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Association between serum total antioxidant capacity and mortality in severe septic patients☆,☆☆

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ABSTRACT

Purpose: Total antioxidant capacity (TAC) in severe septic patients has been analyzed in few studies with limited number of subjects. In addition, no association between TAC serum levels and mortality in patients with sepsis has been investigated. We aimed at assessing a possible relationship between TAC serum levels and mortality using a large cohort of patients with severe sepsis.
Methods: We performed an observational, prospective, multicenter study in 6 Spanish intensive care units. Serum levels of TAC were measured in a total of 213 patients with severe sepsis. End point was 30-day mortality.
Results: Nonsurviving septic patients (n = 75) showed higher serum TAC levels ($P = .006$) than survivors (n = 138). Cox regression analysis showed that TAC serum levels were associated with 30-day survival (hazard ratio = 1.50, 95% confidence interval = 1.16-1.94, $P = .002$). Receiver operating characteristic analysis showed that the area under curve of TAC serum levels to predict 30-day survival was 0.61 (95% confidence interval = 0.545-0.680, $P = .04$).
Conclusions: The most relevant and new findings of our study, the largest cohort of septic patients providing data on circulating TAC levels so far, were that serum TAC levels are associated with mortality and could be used as biomarker to outcome prediction in severe septic patients.

1. Introduction

Sepsis represents a systemic response of the immune system to infection. Severe sepsis is a common, expensive, and frequently fatal condition [1,2]. Severe sepsis is characterized by excessive production of reactive oxygen species and reactive nitrogen species in the circulation and in the affected organs [3,4]. Antioxidants within cells, cell membranes, and extracellular fluids can be upregulated and mobilized to neutralize excessive and inappropriate reactive oxygen species formation [5]. However, when antioxidant defenses are overwhelmed, oxidative stress results, which can cause significant damage to lipids, proteins, carbohydrates, and nucleic acids, within both mitochondria and cells [6]. It has been suggested that mitochondrial dysfunction occurs as a result of oxidative stress, resulting in failure of energy production, organ dysfunction, and finally death [7,8]. Within the strategy to maintain redox balance against oxidant conditions, blood has a central role because it transports and redistributes antioxidants to every part of the body [9]. The way that a chain-breaking antioxidant exerts its function is receiving an electron from a radical or donating an electron to a radical with the consequent formation of stable products. However, these antioxidants do not work alone; but they establish complex interactions with each others [10]. Thus, measurement of total antioxidant capacity (TAC) in serum or plasma may give more biologically relevant information about patient antioxidant status than that obtained from measuring concentrations of individual compounds [5].

Oxidative stress induces peroxidation of membrane lipids, affecting the biological properties of the cellular membrane and impairing normal cellular function [11]. This process generates a variety of relatively

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stable decomposition end products, such as malondialdehyde (MDA), which is frequently used as a marker of peroxidation of polyunsaturated fatty acids [12].

Circulating TAC levels have been assessed in critically ill patients [13–17] and severe septic patients [18–22] in studies with limited number of subjects and with contradictory results. In addition, no association between serum TAC levels and mortality in patients with sepsis has been found. To assess any possible relationship between serum TAC levels and adverse outcome in patients with severe sepsis, we have determined the antioxidant capacity of blood collected at time of diagnosis in the largest cohort of patients analyzed so far.

2. Methods

2.1. Design and subjects

A multicenter, observational, prospective study was carried out in 6 Spanish intensive care units. The institutional review boards of the 6 hospitals approved this study: Hospital Universitario de Canarias (La Laguna, Santa Cruz de Tenerife, Spain), Hospital Universitario Nuestra Señora de Candelaria (Santa Cruz de Tenerife, Spain), Hospital Universitario Dr Negrín (Las Palmas de Gran Canaria, Spain), Hospital Clínico Universitario de Valencia (Valencia, Spain), Hospital San Jorge (Huesca, Spain), and Hospital Insular (Las Palmas de Gran Canaria, Spain). Written informed consent from the patients or from their family members was obtained. The study was conducted between 2008 and 2009.

The inclusion criteria used for severe sepsis were those defined according to the International Sepsis Definitions Conference criteria [23]. Patients with age less than 18 years; pregnancy; lactation; human immunodeficiency virus; white blood cell count less than 1000/μL; solid or hematological tumor; or immunosuppressive, steroid, or radiation therapy were excluded. A total of 213 patients with severe sepsis were included.

2.2. Variables recorded

The following variables were recorded for each patient: sex, age, diabetes mellitus, *chronic renal failure* defined as glomerular filtration rate less than 60 mL/min per 1.73 m^2, chronic obstructive pulmonary disease (COPD), site of infection, microorganism responsible, bloodstream infection, empiric antimicrobial treatment, pressure of arterial oxygen (Pao_2)/fraction inspired of oxygen (Fio_2), creatinine, bilirubin, leukocytes, lactic acid, platelets, international normalized ratio (INR), activated partial thromboplastin time (aPTT), Acute Physiology and Chronic Health Evaluation II (APACHE II) score [24], and Sepsis-related Organ Failure Assessment (SOFA) score [25].

2.3. End point

End point was 30-day mortality.

2.4. Blood samples

Blood samples from 213 patients were collected in citrated tubes within 2 hours of the diagnosis of severe sepsis. Serum was allowed to clot for 20 minutes at room temperature and then centrifuged at 1000*g* for 15 minutes, and supernatant was immediately stored in aliquot at −80°C. Samples were all processed at the same time, at the end of the recruitment process, by the same analyst using the same equipment. Total antioxidant capacity and MDA determinations were performed by a laboratory technician blinded to all clinical data.

2.5. Serum TAC level analysis

Total antioxidant capacity assay was performed in the Genetic Unit of the Instituto de Enfermedades Tropicales y Salud Pública de Canarias of the University of the La Laguna (Tenerife, Spain). Total antioxidant capacity in serum samples was evaluated using antioxidant assay kit (Cayman Chemical Corporation, Ann Arbor, MI). The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of 2,2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) to $ABTS^+$ by metmyoglobin. The capacity of the antioxidants in the sample to prevent ABTS oxidation is compared with that of Trolox, a water-soluble tocopherol analogue, and is quantified as molar Trolox equivalents. All samples were assayed in duplicate at 20-fold dilutions in assay buffer following manufacturer's instructions. Absorbance at 750 nm was measured using the EnSpire multimode plate reader (PerkinElmer,Waltham, MA). The serum concentration of TAC was expressed in millimoles per liter. The detection limit of this assay was 0.04 mmol/L; the intra- and interassay coefficients or variation were 3.4% and 3.0%, respectively.

2.6. Serum MDA level analysis

Malondialdehyde assay was carried out in the Physiology Department of the Faculty of Medicine of the University of the La Laguna (Tenerife, Spain). We used a thiobarbituric acid–reactive substance assay to evaluate plasma lipid peroxidation according to Kikugawa et al [26]. Briefly, plasma (200 μL) was mixed with trichloroacetic acid (1.5 mL, pH 3.5), sodium dodecyl sulfate (200 μL, 8.1%), and thiobarbituric acid (2.5 mL, 0.8%). Fifty microliters of butylated hydroxytoluene (0.8%) was added to the assay mixture to prevent autooxidation of the sample. The mixture was kept at 5°C for exactly 1 hour and was heated only afterwards at 100°C for 1 hour. After *n*-butanol extraction and centrifugation, each sample was placed in duplicate in a 96-well plate and read at 535 nm using a spectrophotometer reader (Benchmark Plus; Bio-Rad, Hercules, CA). The serum concentration of MDA was expressed in nanomoles per milliliter. The detection limit of this assay was 0.079 nmol/mL; the intra- and interassay coefficients or variation were 1.82% and 4.01%, respectively.

2.7. Statistical methods

Continuous variables are reported as medians and interquartile ranges. Categorical variables are reported as frequencies and percentages. Comparisons of continuous variables between groups were carried out using Mann-Whitney *U* test. Comparisons between groups for categorical variables were carried out with χ^2 test. We used χ^2 test as omnibus test to compare site of infection and empiric antimicrobial therapy between surviving and nonsurviving patients, and the *P* value is a global *P* value. We plotted a receiver operating characteristic curve using survival at 30 days as classification variable and TAC serum level as prognostic variable. Analysis of survival at 30 days with Kaplan-Meier method curve and comparisons by log-rank test were carried out using TAC serum levels lower/higher than 2.80 mmol/L as the independent variable and survival at 30 days as the dependent variable. Cox regression analysis was applied to determine the independent contribution of TAC serum levels on the prediction of 30-day mortality, controlling for lactic acid levels, APACHE II, and renal failure. Hazard ratio and 95% confidence intervals were calculated as measures of the clinical impact of the predictor variables. To determine the association between serum TAC and MDA levels, we used Spearman rank correlation coefficient or Spearman ρ coefficient. A *P* value of less than .05 was considered statistically significant. Statistical analyses were performed with SPSS 17.0 (SPSS Inc, Chicago, IL) and NCSS 2000 (NCSS, Kaysville, UT).

3. Results

Comparisons of demographic and clinical parameters between surviving (n = 138) and nonsurviving septic patients (n = 75) are shown in Table 1. We found that nonsurviving septic patients showed higher age, renal failure, lactic acid, INR, aPTT, SOFA scores, and