Thrombosis Research 129 (2012) 797–800

Contents lists available at ScienceDirect

Thrombosis Research

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

Regular Article

Thrombin generation in severe sepsis

S. Petros [a,*], P. Kliem [a], T. Siegemund [b], R. Siegemund [a]

[a] University Hospital Leipzig, Medical ICU, Leipzig, Germany
[b] The Scripps Research Institute, Department of Molecular and Experimental Medicine, La Jolla, CA, USA

ARTICLE INFO

Article history:
Received 10 May 2011
Received in revised form 16 July 2011
Accepted 2 August 2011
Available online 26 August 2011

Keywords:
Thrombin generation
Severe sepsis
Mortality
Calibrated automated thrombography
Coagulation
Inflammation

ABSTRACT

Introduction: Hemostasis and inflammation are two tightly interrelated systems in the host's response to infection. Thrombin generation in sepsis plays a crucial role in enhancing and modulation of inflammation. The present study aimed to investigate the course of thrombin generation in patients with severe sepsis, and its correlation with outcome.
Materials and Methods: Thrombin generation was measured in platelet-poor plasma from 32 healthy controls and 75 patients with severe sepsis using the commercially available Calibrated Automated Thrombography. Samples were taken within 24 hours of the diagnosis of severe sepsis (t1) as well as on day 2 (t2), day 3 or 4 (t3) and between day 6 to 8 (t4), while this was done only once in healthy controls. The assay was run with and without the addition of thrombomodulin. Clinical data were also collected at the same time points.
Results: Except for endogenous thrombin potential, there was significant difference between patients and controls regarding peak thrombin, lag time and time to thrombin peak. Twelve patients (16%) died in the ICU. There was no significant difference in endogenous thrombin potential between survivors and non-survivors of sepsis. Thrombin peak was higher in survivors than non-survivors at all time points, with a significant difference at t2 and t4. The lag time and time to thrombin peak were shorter in non-survivors than in survivors at t1 and t3.
Conclusions: While thrombin peak shows a positive correlation with survival, the lag phase and time to thrombin peak may be signs of impending DIC. The endogenous thrombin potential does not have any prognostic importance.

Introduction

Sepsis is one of the most frequent causes of hospital death. Despite a considerable progress in intensive care medicine, the sepsis-attributed mortality is as high as 30 – 50% [1–3], rising up to 70% in patients with an overt disseminated intravasal coagulation (DIC) [4]. The incidence of sepsis and the number of sepsis-related deaths are increasing [5].

The host response to infection is characterized by a systemic inflammatory response. Bacterial toxins and cytokines lead to expression of tissue factor (TF) on the surface of damaged endothelium and circulating mononuclear cells. TF expression results in activation of the coagulation cascade, which leads to increased thrombin formation. On the other hand, the regulatory physiologic anticoagulation system is impaired. Furthermore, activation of the endogenous fibrinolysis may be insufficient to counteract the ongoing coagulation, so that fibrin clot formed as a result of thrombin burst is not effectively removed, the consequence being intravascular thrombus deposition [6].

Besides its hemostatic activities, thrombin is also involved in perpetuating the inflammatory reaction. It induces cytokine expression on monocytes and endothelial cells via the protease-activated receptors (PAR) [7,8]. The inflammatory reaction and microvascular thrombus formation contribute to organ dysfunction.

Measuring thrombin generation may contribute to our understanding of the hemostatic potential of the organism [9]. Published reports showed that the thrombin generation assay allows to identify hyper- as well as hypocoagulable states [10–14]. However, publications regarding thrombin generation assay in sepsis are sparse. In fact, Medline research yields only one study regarding this issue. In that study, Collins et al. [15] reported for the first time on TG data comparing healthy controls and septic patients. However, the assay was run only once in patients and it was not specified at which time point in the course of sepsis the blood sampling was conducted.

Based on these observations, this study was aimed to investigate the course of thrombin generation in patients with severe sepsis, and its correlation with outcome.

Materials and methods

The study was approved by the ethics commission of the University of Leipzig, and it is conducted in accordance with the ethical standards

Abbreviations: APACHE, Acute Physiology and Chronic Health Score; CAT, Calibrated Automated Thrombography; DIC, disseminated intravascular coagulation; ETP, endogenous thrombin potential; LMWH, low molecular weight heparin; PAR, protease-activated receptors; PPP, platelet-poor plasma; PRP, platelet-rich plasma; SOFA, Sequential Organ Function Assessment; TF, tissue factor; TG, thrombin generation.

* Corresponding author at: University Hospital Leipzig, Medical ICU, Liebigstr. 20, D-04103 Leipzig, Germany. Tel.: +49 3419712700; fax: +49 3419712709.
E-mail address: Sirak.Petros@uniklinik-leipzig.de (S. Petros).

0049-3848/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2011.08.004

laid down in the 1964 Declaration of Helsinki. Patients with the diagnosis of severe sepsis admitted to the medical ICU of the University Hospital Leipzig were included in this study after an informed consent of the patient or legal guardian. Inclusion criterion was the diagnosis of non-surgical sepsis made within 24 hours prior to the study inclusion based on the consensus criteria of the American College of Chest Physicians and the Society of Critical Care Medicine [16]. Exclusion criteria were septic shock on admission, pregnancy, age below 18 years, no consent, active hematologic or malignant disease, platelet count below 80.000/mm^3, known liver failure on admission, need for therapeutic anticoagulation, current oral anticoagulation with vitamin K antagonists, and ongoing or planned treatment with activated protein C. Standard prophylactic anticoagulation with a low molecular weight heparin (LMWH, in our case nadroparin 2850 units once daily) was allowed according to the local protocol with an interval of at least 12 hours between the LMWH administration and the study blood sampling. In general, blood sampling was undertaken immediately before the administration of the daily LMWH thrombosis prophylaxis, so that the maximum possible time interval between the previous thrombosis prophylaxis and blood sampling was maintained. All patients were treated according to the standard of care of the ICU based on the international management guidelines for sepsis [17].

Blood samples for the thrombin generation (TG) assay were collected on the day of study admission (day 1, which was also the day of diagnosis of severe sepsis) (t1), day 2 (t2), day 3 or 4 (t3), and between day 6 to 8 (t4) into Sarstedt tubes containing 3.8% citrate. Blood samples were collected from healthy controls once after informed consent. All samples were then centrifuged within an hour after collection at 18 °C and 1000 rpm for 10 minutes to prepare a platelet-rich plasma, then at another 4000 rpm for 20 min. to prepare a platelet-poor plasma (PPP). Samples were analyzed within two hours of collection. The TG assay was carried out according to the manufacturer's instructions on a Fluoroscan Ascent (Helsinki, Finland) at 390/460 nm wave length using the Calibrated Automated Thrombogram (CAT) and commercially available test kits. In short, a solution containing the buffer and the fluorogenic substrate was prepared according to the instructions of the manufacturer. 20 µl of this solution and 20 µl of the PPP reagent were transferred into each well of a 96-well microtiter plate (Greiner Bio One, Germany) and then mixed with 80 µl of PPP of the patient, with the final solution containing 5 pM TF and 4 µM phospholipids. The assay with thrombomodulin contained 5 pM thrombomodulin in the final solution (all from Thrombinoscope BV, Maastricht, NL). Data for endogenous thrombin potential (ETP), the thrombin peak, the lag time and time to thrombin peak for assays with as well as without the addition of thrombomodulin are presented. The DIC score was calculated at the same time points using the ISTH criteria [18].

The following data were also collected for every patient: age, gender, the Acute Physiology and Chronic Health II (APACHE-II) score and the highest blood lactate level on the day of study admission, C-reactive protein (CRP), procalcitonin, as well as the Sequential Organ Function Assessment (SOFA) score at the time points of study blood sampling, the need for vasopressor administration on the course of the sepsis, and ICU mortality. The TG data were compared between controls and patients as well as between survivors and non-survivors.

The statistical analysis was conducted using the statistical program SPSS for Windows Version 18.0. The Student *t* test was applied for data comparison. Data are presented as mean ± SD, unless stated otherwise. A p value below 0.05 was considered statistically significant.

Results

Seventy five patients (46 males and 29 females) with severe sepsis with a mean age of 65.0 ± 14.6 years were included in the study. Twelve patients (16.0%) died in the ICU, which was after a median of 8 days. The most common cause (74.0%) of sepsis was pneumonia. The mean APACHE-II score for the total study population was 23.7 ± 7.7, with survivors having significantly lower score than non-survivors (22.5 ± 6.8 vs. 30.0 ± 9.4, p = 0.02). The SOFA score for survivors and non-survivors during the study period is shown in Fig. 1. There was no significant difference in the level and the course of serum CRP and procalcitonin between survivors and non-survivors, both variables gradually declining during the study period in both groups (data not shown).

Thrombin generation data from 32 healthy controls (mean age 36.8 ± 13 years) are presented for comparison. Table 1 shows TG data of controls and patients (day 1). Except for ETP, there was significant difference between the controls and patients regarding peak thrombin, lag time and time to thrombin peak.

There was no significant difference in ETP between survivors and non-survivors (ranging between 1839. ± 463 nM on day 4 and 2282 ± 614 nM on day 3 for non-survivors vs. 2160 ± 556 nM on day 4 and 2277 ± 629 nM on day 1 for survivors, n.s.). The thrombin peak was higher in survivors than in non-survivors at all time points, with a significant difference at t2 and t4 in the assay without as well as with thrombomodulin (Fig. 2a and b).

The lag time and time to thrombin peak were significantly shorter in non-survivors than in survivors at t3. In the assay with thrombomodulin, there were also significant differences at t1 for both lag time and time to thrombin peak (Figs. 3 and 4).

In general, the TG course in survivors showed a steadier course, while this was heterogeneous for non-survivors. The DIC score showed a significant difference between survivors and non-survivors at t4 only (Fig. 5).

Discussion

Hemostasis and inflammation are tightly interwoven systems, which influence each other [19–21]. They are indispensible in the host response to infection. The damage to the endothelium and the activation of leukocytes result in an increase in TF expression and activation of the coagulation cascade. Additionally, microparticles also play an important role in enhancing coagulation. A recent study showed that neutrophils enhance coagulation via local proteolysis of tissue factor pathway inhibitor, and this activation of coagulation contributes to compartmentalization of bacteria in liver microvessels and reduction in bacterial invasion into the tissue [22]. The thrombin generated during coagulation activation is not only involved in fibrin clot formation, but also in modulation of inflammation and the immune system [23–25]. On the other hand, thrombin leads to activation of the protein C system that has anti-inflammatory effects. Thus, it is not fully clear to what extent and at which time point in the

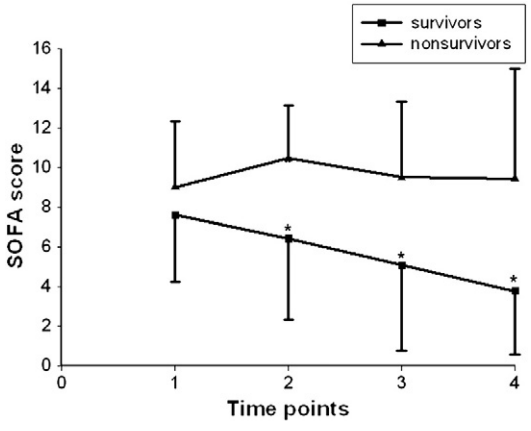

Fig. 1. The SOFA score for survivors and non-survivors (* = p<0.05).