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Regular Article

Serum soluble CD40 Ligand levels are associated with severity and mortality of brain trauma injury patients

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ABSTRACT

Background: Serum soluble CD40 Ligand (sCD40L) levels, which exhibit prothrombotic and proinflammatory properties, have not been studied in patients with traumatic brain injury (TBI). Thus, the objective of this study was to determine whether serum sCD40L levels are associated with severity and mortality in patients with severe TBI.

Methods: This was a prospective, observational and multicenter study carried out in six Spanish Intensive Care Units. Patients with severe TBI defined as Glasgow Coma Scale (GCS) lower than 9 were included, while those with Injury Severity Score (ISS) in non-cranial aspects higher than 9 were excluded. Serum levels of sCD40L were measured on the day of TBI. Endpoint was established in 30-day mortality.

Results: We found higher serum sCD40L levels ($P < 0.001$) in non-surviving TBI patients ($N = 27$) than in survivor ones ($N = 73$). Logistic regression analysis showed that serum sCD40L levels were associated with 30-day mortality (OR = 1.58; 95% CI = 1.12-2.21; $P = 0.008$) controlling for APACHE-II score and computer tomography findings. The area under the curve (AUC) for serum sCD40L levels as predictor of 30-day mortality was 0.79 (95% CI = 0.70-0.86; $P < 0.001$). Survival analysis showed that patients with serum sCD40L levels higher than 2.11 ng/mL presented increased 30-day mortality than patients with lower levels (Hazard ratio = 9.0; 95% CI = 4.25-19.27; $P < 0.001$). We found an association between serum sCD40L levels and APACHE-II (rho = 0.33; $P = 0.001$), and GCS score (rho = -0.21; $P = 0.04$).

Conclusions: To our knowledge, this is the first study reporting data on serum sCD40L levels in patients with severe TBI. The most relevant and newer findings of our study are that serum sCD40L levels in non-surviving patients with severe TBI are higher than in surviving ones, and that there are an association between serum sCD40L levels and TBI severity and mortality.

Introduction

Traumatic brain injury (TBI) is an important cause of death, disability and resources consumption [1]. Initial primary injury is referred to the physical forces applied to brain during the impact. Secondary injury occurs later over a period of hours or days after the initial traumatic injury and is due to different mechanisms [2,3]. Among them, inflammation and thrombosis are mechanisms that play a pivotal role in this delayed response.

CD40 Ligand (CD40L) and its soluble counterpart (sCD40L) are proteins that exhibit proinflamatory [4,5] and procoagulant [6–11] properties on binding to their cell surface receptor CD40 [12,13]. CD40L is a member of the tumor necrosis factor (TNF) family and is expressed as a transmembrane protein in activated platelets [14,15].

Raised levels of sCD40L have been found in patients with acute coronary syndrome [16,17], stroke [18–22] and sepsis [23,24]. However, circulating sCD40L levels has not been studied in patients with TBI.

Abbreviations: sCD40L, soluble CD40 Ligand; TNF, tumour necrosis factor; TF, tissue factor; ICU, Intensive Care Unit; SOFA, Sepsis-related Organ Failure Assessment score.

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Thus, the objective of this study was to determine whether serum sCD40L levels are associated with mortality, severity, and inflammatory and prothrombotic markers in patients with severe TBI.

Methods

Design

This was a prospective, observational, multicenter study carried out in 6 Intensive Care Units of Spain belonging to the following hospitals: Hospital Universitario de Canarias (La Laguna, Tenerife, Spain), Hospital Universitario Nuestra Señora de Candelaria (Tenerife, Spain), Hospital General de La Palma (La Palma, Tenerife, Spain), Hospital Clínico Universitario de Valencia (Valencia, Spain), Hospital Insular (Las Palmas de Gran Canaria, Spain), Hospital Universitario Dr. Negrín (Las Palmas de Gran Canaria, Spain). The study was approved by the Institutional Ethic Review Boards of the 6 participant hospitals. A written informed consent from the patient or from the legal next of kin was obtained.

We included 100 patients with severe TBI. Severity of brain trauma injury was classified according to Glasgow Coma Scale (GCS) [25], and severe TBI was defined as GCS lower than 9 points.

Exclusion criteria were: age less than 18 years, pregnancy, inflammatory or malignant disease, and Injury Severity Score (ISS) [26] in non-cranial aspects higher than 9 points.

The following variables were recorded for each patient: sex, age, ISS, GCS, blood lactate, platelets, international normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen, Acute Physiology and Chronic Health Evaluation II (APACHE II) score [27] and brain lesion according to the Marshall computer tomography (CT) classification [28].

The end-point was 30-day mortality.

Laboratory Determinations

Blood samples were collected on the day of TBI to measure serum sCD40L levels. We also measured serum TNF-alpha levels, and plasma tissue factor (TF) levels.

Venous blood samples were collected in serum separator tubes (SST) for determination of serum sCD40L and TNF-alpha levels; and in citrate collected plasma tubes to determination of plasma TF levels. Blood samples were centrifuged within 30 minutes at 1000*g for 15 min. The serum and plasma were removed and frozen at -80 °C until measurement. The determination of serum sCD40L and TNF-alpha levels, and plasma TF levels were centralized in the Laboratory Department of the Hospital Universitario de Canarias (La Laguna, Santa Cruz de Tenerife, Spain).

Serum sCD40L levels were assayed by specific ELISA (Bender MedSystems GmbH, Vienna, Austria). The intra-assay and inter-assay coefficients of variation (CV) were 4% (n = 8) and 6.8% (n = 8) respectively; and detection limits for the assays was 0.06 ng/mL.

Serum TNF-alpha levels were measured by a solid-phase, chemiluminiscents immunometrics assays kit (Immulite®, Siemens Healthcare Diagnostics Products, Llanberis, United Kingdom). The intra-assay and inter-assay CV were <3.6 % (n = 20) and <6.5% (n = 20) respectively; and detection limits for the assays was 1.7 pg/mL.

Plasma TF levels were assayed by specific ELISA (Imubind® Tissue Factor ELISA, American Diagnostica, Inc, Stanford, CT, USA). The intra-assay and inter-assay CV were <7.2% (n = 20) and <8% (n = 20) respectively; and detection limits for the assays was 10 pg/mL.

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 Wilcoxon-Mann-Whitney test. Comparisons between groups on categorical variables were carried out with chi-square test. Multiple binomial logistic regression analysis was applied to predict 30-day mortality. As number of events was 27 exitus, we constructed two multiple binomial logistic regression models with only three predictor variables in each to avoid an over fitting effect that may lead to choose a final model of order slightly higher than required [29]. In the first regression model were included serum sCD40L, APACHE-II score and CT classification. Previously to include the variable CT classification in the regression analysis, it was recoded according with the risk of death observed in the bivariated analysis as low (CT types 2 and 5) and high risk (CT types 3, 4 and 6) of death. In the second regression model were included serum sCD40L levels, GCS and age. Odds Ratio and 95% confidence intervals were calculated as measurement of the clinical impact of the predictor variables. Receiver operating characteristic (ROC) analysis was carried out to determine the goodness-of-fit of the of serum sCD40L levels to predict 30-day mortality. Kaplan-Meier analysis of survival at 30 days and comparisons by log-rank test were carried out using serum sCD40L levels lower/higher than 2.11 ng/mL as the independent variable and survival at 30 days as the dependent variable. The association between continuous variables was carried out using Spearman´s rank correlation coefficient. A P value of less than 0.05 was considered statistically significant. Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and NCSS 2000 (Kaysville, Utah) and LogXact 4.1, (Cytel Co., Cambridge, MA).

Results

From the 100 TBI patients in the study, non-survivors (n = 27) showed lower GCS, and higher age, female rate and APACHE-II score than survivors (n = 73). CT classification also showed statistically significant differences between non-surviving and surviving patients. In addition, non-survivors showed higher serum sCD40L levels than survivors. However, no significant differences were found between non-surviving and surviving patients in serum TNFalpha and plasma TF levels (Table 1).

As shown in Table 1, mortality rate varied with CT classification types: thus 3/24 patients (12.5%) in type 2, 5/18 (27.8%) in type 3, 6/16 (37.5%) in type 4, 5/31 (16.1%) in type 5 and 8/11 (72.7%) in type 6. Once the CT classification variable was recoded for the regression analysis the new variable considered CT classification types 2 and 5 as low death risk, with a observed mortality rate of 8/55 (14.5%), and CT classification types 3, 4 and 6 as high death risk, with a observed mortality rate of 19/45 (42.2%). Multiple binomial logistic regression analysis showed that serum sCD40L levels could predict 30-day mortality (OR = 1.58; 95% CI = 1.12-2.21; P = 0.008) controlling for APACHE-II and CT classification (Table 2). Multiple binomial logistic regression analysis also showed that serum sCD40L levels could predict 30- day mortality (OR = 1.43; 95% CI = 1.05-1.95; P = 0.02) controlling for GCS and age (Table 2).

The area under the curve (AUC) for serum sCD40L levels as predictor of 30-day mortality was 0.79 (95% CI = 0.70-0.86; P < 0.001) (Fig. 1). Survival analysis showed that patients with serum sCD40L levels higher than 2.11 ng/mL presented higher 30-day mortality than patients with lower levels (Chi-square: 19.8; Hazard ratio = 9.0 (95% IC = 4.25-19.27); P < 0.001) (Fig. 2).

We found an association between serum sCD40L levels and APACHE-II (rho = 0.33; P = 0.001), GCS score (rho = -0.21; P = 0.04) and age (rho = 0.30; P = 0.003) (Table 3).

Discussion

Raised levels of sCD40L related to impaired prognosis have been previously reported in patients with acute coronary artery syndrome and [30] and sepsis [23,24]; however, to our knowledge, this is the first study reporting data on serum sCD40L levels in patients with severe TBI.