proportions, *t* tests for normally distributed data, Kruskal Wallis and Wilcoxon rank sum tests otherwise, with results presented as frequencies with percentages, means with SDs, and medians with minimum and maximum, respectively.

The primary end point SOFA score and secondary end points (PCR and PCT) were analyzed with a mixed linear model and fit to repeated-measures analysis of variance. The model included 1 between-participant factor (group [Vit C, Vit E, NAC, MT, no treatment]), 1 within-participant factor (time [0, 1, 2, 3, 4, and 5

days]), and the interaction between group and time, testing the hypothesis that differences between treatment groups are the same over time. Because of a potential for type I error caused by multiple comparisons, findings for analyses of secondary end points should be interpreted as exploratory. Statistical analysis was performed with Stata version 15.1.

Results

Characteristics of the Patients

From July 2018 to November 2019 a total of 1695 eligible patients were identified, of whom 1598 were excluded (reasons listed in Fig. 1**)**. Ninety-seven patients were randomized, with 18 assigned to each antioxidant and 21 to the control group. Of all patients included none was lost in the follow up. Baseline demographic data (eg, age, sex) were similar between the groups (Table 1).

Table 1
Baseline Participant Characteristics

Characteristics	Vit C (n = 18)	Vit E (n = 18)	NAC (n = 20)	MT (n = 20)	Control (n = 21)
Age, median (min-max), y	62 (22–95)	65.5 (22–91)	67.5 (18–95)	62.5 (46–95)	76 (51–89)
Weight, median (min-max),	71 (33–112)	71.5 (40–120)	69.5 (39–95)	67 (50–106)	68 (50–105)
BMI, median (min-max), weight/height^2	25.4 (14.7–40.4)	25 (15.1–41.4)	22.45 (16.5–30.3)	25.35 (17.3–52)	25.4 (19.6–58)
Sex, No. (%)	6 (6.19)	12 (12.37)	11 (11.34)	10 (10.31)	10 (10.31)
Men	12 (12.37)	6 (6.19)	9 (9.28)	10 (10.31)	11 (11.34)
Women					
Chronic health condition, No. (%)	4 (4.12)	4 (4.12)	3 (3.09)	5 (5.15)	6 (6.19)
Diabetes Mellitus	6 (6.19)	8 (8.25)	9 (9.28)	7 (7.22)	11 (11.34)
Hypertension	5 (5.15)	9 (9.28)	7 (7.22)	7 (7.22)	11 (11.34)
Cancer	1 (1.03)	2 (2.03)	4 (4.12)	3 (3.09)	2 (2.06)
Chronic renal failure					
Admission source, No. (%)	9 (9.28)	12 (13.37)	10 (10.31)	14 (14.43)	9 (9.28)
Emergency department	4 (4.12)	2 (2.06)	3 (3.09)	2 (2.06)	4 (4.12)
Operating room	3 (3.09)	4 (4.12)	7 (7.22)	4 (4.12)	7 (7.22)
Inpatient ward transfer	2 (2.06)	0	0	0	1 (1.03)
Other					
Primary site of infection, No. (%)	7 (7.37)	9 (9.97)	9 (9.97)	8 (8.42)	6 (6.32)
Pulmonary	7 (7.37)	3 (3.16)	4 (4.21)	3 (3.16)	9 (9.97)
Gastrointestinal	2 (2.11)	2 (2.11)	5 (5.26)	5 (5.26)	3 (3.16)
Urinary	0	2 (2.11)	0	0	1 (1.05)
CNS	0	1 (1.05)	0	2 (2.11)	0
Blood					

Characteristics	Vit C (n = 18)	Vit E (n = 18)	NAC (n = 20)	MT (n = 20)	Control (n = 21)
Physiological variables	11 (5.1–39.9)	10.8 (0.4–25.4)	8.6 (0–32.5)	11.7 (5.2–29.6)	12 (0.9–49.8)
White blood cell count, median (min-max), × 103/μL	256 (76–409)	158 (10–363)	155 (22–470)	187.5 (29–543)	225 (24–436)
Platelet count, median (min-max), x103/μL	1.65 (0–4.8)	2.1 (0.82–10.5)	1.74 (0.99–7.8)	2.27 (1–17)	2.52 (1.1–12.4)
Lactate, median (min-max), mmol/L	0.9 (0.5–5.5)	1.35 (0.4–3.8)	0.92 (0.5–6.6)	1.27 (0.57–6.6)	1.2 (0.5–5.2)
Serum creatinine, median (min-max), mg/dL	0.75 (0.23–3.5)	1.05 (0.35–4.4)	0.80 (0.2-4)	1.03 (0.17–3.7)	1.15 (0.2–13.6)
Bilirubin, median (min-max), mg/dL	168.5 (61–408)	215 (39–271)	146 (71–367)	197 (57–261)	197 (131–560)
PaO2/FiO2, median (min-max), mmHg	18.33 (1.9–1.4)	20.12 (0.5–47)	13.34 (0.02–46.7)	21.75 (1.35–36.7)	20.25 (1.36–5.3)
C reactive protein, median (min-max), mg/dL	1.46 (0.16–321)	2.92 (0.08–109)	2.35 (0.06–95.5)	2.32 (0.22–138.7)	8.25 (0.08–100)
Procalcitonin, median (min-max), ng/dL					
Intervention at randomization, No. (%)	11 (11.58)	9 (9.47)	14 (14.47)	12 (12.63)	16 (16.84)
Mechanical ventilation	9 (9–38)	7 (7.29)	12 (12.50)	9 (9.38)	11 (11.46)
Vasopressors	0	1 (1.04)	0	0	0
Norepinephrine	8 (8.33)	10 (10.42)	8 (8.33)	11 (11.46)	10 (10.42)
Vasopressin	0	0	0	0	1 (1.04)
Norepinephrine plus vassopressine	0	5 (5.21)	1 (1.04)	3(3.13)	5 (5.21)
Inotropes	1 (1.04)	0	0	1 (1.04)	0
Dobutamine	1 (1.04)	2 (2.08)	2 (2.08)	1 (1.04)	3 (3.13)
Levosimendan					
Dopamine					
Renal replacement therapy					
Corticosteroid use during the study, No. (%)	6 (6.19)	11 (11.34)	9 (9.28)	8 (8.25)	10 (10.31)

Characteristics	Vit C (n = 18)	Vit E (n = 18)	NAC (n = 20)	MT (n = 20)	Control (n = 21)
SAPS II, median (min-max)	38 (16–62)	40 (24–73)	38.5 (12–97)	41.5 (13–73)	40 (18–79)
APACHE III, median (min-max)	13.5 (5–47)	19 (11–33)	14.5(5–46)	17 (6–39)	15 (5–38)
SOFA score, median (min-max)	8.5 (3–16)	8.5 (5–14)	8.5 (1–17)	8 (3–14)	8 (1–16)
Time from ICU admission to randomization, median (min-max), h	5 (1.5–70)	6 (1–17)	3 (1–140)	9 (3–48)	-

Abbreviations: Vit C: vitamin C; Vit E: vitamin E; NAC: n-acetylcysteine; MT: melatonin; (min-mx): minimum – maximum; BMI: body mass index; CNS: central nervous system; SAPS II: Simplified Acute Physiology Score; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; ICU: intensive care unit.

Treatments

Treatments were given for a median of 5 days. The median of adherence in the 4 different groups of treatment was 100%. There was no difference between groups in the time from meeting eligibility criteria to the first dose, time receiving the treatment and the adherence.

Primary outcome

Patients receiving MT and Vit C showed a significant decrease in SOFA score [-1.27 (95% CI -2.21 to -0.34); p = 0.007 for MT and − 1.94 (95% CI -2.95 to -0.93); p < 0.001 for Vit C] (Fig. 2).

Secondary outcome

OS marker levels before and after 48 hours of antioxidant therapy are shown (Table 2). The LPO levels were significantly reduced in patients treated with MT and there was a significant decrease in NO_3^-/NO_2^- levels in patients with lung infection treated with Vit C.

Table 2
Oxidative stress markers before and after 48 hours of antioxidant therapy.

Lipid Peroxidation, nM MDA/ml			
Group	Pre	Post	*p*
Vit. C (n = 18)	3.44 (0.52–19.62)	2.81 (0.23–8.70)	0.14
Vit. E (n = 18)	4.33 (1.25–15.25)	3.24 (0.38–12.07)	0.17
NAC (n = 20)	3.46 (0.23–9.49)	3.46 (0.38–11.01)	0.77
M**T** (n = 20)	2.13 (0.23–11.68)	2.42 (0.23–7.11)	0.04
Control (n = 21)	3.44 (0.52–9.49)	3.90 (0.23–9.10)	0.75
Nitrates and nitrite, µM/mL			
Group	Pre	Post	*p*
Vit C (n = 18)	2.10 (0.98–2.73)	1.49 (0.03–2–57)	< 0.01
Vit E (n = 18)	1.79 (0.53–3.81)	2.00 (0.76–5.65)	0.36
NAC (n = 20)	2.43 (0.80–7.02)	2.15 (0.01–8.16)	0.81
M**T** (n = 20)	1.72 (0.67–4.77)	1.32 (0.03–7.42)	0.19
Control (n = 21)	2.25 (0.28–2.76)	2.24 (0.01–7.22)	0.97
Total antioxidant capacity, nM/ml			
Group	Pre	Post	*p*
Vit C (n = 18)	2226.2 (747.6–3053.4)	2050.9 (966.6–2551.8)	0.11
Vit E (n = 18)	2148.4 (886.3–3287.6)	2223.1 (618.3–3841.9)	0.90
NAC (n = 20)	1453.6 (621.5–2351.4)	1951 (812.6–3528.7)	0.05
M**T** (n = 20)	1999 (561.3–2519.2)	1747.5 (456.5–2745.6)	0.59
Control (n = 21)	2451.6 (1600–3467.1)	2064.7 (312.4–3501)	0.42
Carbonylation, ng/mL			
Group	Pre	Post	*p*
Vit C (n = 18)	48.85 (10.90–114.53)	44.76 (12.72–98.17)	0.59
Vit E (n = 18)	52.26 (27.27–137.25)	42.723 (21.36–89.53)	0.07
NAC (n = 20)	40.22 (22.27–89.99)	41.13 (22.72–93.17)	0.47
MT (n = 20)	74.76 (8.63–181.34)	62.721 (29.99–142.25)	0.40
Control (n = 21)	46.359 (9.99–106.80)	44.08 (26.36–111.80)	0.28

Lipid Peroxidation, nM MDA/ml			
Glutathione concentration, nM/ml			
Group	Pre	Post	*p*
Vit C (n = 18)	0.10 (0.01–0.24)	0.08 (0.01–0.20)	0.50
Vit E (n = 18)	0.05 (0.00–0.30)	0.07 (0.00–0.32)	0.38
NAC (n = 20)	0.08 (0.00–0.54)	0.10 (0.009–0.57)	0.14
Melat (n = 20)	0.07 (0.00–0.32)	0.07 (0.010–0.51)	0.64
Control (n = 21)	0.06 (0.03–0.20)	0.05 (0.01–0.16)	0.15
Vitamin C, µM/mL			
Group	Pre	Post	*p*
Vit C (n = 18)	0.17 (0.04–0.87)	0.27 (0.06 – .99)	< 0.01
Vit E (n = 18)	0.27 (0.08–0.99)	0.26 (0.12–0.79)	0.58
NAC (n = 20)	0.21 (0.09–0.61)	0.18 (0.00–0.96)	1.00
MT (n = 20)	0.21 (0.04–0.56)	0.21 (0.04–0.43)	0.83
Control (n = 21)	0.22 (0.08–0.77)	0.19 (0.07–0.64)	0.02
Abbreviations: Pre: pre-treatment; Post: post-treatment; Vit C: vitamin C; Vit E: vitamin E; NAC: n-acetylcysteine; MT: melatonin. All values are expressed as median (minimum-maximum). Wilcoxon matched pairs signed rank tests.			

Patients receiving Vit C had a significant decrease in CRP levels per day of treatment (Fig. 3). PCT levels were significantly decreased in patients receiving Vit E, NAC, and MT (Fig. 4). Vit E showed a tendency to reduce levels before and after treatment of LPO and of carbonylation.

Regarding the secondary outcomes, 13 patients (13.68%) required renal replacement therapy, 63 (65.63%) mechanical ventilation and 17 (17.89%) died. There was no statistically significant difference in days free of renal replacement therapy, mechanical ventilation, ICU stay length or hospitalization at 28 days. There was also no statistically significant difference in intrahospital mortality.

Undesired side effects

A patient receiving Vit C presented abdominal pain and another patient underwent a skin rash. Only one patient who received MT reported drowsiness. No adverse events were reported in patients with NAC or Vit E.

Discussion

Treatment with antioxidants as an adjuvant in the standard management of patients with sepsis, septic shock and infection with COVID-19 has been suggested (31–34). We studied critically ill patients with septic shock regardless of the etiology and site of infection. All patients had initial low levels of Vit C, which was related with the severity of organ failure and mortality (18). The decrease in Vit C levels confirms the reported hypovitaminosis (< 0.23 μM ascorbic ac/mL) in septic shock (34–37). It may be due to augmented metabolic demand, since intestinal absorption is not compromised in the patients in our study. (36). Vit C restored the normal values of this vitamin and organ function was improved. The best result was found in subjects with pneumonia with significant difference. This finding is in agreement with previous results (38–40). The combined use of Vit C, thiamine and steroids has recently been suggested. It is still necessary to compare if the use of Vit C alone has worse effects than the combinations (41).

In patients with septic shock, the administration of Vit C and MT improved the organ dysfunction assessed by the SOFA score. This finding could be associated to a decrease in the NO_3^-/NO_2^- and LPO levels.

The CITRIS-ALI study in patients with acute respiratory failure syndrome, ARFS, and organ failure showed no improvement with Vit C (42). The median time before starting treatment with Vit C was of 5 hours in this study, and markers such as CPR were significantly decreased, which was similar to another study (43). The possible difference with our results could be related to the fact that in the CITRIS-ALI study they started the therapy with Vit C later.

The VITAMINS trial showed no significant difference in the SOFA score, or in days without ventilation at 28 day; however, the use of Vit C lowered mortality (44). In that same study, CRP levels were not decreased, which was probably due to the late administration of Vit C in advanced stages of sepsis before developing ARDS (42). In contrast, we found a decrease in the levels of NO_3^-/NO_2^- which is relevant since Vit C inhibits the production of superoxide (O_2^-) and peroxynitrite, thus preventing abundant NO synthesis, inhibiting mRNA expression and decreasing pathological vasoconstriction (17). These effects might be underlying the clinical benefit. A shorter time of use of vasopressors and a decreased intrahospital mortality was found in patient receiving Vit C (45).

This is the first study of the use of MT in humans with septic shock. Recently MT has been applied in subjects with COVID 19 and it has a high safety profile limiting this virus-related disease. Experimental and clinical studies are required to confirm this hypothesis (31). MT possesses free radical scavenging properties thus protecting cell membrane lipids, cytosol proteins, and nuclear and mitocondrial DNA (23, 24). In our findings, LPO was significantly decreased in the group of patients who received MT which was similar to results in the Galley's study (25). MT has beneficial effects in experimental cells, plants, and animals; however, its mechanisms of action remain unknown. The functions of the MT receptor relate to its ability as a detoxification agent, thus protecting molecules from the destructive effects of OS in ischemia/reperfusion (stroke, heart attack), ionizing radiation and drug toxicity. In sepsis, the protective effects of MT are associated with the inhibition of the apoptotic processes and reduction of OS.

Production of ROS is increased in an animal model of septic shock (46, 47). This coincides with a lowering of the TAC and a reduction of the activity of superoxide dismutase and GSH peroxidase (48–55). MT reversed morphological damage and increased the activities of antioxidant enzymes (46, 48, 61, 49, 53, 55–60). Therefore, research through blinded clinical trials (62, 63) and multicenter studies with adequate amounts of MT are needed to determine the potential of MT. In this clinical trial, we found a reduction of LPO and its potentially beneficial effect in organ dysfunction. Its use as an adjuvant in septic shock reduces inflammation and oxidation in animal models with respiratory damage induced by infection. MT has positive physiological actions, it is effective and safe for patients with septic shock of any etiology including those infected with SARS-CoV2 (31).

The use of NAC improved the antioxidant capacity and tended to increase GSH although the difference was not statistically significant. This confirms its antioxidant effect through the replacement of GSH deposits (12). NAC was related to decreases in organ failure, confirming previous findings (14).

Other antioxidants such as polyphenols, MT, β-glucan, antioxidants targeting mitochondria, selenium salts, and selenium organ compounds are effective for improving OS in sepsis. The study of their pathophysiological implications justifies the combined therapy with antioxidants and standard treatments.

Vit E tended to decrease LPO and carbonylation. This vitamin protects cell membranes from LPO, ending their chain reaction. It is also an O_2^- and hydroxyl (OH) sequestrant (64).

In summary, antioxidants benefit subjects with septic shock. Septic shock is triggered by bacterial stimuli, fungi or viruses. In this medical condition, it is necessary to regulate inflammation and other mechanisms that lead to OS (65).

Limitations

The absorption may be altered by the enteral route of administration. However, we found increments on Vit C levels in serum.

The present trial is underpowered to detect differences in mortality and in outcomes between groups because the sample size was calculated for differences of OS.

Conclusion

In patients with septic shock, adding antioxidants to standard therapy regulates inflammation. In pulmonary sepsis, replacement therapy with Vit C increases its serum levels, which is associated with decreased levels of CRP, PCT, and NO_3^-/NO_2^-. MT decreases LPO and SOFA score. NAC reduces LPO and improves antioxidant capacity. Vit E tends to decrease LPO. Each antioxidant has beneficial effect; thus, they might be combined in clinical trials in patients with septic shock. We suggest the use of antioxidants

as an adjuvant to standard therapy in patients with COVID-19, adjusting for comorbidities and drug interaction.

Abbreviations

(COVID-19) = Coronavirus infectious disease,(OS) = Oxidative stress (ICU) = Intensive Care Units,(MOF) = multiple organ failure,(ARDS) = respiratory failure syndrome,(SOFA) score sequential organ failure assessment, (iNOS) inducible nitric oxide, (ROS) reactive oxygen species,(RNS) reactive nitrogen species, (NAC), N-acetylcysteine,(MT) melatonin, (GSH) glutathione, (Vit) Vitamin, (NO_3^-/NO_2^-) nitrate/nitrite, (LPO) Lipoperoxidation, (CRP) C reactive protein

Declarations

Availability of data and materials

The data generated or analyzed during the current study are included in this manuscript and its additional files.

Ethics approval and consent to participate.

This study was approved by the Ethics Committees of American British Cowdray (ABC) Medical Center, I.A.P., and Instituto Nacional de Cardiologia Ignacio Chavez, informed consents were obtained from all patients

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

AAA: Analysis data collection patient care, laboratory work, protocol work in written master's program and article review. IPT: Conceptualization, design, laboratory elaboration, writing, review and approval for publication. GCA: Patient care, article review. JFG: Patients attention and review of the article. VGL: Participation in conceptualization, written laboratory work and review and approval for publication. EMR: Patient care, database management, laboratory work. RG, Laboratory work, review and approval of the writing. MES: Conceptualization, design, thesis tutor, statistical analysis, preparation of the protocol and manuscript revision and approval of the manuscript.All authors read and approved the final manuscript

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Figures

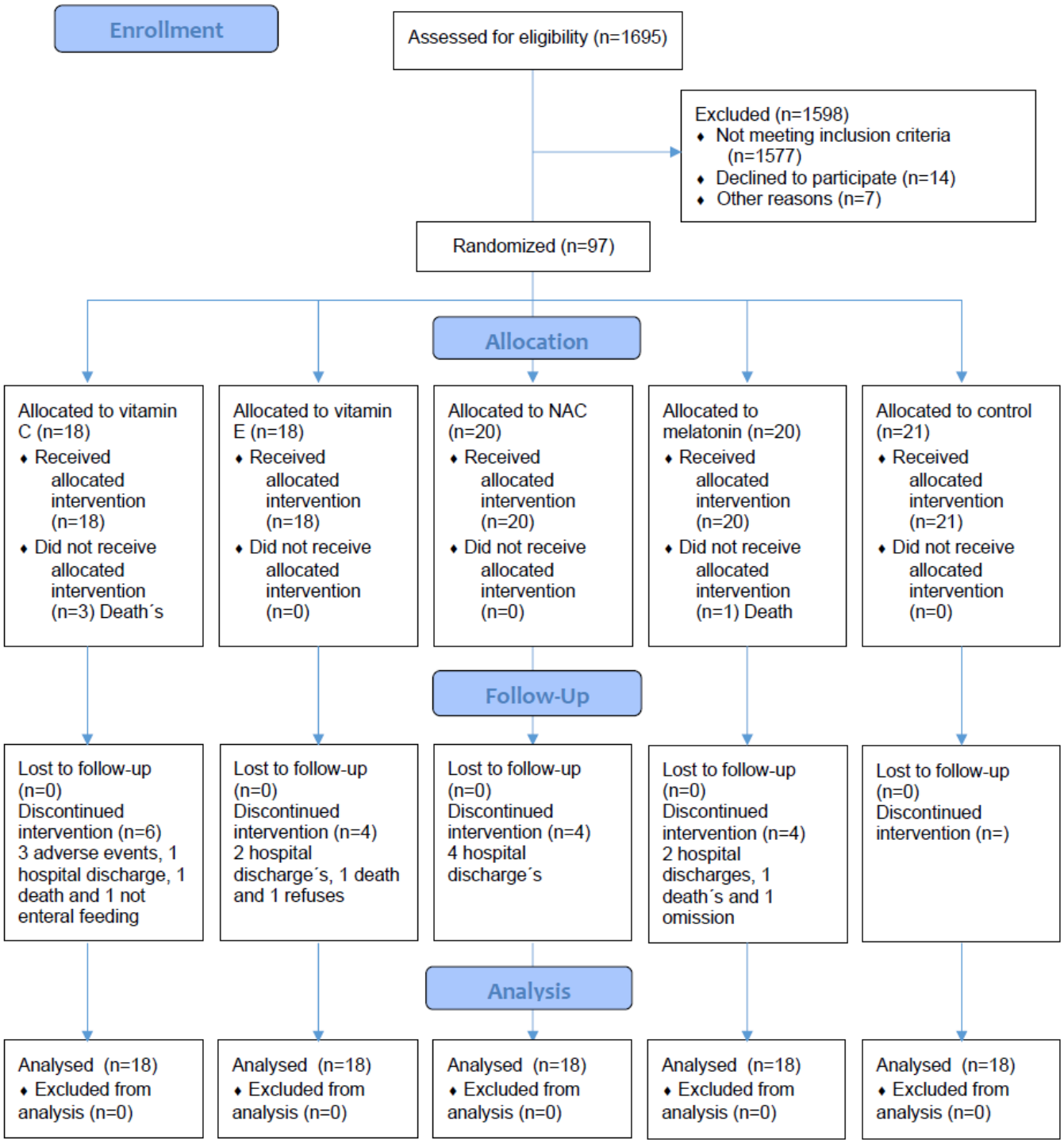

Figure 1

Flow diagram of the study

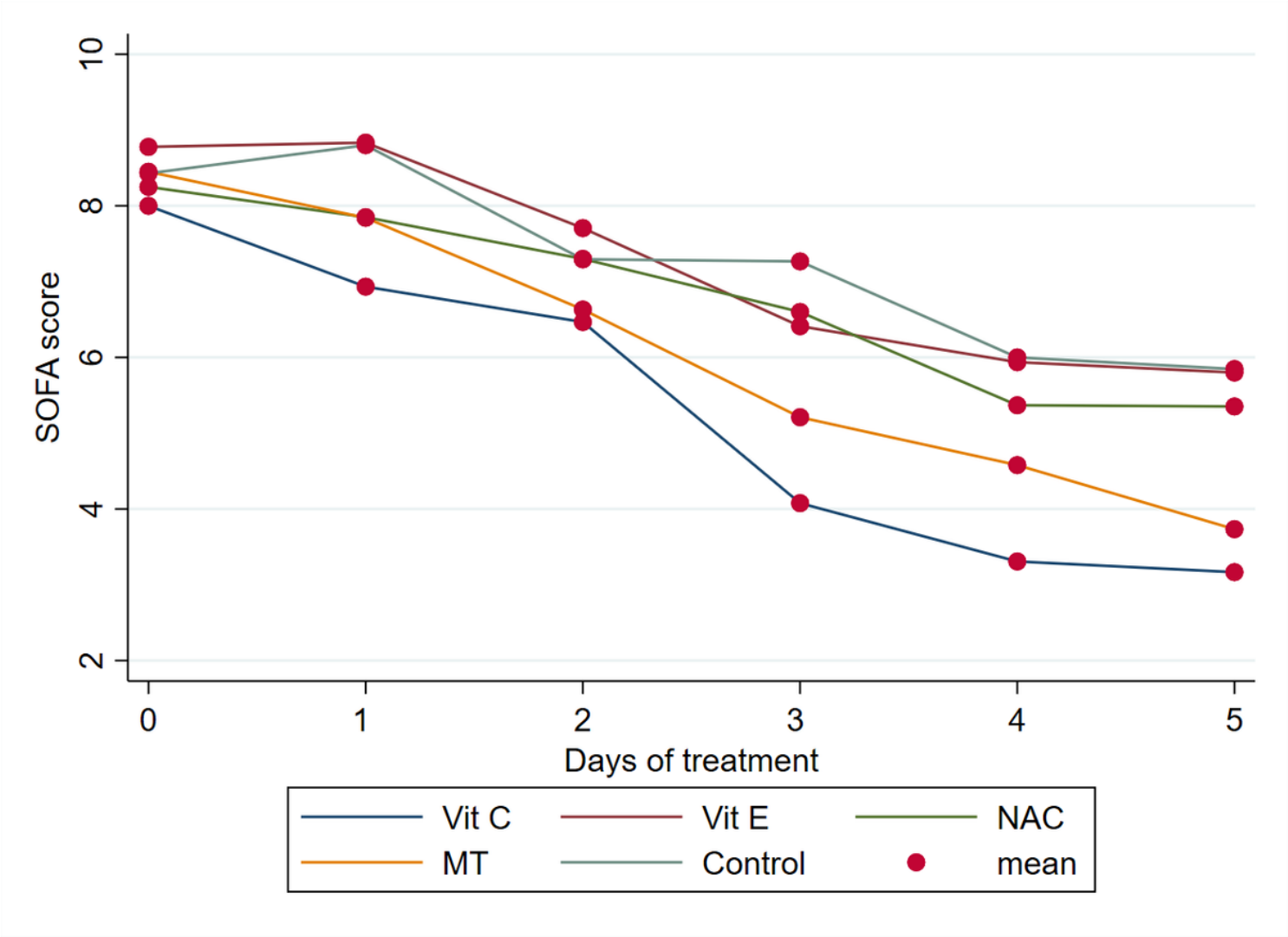

Figure 2

SOFA score in patients: (sequential organ failure assessment); Vit C: vitamin C; Vit E: vitamin E; NAC: n-acetilcisteíne; MT; marginal approximation model taking into account the control group as a base: Melat -1.27 (-2.21 a -0.34; p=0.007); Vit C -1.94 (-2.95 a -0.94; p=<0.001).

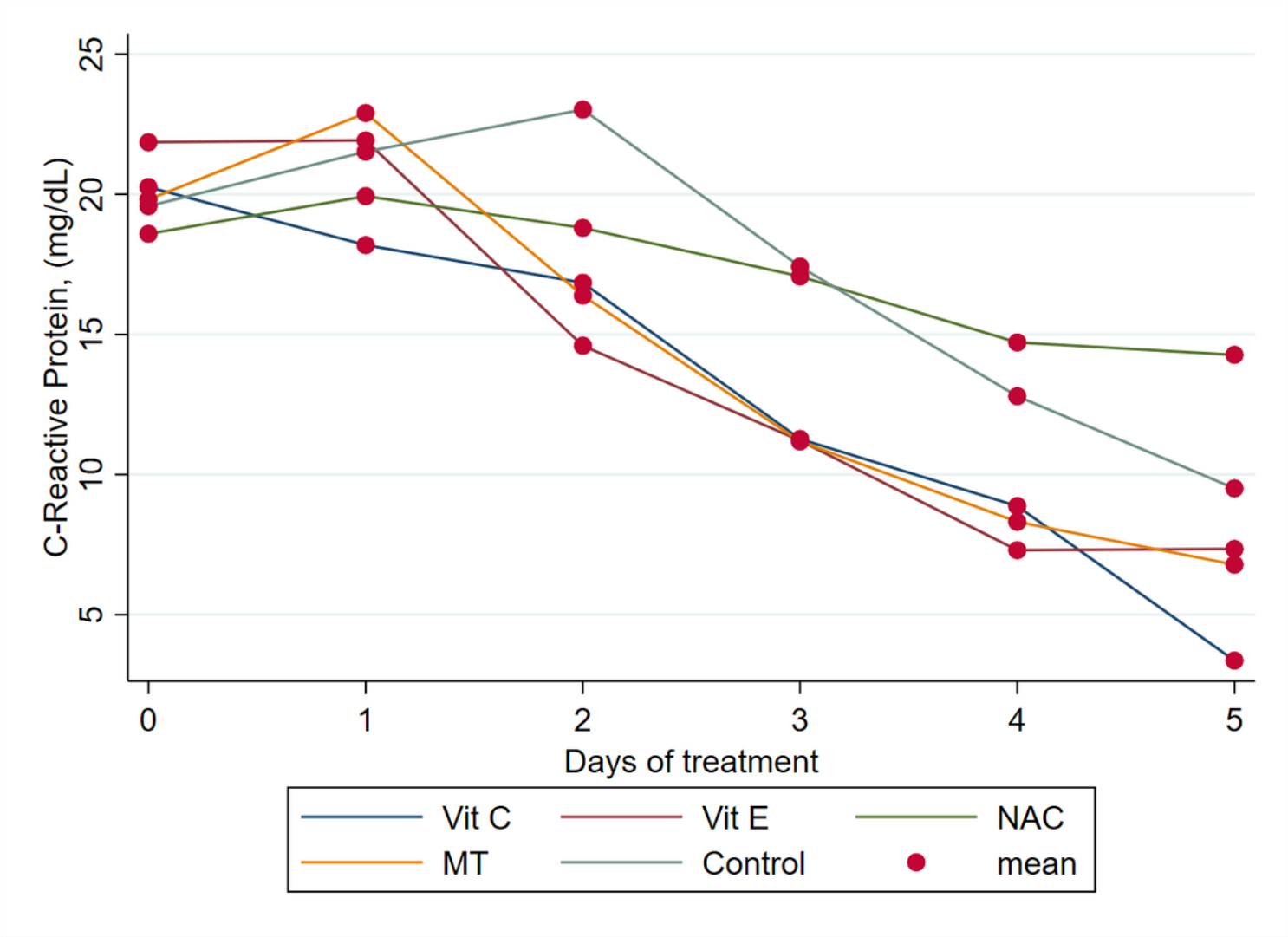

Figure 3

CRP; Vit C; Vit E:; NAC; MT:. Marginal approximation model taking into account the control group as a base: Vit C -3.82 (-7.49 a -0.15; p=<0.041).

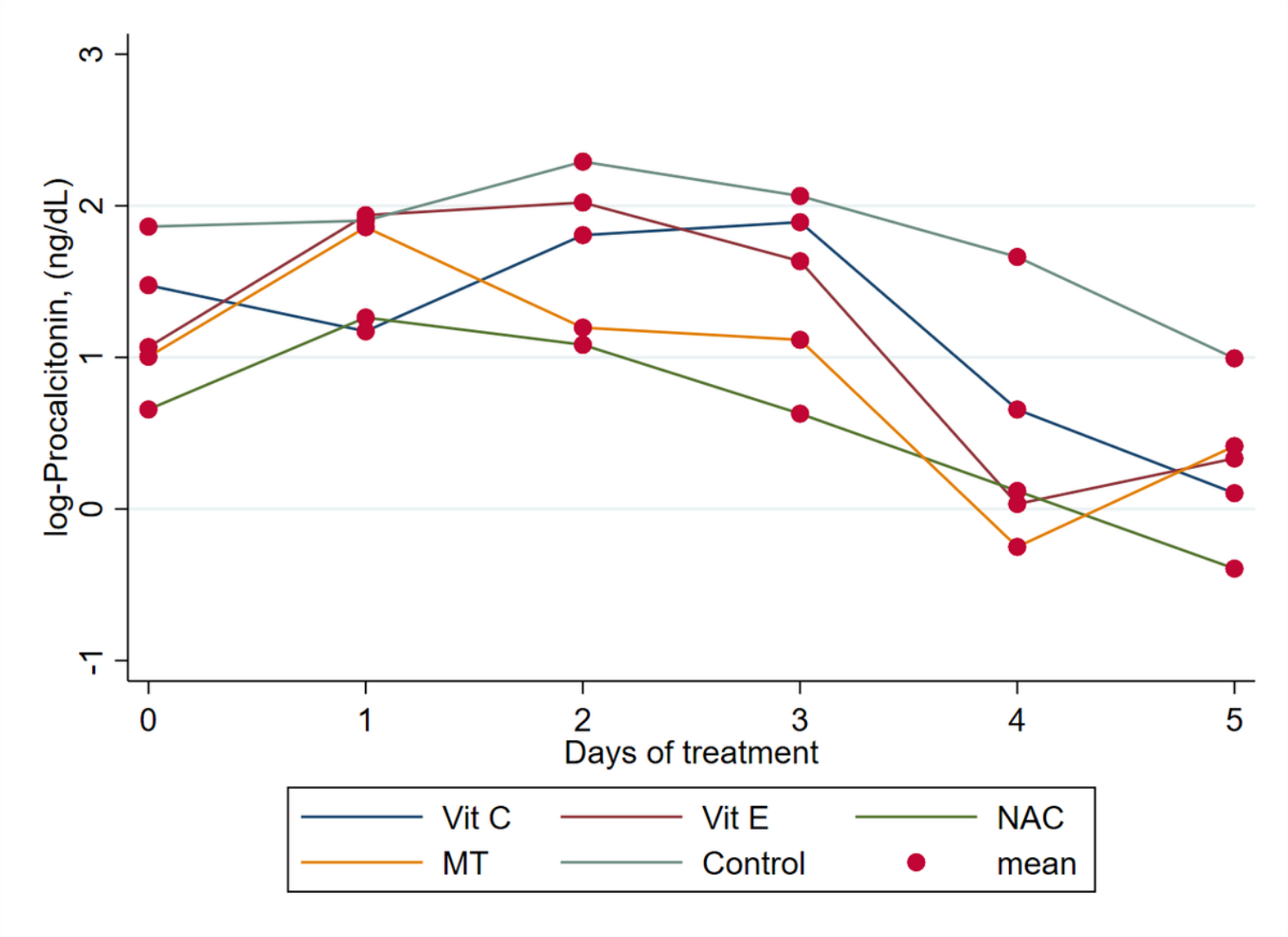

Figure 4

Levels of PCT, Vit C, Vit E,NAC, MT. Marginal approximation model taking into account the control group as a base: Vit E -0.59 (-1.18 a -0.006; p=<0.047); NAC -0.92 (-1.48 a -0.35; p=0.001); MT -0.57 (–1.15 a 0.006; p=0.05).