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Regular Article

Sustained high plasma plasminogen activator inhibitor-1 levels are associated with severity and mortality in septic patients

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ABSTRACT

Background: Higher plasma plasminogen activator inhibitor-1 (PAI-1) levels have been reported in septic patients. However, some questions remain unanswered, such as whether there is an association between plasma PAI-1 levels and sepsis severity and mortality, and inflammation state during the first week.
Methods: Multicenter, observational and prospective study carried out in six Spanish Intensive Care Units of 260 patients with severe sepsis. Circulating levels of PAI-1 and tumour necrosis factor (TNF)-α were measured at day 1, 4 and 8. End-point was 30-day mortality.
Results: Nonsurviving septic patients (n = 89) presented higher PAI-1 levels than surviving (n = 171) at day 1 (58.4 (33.3-83.8) vs 36.5 (21.1-62.5) ng/mL; $p < 0.001$), 4 (34.0 (14.7-53.3) vs 16.2 (10.2-27.4) ng/mL; $p < 0.001$) and 8 (30.6 (16.2-47.8) vs 18.9 (10.4-29.5) ng/mL; $p = 0.004$). We found a positive correlation of PAI-1 levels with SOFA, lactic acid, aPTT, INR and TNF-α, and negative with platelet count at day 1, 4 and 8. Logistic regression analyses showed that PAI-1 levels at day 1 ($p < 0.001$), 4 ($p < 0.001$) and 8 ($p = 0.001$) were associated with 30-day mortality. On ROC curve analysis to predict 30- day survival, the area under the curve of PAI-1 levels at day 1, 4 and 8 were 0.65 (95% CI = 0.58-0.72; $p < 0.001$), 0.69 (95% CI = 0.60-0.78; $p < 0.001$) and 0.65 (95% CI = 0.54-0.75; $p = 0.005$) respectively.
Conclusions: The most interesting findings of our study, to our knowledge the largest series reporting PAI-1 levels during follow-up in septic patients, were that plasma PAI-1 levels during the first week were associated with inflammation, severity and mortality.

Introduction

Sepsis is a complex clinical syndrome that results from a systemic inflammatory response to an infectious event. The coagulation and inflammation systems are interconnected in sepsis; the inflammatory mediators activate coagulation and conversely, intravascular coagulation induces an inflammatory response [1–3].

Microorganisms have cellular components that bind to pattern recognition proteins of the innate immune pathway. This leads to complex intracellular signaling ultimately causing increased generation of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6; and anti-inflammatory cytokines, such IL- 10. Proinflammatory cytokines damage the endothelium initiating a procoagulant response due to the expression of subendothelial tissue factor, and an antifibrinolytic response. These procoagulant and antifibrinolytic pathways lead to microvascular fibrin deposition, resulting in multiorgan failure, and ultimately to patient's death.

Plasminogen activator inhibitor-1 (PAI-1) plays an important role in the fibrinolytic response associated with sepsis. PAI-1 is a member of the serine protease inhibitor (serpin) family, which regulates fibrinolysis by inhibiting the tissue-type plasminogen activator (t-PA) and the urokinase-type plasminogen activator (u-PA) [4]. Activated protein C

Abbreviations: PAI-1, plasminogen activator inhibitor; TNF, tumour necrosis factor; ICU, Intensive Care Unit - SOFA: Sepsis-related Organ Failure Assessment score.

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(APC) can stimulate fibrinolysis by forming a tight 1:1 complex with PAI-1; and in sepsis there is a decrease of APC, thus there are an increase of PAI- 1 and an antifibrinolytic response [2,3].

Experimental studies of endotoxemia in both animal models [5] as well as in healthy subjects [6,7] have demonstrated that intravenous injection of endotoxin, induces biological response characterized by cytokines release (as TNF-α and IL-6), activation of the coagulation system and impairment of the fibrinolytic activity (assessed by increased PAI-1). In a similar way, an increase of PAI-1 plasma levels has been induced after an intravenous challenge with TNF-α in animal models [8] and in healthy subjects [9]. Previous reports have found higher plasma levels of PAI-1 in septic patients compared with healthy controls [10–13], and that PAI-1 levels were associated with higher disease severity [13–25] and mortality [18–25] in sepsis.

However, the following questions remain unanswered: 1) Is there an association between plasma PAI-1 levels and sepsis severity during the first week? 2) Is there an association between plasma PAI-1 levels and sepsis mortality during the first week? 3) Is there an association between plasma PAI-1 levels and inflammation state during the first week? 4) Plasma PAI-1 levels could be used as biomarker of sepsis outcome during the first week? The present study sought to answer these questions.

Methods

Design and Subjects

A multicenter, cohort study was carried out in 260 patients with severe sepsis from six Spanish Intensive Care Units. The study was approved by the Institutional Review Boards of the six hospitals and informed consent from the patients or from the family members was obtained.

The diagnosis of severe sepsis was established according to the International Sepsis Definitions Conference [26]. Exclusion criteria were: age <18 years, pregnancy, lactation, human immunodeficiency virus (HIV), white blood cell count <1,000/mm3, solid or haematological tumour, or immunosuppressive, steroid or radiation therapy.

Variables Recorded and Outcome

The following variables were recorded at ICU-admission for each patient: sex, age, diabetes mellitus, chronic obstructive pulmonary disease (COPD) and site of infection. We also assessed platelet count, lactic acid, INR, aPTT, Sepsis-related Organ Failure Assessment [SOFA] score [27], and PAI-1 levels at the time of ICU admission, and on days 3 and 7 of ICU-admission. The outcome variable was ICU-mortality. End-point was 30-day mortality.

Blood Samples

Blood samples were collected from patients on day 1, 4 and 8 of severe sepsis diagnosis. Day 1 was considered as the first day that severe sepsis was diagnosed (baseline values). Day 4 was considered as the day after 72 hours had elapsed, and day 8 as the day after 7 days had elapsed since the diagnosis of severe sepsis.

Serum separator tubes were used to determine TNF-alpha, and venous citrated plasma tubes to determine PAI-1 concentration. Venous blood samples were taken and centrifuged within 30 minutes at 1000 g for 15 min, and frozen at −80 °C until assayed.

PAI-1 and TNF-Alpha Assays

Assays for PAI-1 and TNF-α were centralized at the Laboratory Deparment of the Hospital Universitario de Canarias (La Laguna, Santa Cruz de Tenerife, Spain).

PAI-1 antigen was assayed by specific ELISA (Imubind Plasma PAI-1 ElisaTM. American Diagnostica, Inc., Stanford, CT, USA) according to manufacturer's instructions. The assay detects latent (inactive) and active forms of PAI-1 and PAI-1 complexes. The interassay coefficient of variation (CV) was <5% (n = 20) and the detection limit for the assay 1 ng/ml.

TNF-α was measured in serum by solid-phase chemiluminescent immunometric assays (Immulite®, Siemens Healthcare Diagnostics Products, Llanberis, United Kingdom). The interassay CV were <6.5% (n = 20) and detection limit for the assays was 1.7 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 by using Wilcoxon-Mann- Whitney test. Comparisons between groups on categorical variables were carried out with chi-square test. Logistic regression analyses were applied to determine the independent contribution of plasma PAI-1 levels at days 1, 4 and 8 on 30-day mortality controlling for diabetes mellitus and chronic renal failure. Odds ratio (OR) and its 95% confidence intervals (CI) were calculated as measurement of the clinical impact of the predictor variables. We plotted receiver operating characteristic (ROC) curves using survival at 30 days as classification variable and plasma PAI-1 levels at days 1, 4 and 8 as prognostic variables. The association between continuous variables was carried out using Spearman's rank correlation test. 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).

Results

Comparison of patients'demographic and clinical characteristics between nonsurviving (n = 89) and surviving septic patients (n = 171) is shown in Table 1. Non- survivor patients were older and showed higher rate of diabetes mellitus than survivors; however, no differences were observed regarding sex, chronic renal failure, COPD, ischemic heart disease, site of infection, microorganism responsible and the development of bloodstream infection between non-surviving and surviving patient group.

Table 1
Patients'demographic and clinical characteristics in 30-day surviving and non-surviving patients.

	Survival (n = 171)	Non-survival (n = 89)	p-value
Sex male – n (%)	114 (66.7)	56 (62.9)	0.58
Age - median years (p 25–75)	57 (47–69)	64 (54–74)	0.005
Diabetes mellitus – n (%)	39 (22.8)	34 (38.2)	0.01
Chronic renal failure – n (%)	10 (5.8)	11 (12.4)	0.09
COPD – n (%)	21 (12.3)	10 (11.2)	0.99
Ischemic heart disease - n (%)	21 (12.3)	9 (10.1)	0.69
Site of infection			0.76
Respiratory - n (%)	92 (53.8)	52 (58.4)	
Abdominal - n (%)	53 (31.0)	25 (28.1)	
Neurological	4 (2.3)	1 (1.1)	
Urinary - n (%)	8 (4.7)	3 (3.4)	
Skin - n (%)	8 (4.7)	3 (3.4)	
Endocarditis - n (%)	6 (3.5)	4 (4.5)	
Osteomyelitis - n (%)	0	1 (1.1)	
Microorganism responsibles			
Unkwon - n (%	88 (51.5)	47 (52.8)	0.90
Gram-positive- n (%)	40 (23.4)	23 (25.8)	0.65
Gram-negative- n (%)	44 (25.7)	19 (21.3)	0.54
Fungii- n (%)	3 (1.8)	4 (4.5)	0.24
Anaerobe- n (%)	2 (1.2)	1 (1.1)	0.99
Bloodstream infection	23 (13.5)	17 (19.1)	0.28

COPD = Chronic Obstructive Pulmonary Disease.