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# Erythrocyte SOD1 activity, but not *SOD1* polymorphisms, is associated with ICU mortality in patients with septic shock

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ABSTRACT

The objective of our study was to evaluate the influence of the superoxide dismutase 1 (*SOD1)* polymorphisms on erythrocyte SOD1 activity and the mortality of patients with septic shock. We prospectively evaluated 175 patients aged over 18 years with septic shock upon ICU admission. However, 38 patients were excluded. Thus, 137 patients were enrolled in the study. Blood samples were taken within the first 24 h of the patient's admission to determine erythrocyte SOD1 activity and nine *SOD1* gene polymorphisms. The mean patient age was 63 ± 16 years, 58% were men, and ICU mortality rate was 66%. The patients who died were older and more severely ill, with higher Acute Physiology and Chronic Health Evaluation (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores, as well as higher lactate, urea, and protein carbonyl levels. In the logistic regression model, erythrocyte SOD1 activity was associated with ICU mortality. This relationship was also maintained in the highest tertile of SOD1 activity (odds ratio [OR]: 0.02; 95% confidence interval [CI]: 0.00–0.78; p = 0.037). Only SNP rs2070424 of the *SOD1* gene influenced erythrocyte SOD1 activity. For patients with the AA allele, the activity of SOD1 was lower in relation to G-carriers (A/G + G/G genotype) (p = 0.019). None of the nine *SOD1* SNPs were associated with ICU mortality. In conclusion, the SNP rs2070424 of the *SOD1* gene interferes with erythrocyte SOD1 activity, and higher activity of SOD1 was associated with decreased mortality in patients with septic shock.

## 1. Introduction

Septic shock is one of the most important causes of intensive care unit (ICU) admissions, and the leading cause of death in critically ill patients worldwide [1,2]. The incidence of septic shock is not well known; however, it is increasing, likely as a result of the progressive aging of the population, the large number of individuals with co-morbidities, and the increasing recognition of sepsis [2]. Sepsis is characterized by life-threatening organ dysfunction caused by a dysregulated host response to infection [1,2]. In this scenario, oxidative stress plays a key role, as it is a major promoter and mediator of systemic inflammation and organ failure [3–5].

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species and the body's antioxidant defenses [5–7]. Oxidative stress can be measured by different products derived from lipids, proteins and DNA. Protein carbonyl groups are markers of protein damage that are formed early during septic shock and are more stable than lipid peroxidation products [8,9]. Regarding the endogenous antioxidant defense system, it can be divided into an enzymatic and a non-enzymatic group [5–7]. Superoxide dismutase (SOD) is the most abundant enzyme of the antioxidant system, which catalyzes the conversion of superoxide into hydrogen peroxide, and it is considered the first line of defense against ROS [5–7]. SOD1 is the cytoplasmic isoform of SOD, and has copper

*Abbreviations:* APACHE II score, Acute Physiology and Chronic Health Evaluation II score; CI95%, confidence interval 95%; CRP, C-reactive protein; ICU, intensive care unit; MDA, malondialdehyde; OR, odds ratio; ROS, reactive oxygen species; SNPs, single nucleotide polymorphisms; SOD, superoxide dismutase; SOFA score, Sequential Organ Failure Assessment score

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and zinc as cofactors.

Recently, our group published a sub-analysis showing that lower erythrocyte activity of SOD1 was an early predictor of the development of acute kidney injury in patients with septic shock [10]. However, it is important to note that cellular responses to ROS reflect a complex integration of ROS type, location, levels, and polymorphisms of antioxidant enzymes.

Single nucleotide polymorphisms (SNPs) are the most common type of variation in the human genome (about 90% of all variations), and refer to the replacement of only one nucleotide in a certain DNA position. The presence of these polymorphisms can affect protein structure and function, leading to altered enzymatic responses. The decreased activity of antioxidant enzymes could lead to oxidative stress and affect the risk and prognosis of several diseases [11]. Variation in the *SOD1* gene has been found to be associated with cardiovascular deaths in the general population, and the SNP rs1041740 might be associated with the development of ascites in patients with cirrhosis [12,13].

Data in the literature are scarce and there have been no studies evaluating the association between erythrocyte SOD1 activity and *SOD1* polymorphisms and mortality in patients with septic shock. Therefore, the aim of the present study was to evaluate the influence of *SOD1* polymorphisms on erythrocyte SOD1 activity as well as on the mortality of patients with septic shock.

## 2. Materials and methods

### 2.1. Study design

This was a prospective observational clinical study, conducted from April 2014 to May 2015. The protocol was approved by the Ethics Committee of Botucatu Medical School (30457414.7.0000.5411). Written informed consent was obtained from all patients or relatives prior to their inclusion in the study. The sample size was calculated using the following variables: mortality rate of septic shock 40–60%, 95% confidence interval (CI), and 10% sample error. The result was a minimum sample size of 96 patients.

The inclusion criteria were all individuals older than 18 years of age, of both sexes, with a diagnosis of septic shock at ICU admission. Septic shock was defined according to the Surviving Sepsis Guidelines [14]. Exclusion criteria were delayed diagnosis of septic shock (longer than 24 h), pregnancy, noradrenaline dose > 2.0 μg/kg/min, confirmed brain death, palliative care, technical problems in the determination of *SOD1* SNPs and association of other types of shock.

At the time of patient enrollment, demographic information, the Acute Physiology and Chronic Health Evaluation (APACHE II) score, and the Sequential Organ Failure Assessment (SOFA) score were recorded. Blood samples were taken within the first 24 h of the patient's admission to determine erythrocyte SOD1 activity, *SOD1* gene polymorphisms, serum malondialdehyde (MDA), protein carbonyl, and zinc and copper levels. The ICU mortality rate was also recorded.

### 2.2. Laboratory analysis

The hemogram was performed with a Coulter STKS hematological auto analyzer (Beckman Coulter, Inc., Brea, CA, USA). Total serum levels of C-reactive protein (CRP), albumin, creatinine, and urea were measured using the dry chemistry method (Ortho-Clinical Diagnostics VITROS 950®, Johnson & Johnson, New Brunswick, NJ, USA). Lactate was measured using the Roche OMNI S™ Blood Gas Analyzer (Roche Diagnostics, Basel, Switzerland).

Serum MDA levels were analyzed based on the reaction with thiobarbituric acid by high-performance liquid chromatography, as previously specified [15]. Protein carbonyl concentration was determined according to the method described by Reznick and Packer [16].

Zinc levels in plasma and erythrocytes was determined by flame atomic absorption spectrophotometry [17]. The same methodology was used for the analysis of plasma copper concentration. The SOD1 activity in erythrocytes was determined in a Lyasis biochemical analyzer according to the methodology recommended by the manufacturer (Ransod kit; Randox Laboratories Ltd., Crumlin, Antrim, UK) [18].

The reference range of normality was 70–110 μg/dL for plasma zinc concentration; 40–44 μg/g Hb for erythrocyte zinc concentration; 63.7–140.12 μg/dL for plasma copper concentration; and 1102–1601 U/g Hb for erythrocyte SOD1 activity according to the Ransod/Randox kit [19,20]. For comparison, the respective levels in 17 normal volunteers were 78.8 ± 18.2 μg/dL for plasma zinc concentration; 68.3 ± 18.1 μg/g Hb for erythrocyte zinc concentration; 53.2 ± 10.0 μg/dL for plasma copper concentration; and 4340.4 ± 1430.2 U/g Hb for erythrocyte SOD1 activity.

### 2.3. SOD1 gene polymorphisms

DNA was isolated from frozen blood samples using a method previously described [21]. DNA integrity was verified using a 1% agarose gel, while DNA concentration was measured using a Nanodrop 8000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The following SNPs of the *SOD1* gene were analyzed: *rs4998557; rs2070424; rs10432782; rs1041740; rs11910115; rs202446; rs2173962; rs202449;* and *rs17880135*. The genotyping assay was performed using the Taqman Open Array® system (Life Technologies Corporation, Carlsbad, CA, USA), with replicates of the SNP analyses of 20% of the samples and following the manufacturer's instructions.

### 2.4. Statistical analysis

Data are expressed as mean ± standard deviation (SD), in cases of a normal distribution, and median (including the lower and upper quartiles) in cases of a non-normal distribution or percentage. Comparisons between two groups for continuous variables were performed using Student's *t*-test or the Mann-Whitney *U* test. Comparisons between two groups for categorical variables were performed using the chi-square test or Fisher's exact test.

The chi-square test with continuity correction was used to determine if genotype frequencies followed the Hardy-Weinberg equilibrium. Linkage disequilibrium between SNPs and haplotype block formation were tested using Haploview software version 4.2 (Broad Institute, Cambridge, MA, USA). Only haplotype blocks with frequencies above 1% were included in the subsequent analysis.

Simple linear regression models were used to evaluate the association between erythrocyte SOD1 activity, demographics, and laboratory data. Simple and multiple linear regression models were designed to test differences in enzyme activity among the *SOD1* SNP genotypes and were adjusted for variables that exhibited a significant difference in the univariate analyses (serum hemoglobin and MDA concentrations, plasma copper levels, and erythrocyte zinc concentration).

To evaluate the influence of erythrocyte SOD1 activity on mortality, two logistic regression models were created. In the first model, the activity of the enzyme was adjusted for sex, age, and APACHE II score, and in the second model, the adjustment was made for age and protein carbonyl concentration (best fit model).

Logistic regression models were also performed to evaluate the association between mortality and each *SOD1* SNP. These models were adjusted by age and serum protein carbonyl concentration (best fit model). Data analysis was performed using Stata version 13.1 SE (StataCorp, College Station, TX, USA). The significance level was 5%.

## 3. Results

During the study, 175 patients diagnosed with septic shock at the time of admission to the ICU were prospectively evaluated. However, 38 patients were subsequently excluded, 26 owing to technical problems in the determination of *SOD1* SNPs and 12 owing to a delayed

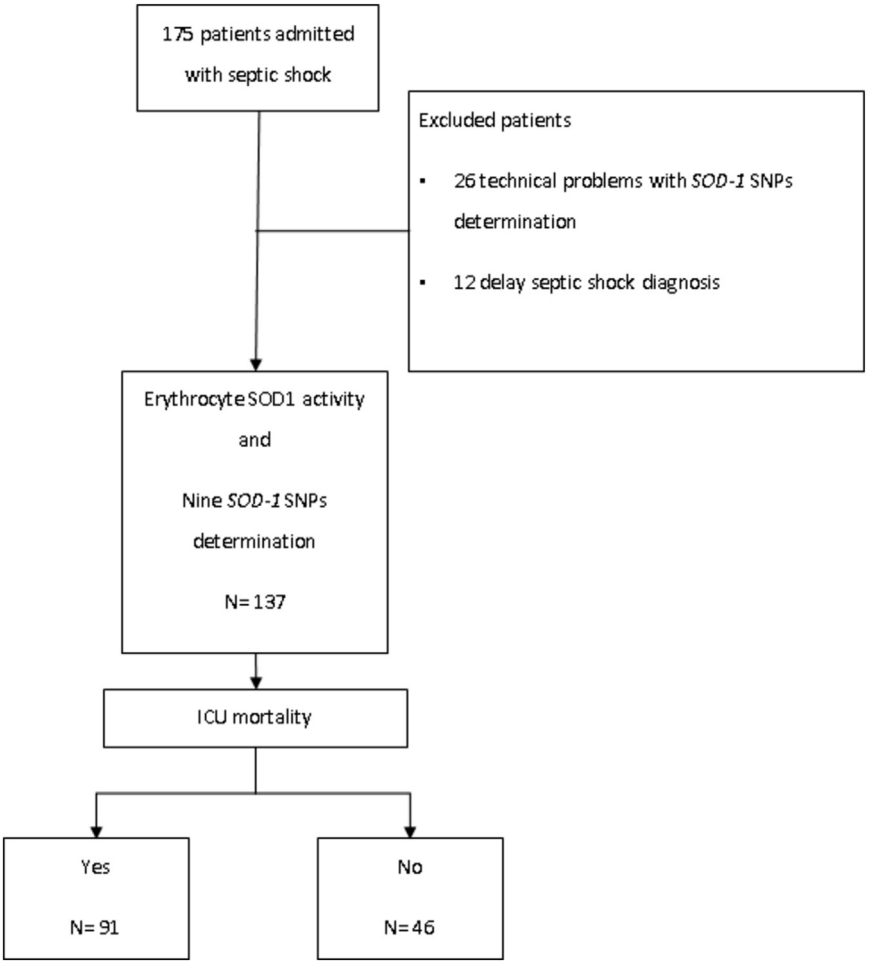


**Fig. 1.** Flow diagram of study patients with septic shock.

diagnosis of septic shock. Thus, 137 patients with septic shock were evaluated and included in the study (Fig. 1).

The mean age of study patients was 63 ± 16 years and 58% were men. The median length of hospital stay and the ICU mortality rate were 8 (4–14) days and 66%, respectively. Concerning the cause of sepsis, 58% of the patients had pulmonary infection, 21% abdominal, 8% urinary, and 13% other types of focal infection. (Table 1)

Regarding laboratory data, the median MDA concentration was 1.5 (0.8–2.2) μmol/L, protein carbonyl concentration was 24.0 (12.5–32.7) nmol/mL, plasma zinc level was 47.2 (35.4–70.3) μg/dL, erythrocyte zinc concentration was 61.0 (50.0–77.2) μg/g Hb, plasma copper concentration was 66.6 ± 18.0 μg/dL, and erythrocyte SOD1 activity was 3191 (2230–4013) U/g Hb.

The individuals who experienced mortality were older and more severely ill, with higher APACHE II and SOFA scores and higher levels of lactate, urea, and protein carbonyl concentrations. There were no differences in sex or in other biochemical variables. (Table 1)

Several demographic and clinical variables were evaluated in relation to SOD1 activity. Erythrocyte SOD1 activity had a positive association with hemoglobin concentration ($p = 0.022$), MDA concentration ($p = 0.016$), erythrocyte zinc level ($p < 0.001$), and plasma copper concentration ($p = 0.029$) (Table 2).

Higher values of erythrocyte SOD1 activity were associated with decreased ICU mortality when adjusted for age and protein carbonyl concentration in the logistic regression model ($p = 0.025$). This relationship was also maintained in the highest tertile of erythrocyte SOD1 activity (odds ratio [OR]: 0.02; 95% CI: 0.00–0.78; $p = 0.037$). The other regression models found no association of SOD1 with ICU mortality (Table 3).

SOD1 activity was also assessed according to the SNPs of the *SOD1* gene. The nine SNPs were genotyped and all showed Hardy-Weinberg equilibrium. The genotypes of each SNP were separated into two groups: individuals with the dominant homozygous allele or individuals with the presence of the recessive allele (heterozygotes and homozygotes). Only the SNP *SOD1* rs2070424 influenced erythrocyte SOD1 activity. For patients with the AA allele, the activity of SOD1 was lower in comparison to G-carriers (A/G + G/G genotype) ($p = 0.019$) (Table 4). On the other hand, there was no difference in SOD1 activity according to haplotype frequency (Table 5).

None of the nine *SOD1* SNPs were associated with ICU mortality, even when adjusted for age and protein carbonyl concentrations. The frequency of haplotypes was also not related to ICU mortality (Tables 6 and 7).

## 4. Discussion

The objective of the present study was to evaluate the influence of *SOD1* gene polymorphisms on the erythrocyte activity of the SOD1 enzyme and on the mortality of patients with septic shock. The results showed that increased activity of SOD1 was associated with a decreased mortality rate in patients with septic shock, and only the *rs2070424* polymorphism interfered with SOD1 activity.

Despite the publication of international guidelines for the early diagnosis and appropriate treatment of septic shock, mortality rates remain high [1,2]. In the present study, 66% of patients with septic shock progressed to death. Although this is a very high mortality rate, it is in

**Table 1**
Demographic and laboratory parameters in patients with septic shock according to ICU mortality.

| Variable | ICU mortality | | |
|---|---|---|---|
| | Yes (n = 91) | No (n = 46) | p value |
| Age (years) | 64.7 (14.6) | 58.9 (18.0) | 0.044 |
| Male, n (%) | 51 (56) | 29 (63) | 0.432 |
| APACHE II score | 20.1 (6.6) | 14.8 (6.5) | < 0.001 |
| SOFA score | 10.6 (2.8) | 8.2 (2.3) | < 0.001 |
| Sepsis focus, n (%) | | | 0.529 |
| Respiratory | 55 (61.1) | 24 (52.2) | |
| Abdominal | 18 (20.0) | 11 (23.9) | |
| Urinary | 5 (5.6) | 6 (13.0) | |
| Other | 12 (13.3) | 5 (10.9) | |
| Hemoglobin (g/dL) | 11.1 (2.2) | 11.4 (2.0) | 0.429 |
| Hematocrit (%) | 33.3 (6.7) | 34.1 (5.7) | 0.495 |
| Leukocytes ($10^3$/mm$^3$)[a] | 15.7 (12.1–22.2) | 16 (12.2–22.9) | 0.545 |
| Albumin (g/dL) | 2.27 (0.57) | 2.43 (0.65) | 0.162 |
| Urea (mg/dL) | 114.4 (69.7) | 88.9 (62.6) | 0.039 |
| Creatinine (md/dL)[a] | 2.0 (0.9–3.6) | 1.4 (0.7–2.2) | 0.169 |
| Lactate (mmol/L)[a] | 2.8 (1.5–3.8) | 1.5 (1.1–2.5) | 0.005 |
| CRP (mg/dL) | 32.2 (16.9) | 30.4 (16) | 0.539 |
| Erythrocyte SOD1 activity (U/g Hb) | 2957 (2205–4157) | 3293 (2302–3936) | 0.757 |
| Protein carbonyl (nmol/mL) | 28.9 (7.1) | 11.1 (4.3) | < 0.001 |
| MDA (nmol/L)[a] | 1.61 (0.92–2.41) | 1.53 (0.70–2.11) | 0.122 |

APACHE - Acute Physiology and Chronic Health Evaluation; SOFA - Sequential Organ Failure Assessment; CRP – C reactive protein; MDA – malondialdehyde; Values are expressed as mean (SD) or median (25–75%). Student´s *t* – test was applied to determine test mean differences in continuous variables and Chi-squared test was applied for association between categorical variables. *p < 0.05 was considered statistically significant.
[a] Log transformed variables.

**Table 2**
Association between demographic or laboratory parameters and erythrocyte SOD1 activity.

| Parameters | β | ρ | p value |
|---|---|---|---|
| Age (years) | 0.003 | 0.11 | 0.198 |
| APACHE II score | − 0.001 | − 0.03 | 0.753 |
| SOFA score | − 0.007 | − 0.05 | 0.606 |
| Hemoglobin (g/dL) | 0.045 | 0.20 | 0.022 |
| Hematocrit (%) | 0.007 | 0.09 | 0.305 |
| Leukocytes ($10^3$/mm$^3$)[a] | − 0.033 | − 0.06 | 0.526 |
| Albumin (g/dL) | 0.050 | 0.06 | 0.468 |
| Urea (mg/dL) | − 0.001 | − 0.08 | 0.334 |
| Creatinine (md/dL)[a] | − 0.031 | − 0.06 | 0.479 |
| Lactate (mmol/L)[a] | − 0.007 | − 0.01 | 0.911 |
| CRP (mg/dL) | − 0.002 | − 0.07 | 0.403 |
| MDA (nmol/L)[a] | 0.135 | 0.21 | 0.016 |
| Plasma zinc (μg/mL) | − 0.001 | − 0.01 | 0.895 |
| Erythrocyte zinc (μg/g Hb) | 0.550 | 0.170 | < 0.001 |
| Plasma copper (μg/dL) | 0.005 | 0.19 | 0.029 |
| Protein carbonyl (nmol/mL) | 0.003 | 0.06 | 0.475 |

APACHE - Acute Physiology and Chronic Health Evaluation; SOFA - Sequential Organ Failure Assessment; CRP – C reactive protein; MDA – malondialdehyde. Simple linear regression models with erythrocyte SOD1 activity as dependent variable. *p < 0.05 was considered statistically significant.
[a] Log transformed variables.

**Table 3**
Logistic regression models for association between ICU mortality and tertiles of erythrocyte SOD1 activity in patients with septic shock.

| Variable | OR (95% CI) | p value | p trend |
|---|---|---|---|
| SOD1 erythrocyte activity[a] | | | 0.881 |
| 1st tertile (957.3–2515.9 U/g Hb)[a] | 1 | | |
| 2nd tertile (2515.9–3807.0 U/g Hb)[a] | 1.11 (0.46–2.68) | 0.822 | |
| 3rd tertile (3807.0–11283.7 U/g Hb)[a] | 0.94 (0.39–2.23) | 0.884 | |
| SOD1 erythrocyte activity[b] | | | 0.754 |
| 1st tertile (957.3–2515.9 U/g Hb)[b] | 1 | | |
| 2nd tertile (2515.9–3807.0 U/g Hb)[b] | 1.14 (0.44–2.98) | 0.792 | |
| 3rd tertile (3807.0–11283.7 U/g Hb)[b] | 0.86 (0.33–2.22) | 0.758 | |
| SOD1 erythrocyte activity[c] | | | 0.025 |
| 1st tertile (957.3–2515.9 U/g Hb)[c] | 1 | | |
| 2nd tertile (2515.9–3807.0 U/g Hb)[c] | 0.15 (0.01–1.53) | 0.109 | |
| 3rd tertile (3807.0–11283.7 U/g Hb)[c] | 0.02 (0.00–0.78) | 0.037 | |

*p < 0.05 was considered statistically significant.
[a] Unadjusted.
[b] adjusted by age, sex, and APACHE II score.
[c] adjusted by protein carbonyl concentration and age.

**Table 4**
Differences in erythrocyte SOD1 activity among *SOD-1* SNP genotypes in patients with septic shock.

| *SOD-1* SNP | SOD1 erythrocyte activity (U/g Hb) Median (25–75%) | p value[a] | p value[b] |
|---|---|---|---|
| *rs4998557* | | 0.618 | 0.117 |
| GG (n = 94) | 3064 (2205–3925) | | |
| GA + AA (n = 43) | 3459 (2438–4312) | | |
| *rs2070424* | | 0.203 | 0.019 |
| AA (n = 111) | 2924 (2188–3925) | | |
| AG + GG (n = 26) | 3798 (2516–4312) | | |
| *rs10432782* | | 0.529 | 0.060 |
| TT (n = 100) | 3064 (2205–3925) | | |
| TG + GG (n = 37) | 3547 (2483–4312) | | |
| *rs1041740* | | 0.749 | 0.406 |
| GG (n = 61) | 3321 (2380–4027) | | |
| GT + TT (n = 76) | 3064 (2205–3942) | | |
| *rs11910115* | | 0.285 | 0.321 |
| AA (n = 129) | 3288 (2344–4074) | | |
| AC (n = 8) | 2663 (1988–2890) | | |
| *rs202446* | | 0.122 | 0.207 |
| GG (n = 100) | 2999 (2032–3942) | | |
| GT + TT (n = 37) | 3459 (2490–4157) | | |
| *rs2173962* | | 0.503 | 0.745 |
| TT (n = 125) | 3288 (2344–3999) | | |
| TC + CC (n = 12) | 2663 (1988–3659) | | |
| *rs202449* | | 0.102 | 0.255 |
| AA (n = 100) | 3350 (2456–4139) | | |
| AT + TT (n = 37) | 2669 (1912–3870) | | |
| *rs17880135* | | 0.405 | 0.538 |
| TT (n = 128) | 3101 (2188–3972) | | |
| TG + GG (n = 9) | 3459 (2870–4157) | | |

Values are expressed as median (25–75%). Multiple linear regressions were applied to test differences in erythrocyte SOD 1 activity among genotypes in a dominant model. *p < 0.05 was considered statistically significant.
[a] Unadjusted model.
[b] Adjusted by hemoglobin, MDA, plasma copper, and erythrocyte zinc.

accordance with the septic shock mortality observed in other studies in Latin America [22,23].

The determining factors for the progressive deterioration of patients with septic shock are not completely clear. However, it is believed that in addition to the profile of the infectious agent, the modulation of the pro and anti-inflammatory response resulting from the individual characteristics of the host is also important [1,2].

In critical patients, especially those with septic shock, there is a drastic increase in ROS production. Therefore, adequate antioxidant defense is essential to combat oxidative stress and improve survival [3–5]. In addition to increased ROS production, decreased antioxidant status has been reported in sepsis. Several causes such as dilution secondary to fluid resuscitation, inadequate nutritional status, redistribution, and losses through body fluids could explain these results [3,24].

When total redox capacity is measured is sepsis, a clear decrease is seen in septic patients compared to healthy controls [25]. In addition, this redox capacity usually remains low in patients who die, whereas it

**Table 5**
Association between *SOD-1* SNP and ICU mortality in patients with septic shock.

| *SOD-1* SNP | ICU mortality | | | |
|---|---|---|---|---|
| | OR (95% CI) | p value | Adjusted OR (95% CI) | p value |
| *rs4998557* | | | | |
| GG (n = 94) | 1 | | 1 | |
| GA + AA (n = 43) | 0.79 (0.37–1.68) | 0.543 | 0.96 (0.18–5.30) | 0.963 |
| *rs2070424* | | | | |
| AA (n = 111) | 1 | | 1 | |
| AG + GG (n = 26) | 0.94 (0.38–2.32) | 0.901 | 0.80 (0.12–5.45) | 0.817 |
| *rs10432782* | | | | |
| TT (n = 100) | 1 | | 1 | |
| TG + GG (n = 37) | 0.66 (0.30–1.44) | 0.295 | 0.95 (0.17–5.26) | 0.950 |
| *rs1041740* | | | | |
| GG (n = 61) | 1 | | 1 | |
| GT + TT (n = 76) | 1.22 (0.60–2.49) | 0.581 | 2.05 (0.43–9.73) | 0.367 |
| *rs11910115* | | | | |
| AA (n = 129) | 1 | | 1 | |
| AC (n = 8) | 0.83 (0.19–3.65) | 0.809 | 2.21 (0.17–28.82) | 0.545 |
| *rs202446* | | | | |
| GG (n = 100) | 1 | | 1 | |
| GT + TT (n = 37) | 0.56 (0.26–1.23) | 0.147 | 1.00 (0.21–4.84) | 0.995 |
| *rs2173962* | | | | |
| TT (n = 125) | 1 | | 1 | |
| TC + CC (n = 12) | 0.47 (0.14–1.55) | 0.215 | 1.16 (0.11–12.40) | 0.904 |
| *rs202449* | | | | |
| AA (n = 100) | 1 | | 1 | |
| AT + TT (n = 37) | 0.77 (0.35–1.70) | 0.521 | 0.23 (0.03–1.52) | 0.126 |
| *rs17880135* | | | | |
| TT (n = 128) | 1 | | 1 | |
| TG + GG (n = 9) | 1.83 (0.37–9.20) | 0.461 | 0.58 (0.03–13.3) | 0.734 |

Simple and multiple logistic regression, adjusted by age and carbonyl, were applied to test the association of SNPs in *SOD-1* gene and ICU mortality. *p < 0.05 was considered statistically significant.

**Table 6**
Association between *SOD-1* haplotypes (rs4998557, rs10432782, rs2070424) and tertiles of erythrocyte SOD1 activity.

| Haplotypes | Erythrocyte SOD1 activity | | | |
|---|---|---|---|---|
| | 1st tertile OR (95% CI) | 2nd tertile OR (95% CI) | 3rd tertile OR (95% CI) | Haplotype frequency (%)[a] |
| AGG | 1 | 1.21 (0.37–3.93) | 2.70 (0.92–7.87) | 9.5 |
| AGA | 1 | 0.32 (0.06–1.66) | 0.51 (0.12–2.18) | 4.0 |
| ATA | 1 | 0.98 (0.19–5.14) | 0.68 (0.11–4.28) | 2.9 |

Haplotype allele sequences correspond to SNP rs4998557, rs10432782, rs2070424, respectively. Multiple logistic regression, adjusted by hemoglobin, MDA, plasma copper, and erythrocyte zinc, was applied to test the association of haplotypes in *SOD-1* gene and erythrocyte SOD1 activity tertiles. Haplotype "000" (prevalence of 83.6%[a]) was the reference haplotype. *p < 0.05 was considered statistically significant.

[a] Generated with Haploview software.

returns to normal in patients who survive sepsis [25–27]. It is also important to observe that mitochondria are a major source of ROS, and at the same time, a target for oxidative damage [3–6]. In addition,

**Table 7**
Association between *SOD-1* haplotypes (rs4998557, rs10432782, rs2070424) and ICU mortality in patients with septic shock.

| Haplotypes | ICU mortality OR (95% CI) | p value | Haplotype frequency (%)[a] |
|---|---|---|---|
| AGG | 1.34 (0.53–3.37) | 0.538 | 9.5 |
| AGA | 0.33 (0.09–1.25) | 0.104 | 4.0 |
| ATA | 3.24 (0.38–27.7) | 0.284 | 2.9 |

Haplotype allele sequences correspond to SNP rs4998557, rs10432782, rs2070424, respectively. Multiple logistic regression, adjusted by age and protein carbonyl concentration, was applied to test the association of haplotypes in *SOD-1* gene and ICU mortality. Haplotype "000" (prevalence of 83.6%[a]) was the reference haplotype. *p < 0.05 was considered statistically significant.

[a] Generated with Haploview software.

damage to mitochondria from oxidative stress appears to be fundamental to the pathophysiology of organ failure and death in sepsis [3–6].

Experimental and clinical studies evaluated the association of SOD and sepsis [27–31]. Macarthur et al. showed in Sprague-Dawley rats that, postinfection treatment with the superoxide dismutase mimetic, protects against hypotension, vascular hyporeactivity to catecholamines, and mortality associated with septic shock [28]. It is also interesting to observe that heterozygous nor homozygous SOD-1 overexpression did not improve the sepsis-related impairment of carbohydrate metabolism nor sustained improvement of the sepsis-related impairment of myocardial-norepinephrine responsiveness, possibly because of the lacking increase of the tissue catalase and the mitochondrial SOD activity [29,30].

Warner et al. showed that the plasma SOD concentration was elevated in septic patients, and levels were higher in non-survivors of sepsis compared to survivors [27]. Recently, we showed that lower erythrocyte SOD1 activity was an early predictor of the development of acute renal injury in patients with septic shock [8]. In the present study, we showed that increased erythrocyte SOD1 activity was associated with decreased ICU mortality in patients with septic shock. Although these results initially appear contradictory, Warner et al. evaluated the plasma levels of SOD1, but not its activity [27]. In addition, they included only 32 patients, with sepsis and septic shock, and they did not evaluated SOD1 polymorphisms or nutrition status of them [27].

It is also important to note that in the present study, the higher activity of SOD1 was related to erythrocyte zinc and plasma copper concentrations. Thus, adequate nutritional status of these minerals, known as cofactors of SOD1, contributes to improved action of the enzyme [7]. However, despite their relevant role, the concentrations of these minerals were not predictors of mortality in patients with septic shock. These results suggest that other factors, such as gene polymorphisms, contribute to erythrocyte SOD1 activity.

Polymorphisms of *SOD1* gene may affect the enzymatic activities, leading to altered antioxidant defense. Several studies have evaluated the influence of *SOD1* polymorphisms in patients with cirrhosis, cardiovascular disease, and cancer; however, there have been no previous studies in patients with septic shock [11–13,32].

The Yamagata study genotyped 639 SNPs of 2799 healthy individuals and followed them for 10 years. The authors found that the SNPs rs1041740 and rs17880487 of the *SOD1* gene were associated with cardiovascular mortality [12]. In the present study, we found no association of SNP rs1041740 with erythrocyte SOD1 activity or with mortality in patients with septic shock. This study was the first that evaluated nine polymorphisms of the *SOD1* gene. Among these SNPs, only one showed influence on SOD1 activity. The presence of the dominant homozygous (AA) allele of the SNP rs2070424 was responsible for the lower activity of SOD1.

Thus, in addition to the nutritional status of copper and zinc, the presence of *SOD1* gene polymorphisms may have directly interfered

with the enzyme activity in our patients. Although one SNP was related to differences in SOD1 behavior, none were associated with ICU mortality. These results reinforce the complexity of the oxidative stress response and the participation of other antioxidants in the mortality of patients with septic shock.

Some limitations of this study should be considered. Only patients from a single medical center were included. The timing of antibiotic dosing was not recorded, and blood samples were taken within the first 24 h, which is a relatively large window. In addition, we could not measure erythrocyte copper concentration owing to technical problems. Despite these limitations, we strongly believe that our data contribute relevant knowledge regarding the role of the polymorphisms of the *SOD1* gene in the activity of the SOD enzyme as well as its relationship with mortality in patients with septic shock.

In conclusion, the SNP rs2070424 of the *SOD1* gene interferes with erythrocyte SOD1 activity, and higher activity of SOD1 is associated with decreased mortality in patients with septic shock.

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## Declarations of interest

None.

## Role of funding source

The funding source had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication

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