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Original article

Circulating sICAM-1 and sE-Selectin as biomarker of infection and prognosis in patients with systemic inflammatory response syndrome ☆

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ABSTRACT

Background: Vascular endothelium activation is a key pathogenic step in systemic inflammatory response syndrome (SIRS) that can be triggered by both microbial and sterile proinflammatory stimuli. The relevance of soluble adhesion molecules as clinical biomarkers to discriminate between infectious and non-infectious SIRS, and the individual patient prognosis, has not been established.
Methods: We prospectively measured by sandwich ELISA, serum levels of soluble E-Selectin (sE-Selectin), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble intercellular adhesion molecule-2 (sICAM-2) at ICU admission and at days 3, 7, 14 and 28 in patients with sepsis and at days 3 and 7 in patients with non-infectious SIRS.
Results: At ICU admission, sE-Selectin, sVCAM-1 and sICAM-1 in patients with infectious SIRS were significantly higher than those found in patients with non-infectious SIRS. ROC analysis revealed that the AUC for infection identification was best for sICAM-1 (0.900 ± 0.041; 95% CI 0.819-0.981; $p<0.0001$). Moreover, multivariate analysis showed that 4 variables were significantly and independently associated with mortality at 28 days: male gender (OR 15.90; 95% CI, 2.54–99.32), MODS score (OR 5.60; 95% CI, 1.67–18.74), circulating sE-Selectin levels (OR 4.81; 95% CI, 1.34–17.19) and sVCAM-1 concentrations (OR 4.80; 95% CI, 1.34–17.14).
Conclusions: Patients with SIRS secondary to infectious or non-infectious etiology show distinctive patterns of disturbance in serum soluble adhesion molecules. Serum ICAM-1 is a reliable biomarker for classifying patients with infectious SIRS from those with non-infectious SIRS. In addition, soluble E-Selectin is a prognostic biomarker with higher levels in patients with SIRS and fatal outcome.

1. Introduction

Vascular endothelium plays a critical role in the regulation of the systemic leukocyte migration and homing [1]. Both the innate and adaptive immune responses depend on the migration of leukocytes across endothelial cells. This interaction between the blood circulating leukocytes and the endothelial cells is a complex process involving several cell surface molecules whose expression is directly related to the leukocyte and endothelium activation stage [2]. Activation of endothelial cells induces the up-regulation and expression of different adhesion molecules provoking the subsequent impact on the adhesive intercellular interactions with circulating leukocytes. Briefly, the interaction of the microvilli that bear L-Selectin (or CD62L) on the leukocyte membrane with vascular cell adhesion molecule (VCAM)-1 on endothelial luminal surface constitutes the first step of the migration process. On the following steps of rolling, activation and adhesion of the migrating cell to the endothelium several adhesion molecules are implicated, including E-Selectin (or CD-62E), intercellular adhesion molecule (ICAM) 1 and 2, and VCAM 1 on the endothelial cell membrane and the leukocyte integrins. Finally, leukocyte diapedesis and basal membrane cross occur [3]. Interestingly, these membrane bound adhesion molecules may suffer a proteolytic cleavage of the cytoplasmic domain and soluble forms are generated that can be measured in the blood [4].

Multiorgan universal endothelium injury is a key pathogenic step in many severe systemic inflammatory diseases including the systemic inflammatory response syndrome (SIRS) [5]. SIRS has a common endothelium damage pathway which is the result of an inappropriate

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generalized inflammatory response of the host to a variety of acute insults [5]. Sepsis, one of the major causes of mortality in critically ill patients, is defined as SIRS due to infection [6]. Moreover, precipitating insult of non-infectious nature such as severe pancreatitis, burns or trauma can also be the cause of SIRS [6]. This wide number of causes of SIRS is explained by the different and diverse biological defense mechanism and inflammation mediators such as cytokines, complement activation, coagulation and fibrinolysis systems, kinin generation, which are involved in the induction of anomalous activation and injury of the endothelial cells [7]. Furthermore, bacterial products can also directly interact and activate endothelial cells [7].

From a clinical point of view, monitoring soluble adhesion molecule levels may be not only of interest to understanding the pathophysiology of SIRS, but might also be a reliable diagnostic laboratory test to distinguish between infection and non-infectious states in critically ill patients with clinical signs of SIRS. On the other hand, the measurement of soluble adhesion molecules in serum have uncovered individualized prognostic value in critical ill patients with SIRS [8–10].

Thus, we have centered our interest on the study of the levels and the kinetics of variation of adhesion molecules in serum obtained from SIRS patients caused by infectious and non-infectious etiologies whose clinical status requires admission at an Intensive Care Unit (ICU). We hypothesized that the endothelium damage induced by infectious and non-infectious pathogenic mechanisms might be associated with different inflammation patterns of damage and subsequent release of endothelial adhesion surface molecules to the serum. A prediction of the hypothesis was that circulating levels of soluble adhesion molecules might also have a distinctive survival prognostic value in patients with SIRS.

2. Materials and methods

2.1. Patient population and healthy controls

Approval from Hospital Príncipe de Asturias Ethics Committee was obtained for the study protocol and written informed consent was obtained from the next of kin and from healthy controls. The study was performed over a period of 36 months.

In this study, we included patients 18 years or older admitted at the ICU with diagnostic criteria of SIRS, which includes two or more of the following conditions: (1) temperature > 38 °C or < 36 °C; (2) heart rate > 90 beats per minute; (3) respiratory rate > 20 breaths per minute or PaCO2 < 32 mm Hg; and (4) white blood cell count > 12,000/mm^3, < 4000 mm^3, or > 10% immature (band) forms [6]. Patients diagnosed with sepsis had SIRS and clinical evidence of infection, defined as the presence of a known source of infection and/or a positive blood culture and had been started on parenteral antimicrobial therapy [6]. We will use sepsis and infectious SIRS indistinctly, like synonymous. Non-infectious SIRS was established by the absence of microbiological documentation or radiological evidence of foci within 7 days after admission in ICU. Exclusion criteria included: a) subjects with immunodeficiency or who were under corticosteroids or any other form of immunomodulation therapy; b) autoimmune or hypersensitivity diseases; c) disseminated malignancy; d) renal failure requiring hemodialysis or hemofiltration; e) participation in another research study. All the patients received conventional intensive care and included patients were treated by physicians who are not involved in this study. Patients treated with hydrocortisone for refractory hypotension were withdrawn from the study. No patient was treated with activated protein C. We prospectively designated a control group to set normal adhesion molecule values. This group was formed by healthy volunteers age- and sex-matched with the patients.

2.2. Measurements and protocol

In patients with sepsis, blood samples were collected using an non-heparinized arterial catheter at admission to the ICU and after informed consent was obtained as well as 48 h and on days 7, 14 and 28. In non-infectious SIRS group, blood samples were taken at ICU admission, at 48 h and on day 7. Immediately after blood samples were taken, the blood was put into sterilized, silicone-coated glass tubes. Samples were centrifuged at 2000 rpm for 20 min, and the serum was stored at −70 °C until assay. The etiology of SIRS was categorized as sepsis or as non-infectious SIRS. The reviewers were blinded to circulating soluble adhesion molecule results. We measured serum levels of soluble E-Selectin (sE-Selectin), soluble VCAM-1 (sVCAM-1), soluble ICAM-1 (sICAM-1) and soluble ICAM-2 (sICAM-2) by a modification of the enzyme-linked immunosorbent assay procedure (ELISA) known as a sandwich ELISA, using commercially available kits from Diaclone Research (Connecticut, USA). For sE-Selectin, sVCAM-1, sICAM-1 and sICAM-2, the lower detection limits of the assay were 0.50, 0.60, 8.00 and 0.0002 ng/ml, respectively. In the 36 healthy controls, the samples serve as a control of the accuracy of our laboratory procedures and for establishing the normal range of these immuno-inflammatory parameters.

2.3. Statistical analysis

All measurements are expressed as mean ± S.E. mean. First, the Kolmogorov–Smirnov test was used for testing data against the normal distribution. All the variables always fulfilled the normality hypothesis, thus, differences between groups were analyzed using the value of *t*-Test for the difference between the means of two independent samples. Bonferroni correction for multiple tests was used. The reliability of the use of serum soluble molecule adhesion concentrations to predict death due to SIRS or for diagnostic accuracy to evaluate infection was calculated by plotting receiver-operating characteristic (ROC) curves and the respective areas under the curves (AUC).

A Cox proportional-hazards regression analysis with backward stepwise selection was performed to estimate the variables associated with mortality at 28 days. Predetermined variables, or those significantly associated with mortality at 28 days in the univariate model ($p < 0.10$) were included in the multivariate model, including: age, gender, APACHE II score, SOFA score, MODS score, acute renal failure, mean arterial pressure, sE-Selectin, sICAM-1 and sVCAM-1 concentrations. A $p < 0.05$ was considered statistically significant.

Statistical analyses were performed with SPSS Statistics 19.0 statistical software (IBM Inc., Armonk, NY).

3. Results

3.1. Study patient characteristics

Of 132 SIRS patients who underwent screening for eligibility, we excluded 40 (35%) because they had a previous underlying condition associated with recognized alterations in immunological patient status and 92 were enrolled in this study. We included 52 patients with sepsis and forty patients who had non-infectious SIRS. Also, 36 healthy volunteers were included (Fig. 1). The source of infection in patients with sepsis was: intra-abdominal sepsis [n = 22], pneumonia [n = 16], primary bacteremia [n = 4], urinary tract infection [n = 4], soft-tissue infection [n = 3], surgical site infection [n = 2] or mediastinitis [n = 1]. The etiologies of non-infectious SIRS were non-infected severe acute pancreatitis [n = 19], resuscitated cardiac arrest [n = 10], intracranial hemorrhage [n = 5], hemorrhagic shock [n = 5] and acute ischemic stroke [n = 1]. The baseline and outcome characteristics of the study participants are summarized in Table 1.

Infectious and non-infectious SIRS patients show different patterns of circulating soluble adhesion molecules. First, we investigated