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Association between serum substance P levels and mortality in patients with severe sepsis☆,☆☆

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ABSTRACT

Background: Substance P (SP) is a peptide of the tachykinins family involved in the inflammatory response. Circulating SP levels have been assessed in septic patients in 2 previous studies with a small number of subjects (61 and 42 patients, respectively), and there were no significant differences in SP levels at the moment of sepsis diagnosis between surviving and nonsurviving patients. The main goal of this study was to determine a possible relationship between serum SP levels and patient outcome in the largest cohort of severe septic patients analyzed so far.
Methods: We performed an observational, prospective, multicenter study in 6 Spanish intensive care units. Serum SP levels were measured at the moment of severe sepsis diagnosis in 238 patients. The end point of the study was 30-day mortality.
Results: We found that surviving septic patients (n = 153) showed higher serum SP levels than did nonsurvivors (n = 85). Multiple logistic regression analysis showed that serum SP levels higher than 350 pg/mL were associated with survival at 30 days (odds ratio, 0.43; 95% confidence interval, 0.24-0.77; $P = .005$) after controlling for serum lactic acid levels and Sepsis-related Organ Failure Assessment score.
Conclusions: The major new finding of our study was that serum SP levels were associated with mortality 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].

The *TAC1* gene encodes substance P (SP), a member of the tachykinin family largely considered a peptide of neuronal origin [3]. However, SP and other tachykinins are also widely distributed in nonneuronal tissues [4–7] where they mediate multiple homeostatic functions, including nociception and neurogenic inflammation [3,8]. The effects of SP are mainly mediated by the NK_1receptor (NK_1R), a G-protein–coupled receptor that is widely expressed in many tissues and cells [7,9].

The role of tachykinin in the host defense system has been extensively studied. Substance P is considered one of the major initiators of neurogenic inflammation because not only does it relay sensory information to the central nervous system but also plays an effector role in the inflammatory, proliferative, and reparative processes [3,8,10–13]. Primary sensory neurons innervate most tissues, and their activation by bacteria products triggers SP release [14]. Subsequent activation of NK_1R in tissue cells (epithelial, fibroblast, and smooth muscle cells), capillary endothelial cells, and leukocytes decreases vascular tone, increases endothelial permeability, and promotes local inflow of inflammatory and immune cells [3,8]. Moreover, eosinophils, neutrophils,

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monocytes, macrophages, mast cells, dendritic cells, and T lymphocytes produce and secrete SP and they also express NK_1R on their membranes [15–18]. Stimulation of NK_1R in leukocytes leads to synthesis and secretion of chemokines, histamine, and cytokines [5,19,20], thereby enhancing the inflammation induced by the original stimulus.

The role of SP in sepsis remains unclear. Serum or plasma SP levels have been assessed in septic and septic shock patients in 2 previous studies, with a small number of subjects showing contradictory results [21,22]. Arnalich et al [21] observed lower SP levels in plasma of both septic and septic shock patients (n = 42) compared with controls for all time points analyzed (onset, 12 hours, and 24 hours). Beer et al [22] collected serum samples daily in 61 septic patients, beginning at the day of diagnosis of sepsis and several weeks later. They observed higher serum SP levels in septic patients than in control subjects on all days analyzed. In addition, when they compared survivors and nonsurvivors, they found increased SP levels during the final phase of lethal sepsis [22]. Thus, because of the controversial results of previous studies with a small number of subjects, the aim of this study was to assess the possible relationship between serum SP levels and outcome of patients with severe sepsis in the largest cohort of patients analyzed so far.

2. Methods

2.1. Design and subjects

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

The inclusion criteria used for severe sepsis were those defined according to the International Sepsis Definitions Conference criteria [23]. Patients with age <18 years, pregnancy, lactation, human immunodeficiency virus, white blood cell count <1000/μL, solid or hematologic tumor, or immunosuppressive, steroid, or radiation therapy were excluded. A total of 238 patients with severe sepsis were included.

2.2. Variables recorded

The following variables were recorded at baseline for each patient: age, Acute Physiology and Chronic Health Evaluation II (APACHE II) score [24], activated partial thromboplastin time (aPTT), empiric antimicrobial treatment, bilirubin, bloodstream infection, chronic renal failure (CRF) defined as glomerular filtration rate less than 60 mL/min per 1.73 m^2, chronic obstructive pulmonary disease, creatinine, diabetes mellitus, sex, international normalized ratio (INR), ischemic heart disease, lactic acid, leukocytes, microorganism responsible, pressure of arterial oxygen/fraction inspired of oxygen, platelets, site of infection, and Sepsis-related Organ Failure Assessment (SOFA) score [25]. The end point of the study was 30-day mortality.

2.3. Serum SP level analysis

Blood samples were collected in citrated tubes from 238 patients 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 store in aliquot at −80°C. Samples were all processed at the same time, at the end of the recruitment process, by the same laboratory technician using the same equipment and blinded to all clinical data.

Substance P assay was performed in the Genetic Unit of the Instituto de Enfermedades Tropicales y Salud Pública de Canarias of the University of La Laguna (Tenerife, Spain). Serum SP levels were assayed by specific enzyme linked immunosorbent assay according to the manufacturer's instructions (R&D Systems, Abingdon, UK). All samples were assayed in duplicate at 2-fold dilutions in assay buffer following the manufacturer's instructions. Absorbance at 450 nm was measured using the EnSpire multimode plate reader (PerkinElmer, Waltham, Mass). The serum concentration of SP was expressed in pg/mL. The detection limit of this assay was 25 pg/mL; the intra-assay and interassay coefficients of variance were 9% and 15%, respectively.

2.4. 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. Analysis of survival at 30 days with Kaplan-Meier method curve and comparisons by log-rank test were carried out using serum SP levels lower/higher than 350 pg/mL as the independent variable and survival at 30 days as the dependent variable. Multiple logistic regression analysis was applied to determine the independent contribution of serum SP levels higher than 350 pg/mL on the prediction of 30-day mortality, controlling for serum lactic acid levels and SOFA score. We used the maximum likelihood ratio between sensitivity and 1 − specificity as criteria to select the cutoff of serum SP levels of 350 pg/mL. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated as measures of the clinical impact of the predictor variables. A *P* value less than .05 was considered statistically significant. Statistical analyses were performed with SPSS 17.0 (SPSS Inc, Chicago, Ill) and NCSS 2000 (Kaysville, UT).

3. Results

Comparisons of demographic and clinical parameters between surviving (n = 153) and nonsurviving septic patients (n = 85) are shown in Table 1. We found that the nonsurviving septic patients showed higher age, creatinine, lactic acid, INR, aPTT, and SOFA and APACHE II scores, and lower platelet count than did the surviving ones. In addition, the nonsurviving septic patients showed lower serum SP levels ($P = .001$) than did the surviving ones.

Kaplan-Meier survival analysis showed that the patients with serum SP levels lower than 350 pg/mL had a higher probability of death at 30 days (log-rank, 12.1; hazard ratio, 2.1 [95% CI, 1.37-3.24]; $P < .001$) than did the patients with higher levels (Figure).

Multiple logistic regression analysis showed that serum SP levels higher than 350 pg/mL were associated with survival at 30 days (OR, 0.43; 95% CI, 0.24-0.77; $P = .005$) after controlling for lactic acid levels and SOFA score (Table 2).

4. Discussion

To our knowledge, this study includes the largest series providing data on serum SP levels in severe septic patients. The most relevant and new findings of our study show that (1) serum SP levels were higher in survivors compared with nonsurvivors at the moment of severe sepsis diagnosis, and (2) serum SP levels were associated with mortality in severe septic patients.

Previous studies have found that sepsis mortality is associated with lactic acid levels [26,27] and SOFA score [1]. Thus, we included in multiple logistic regression analysis serum SP levels higher than 350 pg/mL, lactic acid levels, and SOFA score. Interestingly, we observed that serum SP levels higher than 350 pg/mL were associated with survival at 30 days after controlling for lactic acid levels and SOFA score.