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Impairment of thrombin generation in the early phases of the host response of sepsis☆,☆☆

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ABSTRACT

Purpose: The purpose was to investigate the presence of hypercoagulability in the very early phase of the host response to an infection in the clinical course of sepsis and septic shock.
Material and Methods: Twenty-four patients with chemotherapy-associated febrile neutropenia were evaluated at baseline, at the time of fever onset, and 48 hours thereafter using the thrombin generation test, a more physiological and global assay of hemostasis.
Results: The rate of thrombin generation was decreased and no signals of systemic hypercoagulability could be observed during the first 48 hours of sepsis. Moreover, patients that evolved to septic shock presented a more significant impairment in thrombin generation than those with noncomplicated sepsis.
Conclusions: Patients with sepsis and febrile neutropenia present an impairment in thrombin generation from very early stages of their disease course. These results suggest that the procoagulant in vitro alterations described during sepsis do not necessarily translate into a clinically relevant systemic hypercoagulable state. These findings could help explain why treatment with systemic anticoagulants did not translate to clinical benefits in human sepsis and highlight the need for a better understanding of the hemostatic alterations in sepsis before new treatments targeting coagulation activation are developed.

1. Introduction

According to the current model of the interplay between hemostasis and inflammation, the early phase of the host response to sepsis is characterized by a series of procoagulant changes that, in some patients, eventually develop into the clinical and laboratory syndrome of disseminated intravascular coagulation (DIC) [1]. The presence of this hypercoagulable state in sepsis is supported by unequivocal evidence showing increased expression of tissue factor [2], impaired function of natural anticoagulant systems [3,4], and hypofibrinolysis [5]. In addition, studies with experimental sepsis, as well as clinical studies with patients from intensive care units (ICUs), provide additional indirect evidence that DIC could be involved in the pathogenesis of sepsis complications such as multiorgan failure [5]. Based on this model, systemic hypocoagulation is considered a much later finding in the course of DIC, caused by uncontrolled consumption of coagulation factors and platelets.

Global hemostasis assays such as the thrombin generation (TG) test and thromboelastometry are attractive tools to obtain more comprehensive and biologically relevant evaluations of hemostasis compared to assays that evaluate discrete compartments of hemostasis, such as coagulation factors and inhibitor levels. These assays have been successfully used in the evaluation of conditions characterized by complex alterations of hemostasis such as chronic liver disease [6], and the coagulopathies of trauma [7] and liver transplantation [8]. These conditions share a common characteristic with sepsis and DIC in that multiple elements of hemostasis are dramatically and simultaneously altered, limiting the laboratory characterization of their net effect on hemostasis. Accordingly, several authors have recently used global assays of hemostasis to evaluate critical patients with sepsis. However, as opposed to what would be expected according to the classical model of DIC, all of these studies failed to demonstrate the presence of systemic hypercoagulability in sepsis [9–13]. These consistent, but somewhat unexpected, results could indicate that, although the in vitro evaluation of discrete compartments of hemostasis points to the hypercoagulation in the early stages of sepsis, the net systemic effect might be different. Given the clinical relevance of this issue, which supports at least in part the

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use of systemic anticoagulants in sepsis, we wanted to investigate whether the failure to demonstrate a systemic hypercoagulable state in the early stages of sepsis could be related to the fact that, in studies performed in ICUs, samples are obtained at a relatively late time point in the course of sepsis. In our laboratory, we have been studying sepsis biomarkers in inpatients with chemotherapy-induced febrile neutropenia (FN), a population in which the collection of a very early blood sample in the time course of the host response to an infection (immediately after fever onset), as well as a baseline sample (before the contact with a pathogen), is feasible [14,15]. Therefore, the aim of our study was to evaluate hemostasis with classical and global assays (TG) during the very early stages of sepsis.

2. Material and methods

2.1. Patients

Recruitment of patients took place at the Bone Marrow Transplantation Unit of the University of Campinas. All patients that were admitted for intensive chemotherapy were invited to participate in the study immediately before the initiation of chemotherapy. Fever (temperature ≥38.0°C) at admission was the only exclusion criterion. The study was performed in accordance with the Declaration of Helsinki and approved by the local Ethics Committee. Informed written consent was obtained from all patients before any study procedure. Descriptive data consisting of demographics, diagnosis, clinical and laboratory data, and sepsis severity scores were obtained from the medical records.

2.2. Patient management and sample collection

After fever onset, an infectious etiology was assumed for all patients with postchemotherapy severe neutropenia (absolute neutrophil count <500/μL) with new-onset fever in accordance with FN management protocols. Blood and urine cultures were immediately obtained, and broad-spectrum antibiotics were initiated [16]. Patients were managed according to previously published guidelines [17]. When necessary, intensive care was provided in the Bone Marrow Transplantation Unit. The development of sepsis or septic shock within the next 28 days was recorded. Venous blood was drawn at enrollment (baseline) from all patients. Patients that developed fever associated with severe neutropenia had additional blood samples collected within 12 hours after the first episode of fever and 48 hours thereafter. Samples were immediately centrifuged at 2500*g* (4°C, 20 minutes), and plasma was stored at −80°C until analysis. All samples were processed by the same investigator.

2.3. Sepsis definitions and risk stratification scores

Sepsis was defined by the presence of 2 or more of the following: (*a*) temperature greater than 38.0°C, (*b*) heart rate greater than 90 beats per minute, (*c*) respiratory rate greater than 20 breaths per minute or $Paco_2$ less than 32 mmHg, and a microbiologically proven or clinically evident source of infection. Septic shock was present in patients in which sepsis was complicated with hypotension (systolic arterial pressure <90 mm Hg or a reduction in systolic blood pressure of >40 mm Hg from baseline), despite adequate volume resuscitation, as previously defined [17]. Severity of illness was assessed by calculating the Sequential Organ Failure Assessment (SOFA) score [18] at the time of each blood collection.

2.4. Laboratory measurements

All individual laboratory assays were performed on the same day by the same investigator. The laboratory evaluation consisted of classical assays used in DIC such as fibrinogen (Fib), prothrombin time (PT), and D dimer (DD). These assays were performed in an automated coagulometer (BCS XP; Siemens Healthcare, Marburg, Germany) and are externally quality controlled through the UK National External Quality Assessment Schemes. The DD was performed using the Innovance kit from the same manufacturer. The TG test was performed in platelet poor plasma using the Technothrombin TGA kit (Technothrombin; Vienna, Austria) and a validated plate reader (Biotek FLx800, Brunner) according to the manufacturer's instructions. This methodology is based on the monitoring of the fluorescence generated by cleavage of a fluorogenic substrate by thrombin over time, yielding a TG curve known as a *thrombogram*, which reflects the different moments of the cellular model of coagulation [19]. In our study, coagulation was activated by a low concentration of negatively charged phospholipids and tissue factor (5 pmol/L), more closely resembling the physiological conditions of hemostasis activation. The following parameters were registered: (*a*) lag phase: time to generate detectable levels of thrombin, in seconds; (*b*) peak concentration of thrombin (Peak), in nanomoles per liter; (*c*) time to peak thrombin generation, in seconds; (*d*) velocity index (Vel Index), expressing the speed of thrombin generation; and (*e*) and the area under the TG curve (AUC), which reflects the total thrombin potential of the patient. Each sample was measured in duplicate.

2.5. Statistical analysis

Differences in continuous variables between patients at different time points, as well as between patient with or without septic shock, were analyzed using the Mann-Whitney and the Kruskal-Wallis tests followed by the Dunn multiple-comparison test. Data are expressed as median and range unless otherwise stated. Correlation (Spearman rank correlation) analysis was performed between sepsis severity scores and TG parameters. A P value ≤ .05 was considered statistically significant. All statistical analyses were performed with the GraphPad Prism Software (GraphPad Prism Software Inc; San Diego, CA).

3. Results

In total, 24 patients were evaluated in this study, from the original cohort of 41 patients with FN, for whom plasma samples were available for the TG assays. Additional details of the complete cohort are described elsewhere [14,15]. The demographic and clinical characteristics of these patients are shown in Table 1. No statistically significant difference was observed in the demographic and clinical data between the original cohort and the 24 patients evaluated in the present study. All 24 patients fulfilled the criteria for sepsis at the time of sample collection. Eight patients (33.3%) developed septic shock within a median time of 4 days (range, 1-7) from fever onset, and 7 (29.2%) died from this complication. The median SOFA score on the

Table 1
Patient characteristics (n = 24)

Sex (male:female)	15:9
Age (median, range)	42 (16-62)
Baseline diagnosis	
Acute leukemia	14
Others diseases[a]	10
Neutrophil/μL, fever onset (median, range)	125 (20-500)
SOFA, fever onset (median, range)	3 (0-13)
SOFA, 48 h (median, range)	4 (1-12)
Complications of FN (n)	
Sepsis	24/24
Septic shock	8/24
Thromboembolic events	0/24
Sepsis-related death	7/24
Time to septic shock in days (median, range)	4 (1-7)

[a] Lymphoma (n = 4), multiple myeloma (n = 1), chronic myeloid leukemia (n = 4), paroxysmal nocturnal hemoglobinuria (n = 1).