Clinica Chimica Acta 483 (2018) 275–280

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Concentration and value of endocan on outcome in adult patients after severe sepsis

Sheng-Yuan Hsiao[a,b], Chia-Te Kung[b], Nai-Wen Tsai[c], Chih-Min Su[b], Chin-Cheng Huang[c], Yun-Ru Lai[c], Hung-Chen Wang[d], Ben-Chung Cheng[a,e], Yu-Jih Su[e], Wei-Che Lin[f], Yi-Fang Chiang[c], Cheng-Hsien Lu[a,c,g,*]

[a] Department of Biological Science, National Sun Yat-Sen University, Kaohsiung, Taiwan
[b] Departments of Emergency Medicine, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan
[c] Departments of Neurology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan
[d] Departments of Neurosurgery, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan
[e] Departments of Medicine, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan
[f] Departments of Radiology, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan
[g] Departments of Neurology, Xiamen Chang Gung Memorial Hospital, Xiamen, Fujian, China

ARTICLE INFO

Keywords:
Outcome
Severe sepsis
Endothelial dysfunction
Endocan

ABSTRACT

Background: Endothelial dysfunction plays a central role in severe sepsis. Endocan is constitutively expressed in human endothelial cells when sepsis occurs. We tested the hypothesis that endocan concentrations are substantially increased in severe sepsis and decrease after antimicrobial therapy, and that endocan concentrations can predict treatment outcomes.

Methods: Biomarkers of the endothelium including endocan and cell adhesion molecules were prospectively evaluated in 153 patients with severe sepsis on days 1, 4, and 7 after admission along with biochemical and clinical data.

Results: Sepsis non-survivors had significantly higher endocan, ICAM-1, and VCAM-1 concentrations and lower platelet concentrations upon admission than the survivors. Non-survivors had significantly higher endocan and VCAM-1 concentrations than the survivors on serial analysis (days 1, 4, and 7). After stepwise logistic regression model and AUC analysis, endocan was revealed as a good predictor of outcome in severe sepsis, and the cut-off value for predicting fatality was 6.28 ng/ml. An increase in the endocan concentration by one ng/ml indicated an increase in fatality rate by 11.1%.

Conclusions: Based on our results, serial endocan concentration meets the major requirements for predicting outcome in patients with severe sepsis. An assay of endocan concentration may be a good prognostic biomarker in the clinic for severe sepsis.

1. Introduction

Sepsis is a potentially life-threatening disease and endothelium dysfunction plays a central role in fatality and organ dysfunction. Several mechanisms are implicated in the development of endothelium dysfunction in severe sepsis, including endothelial barrier-produced contraction during infection, induction of the protective regulatory imbalance, and altered endothelial hyperpermeability [1,2]. Thus, access to inflammatory tissues is provided by leukocytes [3] and several factors such as endocan and cell adhesion molecules (CAMs), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin, and L-selectin, are released from the endothelium. Finally, endothelial dysfunction is reflected by vasodilatation and consequent hypotension, contributing to decreased organ perfusion and eventually multiple organ failure [4,5].

Endocan, also called endothelial cell-specific molecule-1 (ESM-1), is a soluble proteoglycan that is expressed constitutively in human endothelial cells and also in response to proinflammatory cytokines and vascular endothelial growth factor when sepsis occurs. Several studies have shown that endocan has a predictive value in some human diseases such as cancer, acute respiratory distress syndrome, and sepsis [6,7]. Although the exact mechanisms have not been completely clarified, endocan has been shown to bind to a wide range of molecules associated with cellular signaling and adhesion, enhance the production

* Corresponding author at: Department of Neurology, Chang Gung Memorial Hospital, 123, Ta Pei Road, Niao Sung District, Kaohsiung, Taiwan.
E-mail addresses: chlu99@ms44.url.com.tw, chlu99@adm.cgmh.org.tw (C.-H. Lu).

https://doi.org/10.1016/j.cca.2018.05.007
Received 8 March 2018; Received in revised form 25 April 2018; Accepted 7 May 2018
Available online 08 May 2018
0009-8981/

of proinflammatory cytokines, increase microvascular permeability, and modulate leukocyte migration [8].

Previous studies on the role of endocan on the outcome of patients with severe sepsis had small sample sizes, less strict inclusion criteria [9,10], or patients with several comorbidities. These studies involved only one blood sample taken from each patient, and considerable variation was evident among patients [9–11]. If such a biomarker was measured using our standard protocol (day 1, day 4, and day 7), it would improve our ability to predict the prognosis.

2. Patients and methods

2.1. Study participants and definition

In this prospective study, we enrolled 153 non-surgical and non-trauma adult severe sepsis patients over a 3–7 period from January 2015 to December 2017 at Kaohsiung Chang Gung Memorial Hospital, an acute care teaching hospital that provides both primary and tertiary referral care. The hospital's Institutional Review Committee on Human Research approved the study protocol (103-5216B and 104-9397B), and written informed consent was provided by first-degree relatives or patients. For comparison, 52 sex- and age-matched volunteers without clinical evidence of infection were recruited as the control group.

The definition of severe sepsis and septic shock used was in accordance with the criteria defined by the American College of Chest Physicians/Society of Critical Care Medicine and our previous studies [12–15]. Septic shock was defined as severe sepsis accompanied with hypotension not controlled by intravascular volume expansion and requiring vasopressive dosage (e.g., dopamine, epinephrine, or norepinephrine) to maintain systolic blood pressure > 100 mmHg. All patients enrolled in this study met three criteria including: a) suspicion or confirmed infection; b) ≥2 manifestations of systemic inflammatory response syndrome; and c) at least 1 sepsis produced acute organ dysfunction or features of hypo-perfusion (i.e., encephalopathy, lactic acidosis, and oliguria). Exclusion criteria were: (a) hematologic disease or those under chemotherapy; (b) multiple simultaneous comorbidities which may influence our results, such as malignancy or stroke; (c) or hospitalization within the past 28 days.

2.2. Clinical assessment and treatment

We collected demographic data and used standardized assessment scales to record disease severity scores, such as acute physiology, chronic health evaluation II (APACHE II) score, and sequential organ failure assessment (SOFA) within the first 24 h of ED admission (day 1). Information about the infection source, antibiotic administration, and the progression of various organ dysfunctions and other treatments, including vasoactive agent supplementation, ventilator support, and renal replacement therapy, were recorded. Furthermore, consultation with an infectious disease expert to identify an appropriate antimicrobial treatment based on the guidelines for infection cause during the first 24 h is an institutional practice.

2.3. Assessment of infection biomarkers

We examined basic laboratory tests, including lactate concentration and inflammatory markers (e.g., C-reactive protein (CRP)) upon admission to the ED. Five milliliters of blood were collected after venipuncture of 1 forearm vein using aseptic technique. Then, the specimen was poured into pyrogen-free tubes (Vacutainer, Becton Dickinson) and centrifuged at ambient temperature. The supernatant serum was immediately aliquoted and aliquots were shipped in dry ice within several hs into the central lab at the Laboratory of Kaohsiung Chang Gung Memorial Hospital. Aliquots were stored there at −80 °C until measurement. All tests were conducted at the hospital's quality-controlled central laboratory. Our infection biomarker test methods were described in previous studies [5,12,15]. CRP concentration was determined using an enzyme immunoassay (EMIT, Merck Diagnostica), while procalcitonin concentration was determined using an enzyme-linked fluorescent assay (VIDAS, Biomerieux). Lactate concentration was estimated using a serum-based assay catalyzed by lactate oxidase (UniCel Integrated System, Beckman Coulter Inc).

2.4. Blood sampling and assessment of endothelium biomarkers

Concentrations of endothelium biomarkers including endocan, ICAM-1, VCAM-1, E-selectin, P-selectin, and L-selectin were checked within the first 24 h and blood sampling was repeated at follow-up time points, day 4 and day 7. The selection of a minimum 72-h time interval between time points could increase the likelihood that the changes described in the studied mediators over time were associated with sepsis-related endothelium dysfunction. Endocan, serum ICAM-1, VCAM-1, E-selectin, L-selectin, and P-selectin concentrations were measured by commercially available ELISA kits (R & D Systems). The detailed methodology was described in our previously published studies [13,15].

2.5. Microbiology

Based on clinical indication, blood samples were taken for culture examination upon admission to the ED prior to initiating antibiotic treatment. Two pairs of aerobic and anaerobic bottles were routinely obtained and incubated for at least 5 days (ID GN 32 System; Biomérieux Vitek Inc.). All isolates were analyzed by standard microbiologic methods. This study group enrolled bacteremic patients, defined as those with the same pathogen growth from both sets of different blood cultures, or from one or more bottles in each of two or more blood culture sets, with at least one positive culture obtained. Growth of coagulase-negative *Staphylococcus* or *Corynebacterium* species were assumed to be contaminants. Mixed cultures were significant when organisms isolated were non-contaminants.

2.6. Outcome and organ dysfunction

Patients were classified in groups according to organ dysfunction in the first 48 h upon arrival to the ED. Physicians evaluated the relationships between severe sepsis and organ failure progression daily. In this study, acute kidney injury (AKI) was defined according to KDIGO guidelines [16] as: 1. Elevation in sCr ≥ 1.5-fold of baseline value; 2. Elevation in sCr by ≥0.3 mg/dl (≥26.5 μmol/l) within 2 days; 3. Urine volume < 0.5 ml/kg/h for 6 h. Ventilator use entails intubation within 48 h due to respiratory failure or impending respiratory failure after severe sepsis.

2.7. Statistical analysis

Four separate statistical analyses were performed. Data were expressed as mean ± SD. If continuous variables were not normally distributed, data were logarithmically transformed to improve its normality. First, we used a Student's *t*-test to compare univariate analysis and used χ^2 test or Fisher's exact test to conduct categorical variables. Second, a correlation analysis was conducted to explore the association among 24-h APACHE II scores; SOFA scores; and the concentrations of ICAM-1, VCAM-1, E-selectin, and endocan in severe sepsis patients upon admission to the ED. Repeated measurements of analysis of variance (ANOVA) were conducted to compare results of endothelium biomarkers at 3 intervals (Day 1, 4, and 7). We used analysis of covariance (ANCOVA) to compare results while controlling for potential confounding variables. Third, we used stepwise logistic regression to explore the associations between significant variables and therapeutic outcomes, with adjustments for potential confounding factors. Finally, receiver operating characteristic (ROC) curves were generated for the