Microvascular Research 93 (2014) 92–97

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Endocan is useful biomarker of survival and severity in sepsis

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ARTICLE INFO

Article history:
Accepted 11 April 2014
Available online 21 April 2014

Keywords:
Sepsis
Endothelium
Hemostasis
Coagulation
Prognosis
APACHE
Infection
Shock
MODS
Endocan

ABSTRACT

Introduction: Coagulation abnormalities which occur as a consequence of endothelial changes are recognized as diagnostic criteria for sepsis, but significance of these changes in the outcome prognosis and prediction of the course of sepsis is still not accurately defined.
Materials and methods: 60 patients who fulfilled the criteria for diagnosis of sepsis were included in our study. Patients were categorized in two groups according to sepsis severity and organ failure and MODS development was assessed in the first 48 h from ICU admission. Prothrombin time (PT), activated partial thromboplastin time (aPTT) and endothelial cell specific molecule-1(endocan) levels, as well as procalcitonin (PCT) and C-reactive protein (CRP) were determined within the first 24 h of the onset of the disease. Predictive APACHE II (Acute Physiology and Chronic Health Evaluation II) and SOFA (Sequential Organ Failure Assessment) scores were calculated on the day of ICU admission. Data were used to determine an association between day 1 biomarker levels, organ dysfunction score values and the development of organ failure, multiple organ dysfunction syndrome (MODS), and mortality during 28 days. These connections were determined by plotting of receiver operating characteristic (ROC) curves. Differences between groups were assessed by Mann–Whitney *U* test. Categorical variables were compared using chi-square test.
Results: Concentration of endocan was significantly higher in the group of patients with sepsis induced organ failure, MODS development and in the group of non- survivors in contrast to group with less severe form of the disease, without multiorgan failure, and in contrast to group of survivors ($p < 0.05$). Values of areas under the ROC curves showed that endocan levels had good discriminative power for more severe course of sepsis, MODS development and possible discriminative power for mortality prediction (AUC: 0.81, 0.67, 0.71 retrospectively), better than PCT for fatality (AUC:053) and better than APACHE II (AUC:0.55) and SOFA (AUC: 0.57) scores for organ failure.
Conclusions: Results of our study show that endocan can be used as strong and significant predictor of sepsis severity and outcome, perhaps even better than SOFA and APACHE II scores.

Introduction

Sepsis is one of the main causes of death in intensive care units and other hospital wards (Dombrovskiy et al., 2007; Moore et al., 2011), in spite of implementation of new sepsis treatment guidelines in everyday hospital practice worldwide (Dellinger et al., 2013; Dombrovskiy et al., 2007; Moore et al., 2011). Changes that occur in the microvasculature, affecting primarily endothelial cell, are the basis of the pathophysiology of multiorgan dysfunction (MODS) in sepsis (Harlen, 2010; Hoesel and Ward, 2007). Proinflammatory cytokines that are released during the systemic inflammation are responsible for morphological and functional changes of endothelial cells (Levi et al., 2002; Schouten et al., 2008; Semeraro et al., 2012). These changes also modify inflammatory

Abbreviations: PT, Prothrombin time; aPTT, Activated partial thromboplastin time; Endocan, endothelial cell specific molecule-1; MODS, Multiple organ dysfunction syndrome; PCT, Procalcitonin; CRP, C-reactive protein; ROC, Receiver operating characteristic; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II; DIC, Disseminated intravascular coagulation; TNF-α, Tumor necrosis factor alpha; IL-1β, Interleukin-1 beta; PaO2, Partial pressure of oxygen in arterial blood; FiO2, Fraction of inspired oxygen; ICU, Intensive care unit; ELISA, Enzyme-linked immunosorbent assay; SD, Standard deviation; CNS, Central nervous system.

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http://dx.doi.org/10.1016/j.mvr.2014.04.004
0026-2862/

response which leads to clinical manifestations of organ hypoperfusion, as well as to clinical manifestation of septic shock, MODS and other complications like disseminated intravascular coagulation (DIC) (Levi and Schultz, 2010; Petaja, 2011). Coagulation abnormalities are recognized as diagnostic criteria for sepsis and septic shock, in the latest surviving sepsis guidelines (Dellinger et al., 2013), but the significance of these changes in the outcome prognosis and prediction of the course of sepsis is still not accurately defined (Charalampos and Vincent, 2010; Kinasewitz et al., 2004; Levi and van der Poll, 2004; Xing et al., 2012; Zakariah et al., 2008). Signs of endothelial damage, together with the markers of the dysfunction of endothelial cells, are not recommended for routine laboratory investigations, according to latest sepsis guidelines (Paulus et al., 2011).

In sepsis, the activation of endothelial cells by mediators of infection such as lipopolysaccharide (LPS) in Gram-negative or lipoteichoic acid in Gram-positive infections, or cytokines, for instance interleukin-1-beta (IL-1 β) and tumor necrosis factor-alpha (TNF- α), result in a complex proinflammatory and prothrombotic phenotype (Harlen, 2010). Endothelial cells express many molecules involved in pathophysiological processes in sepsis (Schlichting et al., 2011.), and one of these molecules is endothelial cell specific molecule-1 (endocan) (Bechard et al., 2001).

Endocan is a proteoglycan that can be detected in human blood and is expressed on the surface of endothelial cells of lungs and kidneys (Bechard et al., 2001). Synthesis and release of this molecule are promoted by proinflammatory cytokines like TNF-α and IL-1β (Bechard et al., 2001; Bechard et al., 2000; Lassalle et al., 1996).

A few studies show that endocan can be acknowledged as a good marker of endothelial dysfunction and multiorgan failure in sepsis, and it can be accepted as a good marker of survival prognosis in sepsis (Scherpereel et al., 2006). The investigation of markers of endothelial dysfunction and coagulation abnormalities in sepsis could contribute not only to better understanding of sepsis pathophysiology, but also to prediction of clinical course and outcome of sepsis, all for the purpose of starting an appropriate early goal directed therapy.

Our primary objective was to determine whether endocan level measured within 24 h of onset of sepsis is associated with organ failure and mortality in patients with sepsis.

The secondary aim of this study was to investigate the use of coagulation biomarkers measured in the first 24 h of sepsis (activated partial thromboplastin (aPTT) and prothrombin time (PT)) for the prediction of the clinical course and outcome prognosis in septic patients.

Material and methods

Patients

Data were collected prospectively (and analyzed retrospectively) over 1-year study period. Any patient treated in the Department of Anesthesia and Reanimation of Emergency Centre of Clinical Centre of Vojvodina, as well as at the Clinic of Infectious Disease of Clinical Centre of Vojvodina with a clinical diagnosis of sepsis defined by The ACCP/SCCM Consensus Conference Committee American College of Chest Physicians/Society of Critical Care Medicine 1992, revised on SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference 2001 (Bone et al., 1992; Levy et al., 2003) and in latest Surviving Sepsis Campaign Guidelines (Dellinger et al., 2013) was eligible for inclusion.

Patients who were excluded from our study were patients with systemic inflammation caused by some other conditions except sepsis (e.g. burns, pancreatitis). Also polytraumatized patients, patients that were in hemorrhagic shock, patients with malignancy, patients with liver failure, pregnant women, patients with cardiac failure, patients with antiphospholipid syndrome, and patients who receive anticoagulant therapy or per oral therapy with vitamin K antagonists were excluded from the study.

The study was approved of by the relevant ethical committee of Clinical Centre of Vojvodina and Medical Faculty of the University of Novi Sad and informed consent was obtained from patients or authorized persons.

Basic laboratory tests were performed within the first hour of ICU admission (total blood count, urea and creatinine level, bilirubin level and concentration of C-reactive protein (CRP) and procalcitonin (PCT)).

Patients were categorized in groups retrospectively according to sepsis severity and organ failure in the first 48 h from ICU (intensive care unit) admission. Severe sepsis with organ failure on admission was diagnosed according to Surviving Sepsis Campaign Guidelines criteria (Dellinger et al., 2013) accessing respiratory, cardiovascular, hematologic, metabolic, hepatic and central nervous system failure. Severe sepsis with organ dysfunction was defined as: sepsis induced hypotension; altered mental status; lactate above normal upper laboratory limits; urine output < 0.5 ml/kg/1 h for more than 2 h despite adequate fluid resuscitation; acute lung injury with PaO_2/FiO_2 < 250 in the absence of pneumonia as infection source; acute lung injury with PaO_2/FiO_2 < 200 in the presence of pneumonia as infection source; creatinine > 2.0 mg/dl (176.8 mol/L); bilirubin > 2 mg/dl (34.2 mol/L); platelet count < 100,000 L; coagulopathy (international normalized ratio > 1.5). The development of organ failure was first assessed in the first 24 h of ICU admission. Severe MODS was defined as the development of more than two organ dysfunctions in the first 48 h. Predictive APACHE II (Acute Physiology and Chronic Health Evaluation II) and SOFA (Sequential Organ Failure Assessment) scores were calculated on the day of ICU admission.

To assess the development of complication and the outcome of the disease, we monitored patients for 28 days from the onset of sepsis.

Biomarkers of endothelial and coagulation integrity

After inclusion in the study, in the first 24 h from the onset of sepsis, 5 mL of venous blood was obtained from each patient and samples were immediately centrifuged. Plasma samples were then refrigerated at −70 °C until assayed.

Laboratory tests were performed in a blind manner, without any knowledge of health condition and prediction mortality scores.

The level of endocan was measured using USCN Life Sciens, USA, ELISA endocan commercial kits. The manufacturer revised protocol was followed, and the values are expressed in ng/mL.

PT and aPTT were determined by coagulation method with IL reagents (Instrumentation Laboratory Milan, Italia) on ACL 9000 machines (PT and aPTT ratio). CRP concentration was determined by immunoturbidimetric measurement (ABX Micros) and concentrations were expressed in mg/L. PCT levels were measured via the automatic analyzer (mini Vidas), and the lower limit of detection of the assay was 0.05 ng/mL.

Statistical analysis

Data were analyzed using SPSS 20.0 software. Data are expressed as mean ± SD or numbers (%). Non-parametric approaches were used since most continuous variables were skewed. Differences between variables were assessed by the Mann–Whitney *U* test. Categorical variables were compared using chi-square test and Fischer's exact test for small samples. Receiver operating characteristic (ROC) curves for day 1 APACHE II and SOFA scores, endocan levels, aPTT and PT ratio values, PCT and CRP concentrations for the prediction of the development of organ dysfunction and mortality during 28 days were plotted and the respective areas under the curves were calculated. Statistical significance (p) was set at a value of 0.05.