Critical Care

CRITICAL CARE

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Critical Care 2009, **13**:R42 doi:10.1186/cc7765

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ISSN 1364-8535

Article type Research

Submission date 7 November 2008

Acceptance date 30 March 2009

Publication date 30 March 2009

Article URL http://ccforum.com/content/13/2/R42

Thromboelastometry for the assessment of coagulation abnormalities in early and established adult sepsis: a prospective cohort study

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Abstract

Introduction: The inflammatory response to an invading pathogen in sepsis leads to complex alterations of hemostasis by dysregulation of pro- and anticoagulant factors. Recent treatment options to correct these abnormalities in patients with sepsis and organ dysfunction have led to conflicting results. Using thromboelastometry (ROTEM®), we assessed the course of hemostatic alterations in patients with sepsis and related these alterations to the severity of organ dysfunction.

Methods: This prospective cohort study included 30 consecutive critically ill patients with sepsis admitted to a 30-bed multidisciplinary intensive care unit (ICU). Hemostasis was analyzed with routine clotting tests as well as by thromboelastometry every 12 hours for the first 48 hours, and at discharge from the ICU. Organ dysfunction was quantified with the sequential organ failure assessment (SOFA) score.

Results: Simplified acute physiology score II (SAPS II) and SOFA scores at ICU admission were 52 ± 15 and 9 ± 4, respectively. During the ICU stay, clotting time decreased from 65 ± 8 seconds to 57 ± 5 seconds ($P = 0.021$), and clot formation time (CFT) from 97 ± 63 seconds to 63 ± 31 seconds ($P = 0.017$), while maximal clot firmness (MCF) increased from 62 ± 11 mm to 67 ± 9 mm ($P = 0.035$). Classification by SOFA score revealed that CFT was slower ($P = 0.017$) and MCF weaker ($P = 0.005$) in patients with more severe organ failure (SOFA ≥10; CFT 125 ± 76 seconds, MCF 57 ± 11 mm) as compared to patients with lower SOFA scores (SOFA < 10; CFT 69 ± 27, MCF 68 ± 8). Along with increasing coagulation factor activity, the initially prolonged routine coagulation tests international normalized ratio (INR) and activated partial thromboplastin time (aPTT) shortened over time.

Conclusions:

Key variables of ROTEM® remained within the reference ranges during the phase of critical illness in this cohort of patients with severe sepsis and septic shock without bleeding complications. Improved organ dysfunction upon discharge from the ICU was associated with shortened coagulation time, accelerated clot formation and increased firmness of the formed blood clot when compared to values on admission. With increased severity of illness, changes of ROTEM® variables were more pronounced.

Introduction

Organ failure contributes cumulatively to mortality of patients with sepsis [1]. One of the mechanisms that is believed to contribute to the pathogenesis of organ failure in sepsis is microvascular thrombosis [2-5]. Pathways involved in the prothrombotic state of critically ill patients include tissue factor-mediated thrombin generation and impaired anticoagulant and fibrinolytic mechanisms [6]. Continuing consumption of platelets and coagulation factors may cause overt disseminated intravascular coagulation (DIC) and bear a risk of bleeding diathesis [7]. In severe inflammation, coagulation-regulating systems seem to be defective primarily as a result of endothelial dysfunction [8, 9]. However, the effect of anticoagulant therapies on outcome is controversial [10-13].

In clinical practice, routinely performed blood coagulation tests only incompletely mirror sepsis-induced coagulation abnormalities, and hypercoagulation in particular is not detected. More advanced laboratory analyses or experimental methods to monitor coagulation in critically ill patients, including tissue factor levels [14], prothrombin fragments F1 and F2 [15], TAT [16, 17] and thrombomodulin expression [18], have not been introduced into routine clinical management.

While endpoints of routine coagulation tests occur early in the hemostatic process, thromboelastography measures the viscoelastic characteristics of blood clot formation in a whole blood assay, and may therefore provide additional information on coagulation. Thromboelastography assesses the influence of plasmatic factors and platelets during all phases of the coagulation process. Thus it is able to evaluate the initiation of coagulation, the propagation of clot formation, and the final firmness of the blood clot.

Thromboelastography has gained importance in the management of bleeding disorders in trauma and surgical patients [19-21]. It has also been used to evaluate alterations of hemostasis in *in vitro* models of endotoxemia or at a single time point in patients with sepsis [22-25]. However, the evolution of disorders of hemostasis measured by thromboelastography during severe sepsis has not been investigated.

The aim of this study was to evaluate the evolution of coagulation abnormalities using thromboelastometry (ROTEM® thromboelastometry, Pentapharm, Munich, Germany) in parallel with routine coagulation tests during the early phase of severe sepsis and septic shock, and to relate these abnormalities to organ dysfunction. We hypothesized that changes in ROTEM® variables may be related to the evolution of organ dysfunction. ROTEM® is a point-of-care device with limited susceptibility to shock and vibrations.

Materials and Methods

The study protocol for the present prospective cohort study was approved by the regional governmental ethics committee (Ethik Kommission des Kantons Bern). Written informed consent was obtained from the patients or their relatives. Thirty patients admitted to a 30-bed multidisciplinary intensive care unit (ICU) with a diagnosis of sepsis, as defined by the recommendations of the SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference [26], were enrolled in the study. Exclusion criteria were age < 18, patients with pre-existent hematological disorders, current oral anticoagulants, or therapy to inhibit platelet aggregation.

General treatment

All patients with sepsis were monitored with a radial arterial line and a central venous line, and with a pulmonary artery catheter if not responsive to initial volume loading. Treatment protocols were used for hemodynamic management, weaning from mechanical ventilation, analgesia and sedation, and insulin therapy. Thromboprophylaxis in all patients was achieved with graduated compression stockings or intermittent pneumatic compression devices in addition to low-dose unfractionated heparin 10,000 units/day according to the guidelines of the American College of Chest Physicians for critically ill patients [27]. The heparin was administered as a continuous intravenous infusion.

Volume resuscitation with colloids in our patients was achieved with hydroxyethyl starch solution (Voluven®), a 6% HES 130/0.4. Patients in the low SOFA group were administered a mean of 1373 ±1929 mL over the whole study period and 2523 ± 1914 mL in the high SOFA group.

Data acquisition

Demographic data, source of infection, and length of stay in the ICU were recorded. The Simplified Acute Physiology Score II (SAPS II) was assessed on admission. The Sequential Organ Failure Assessment (SOFA) score was assessed daily for the first three days after inclusion in the study and on ICU discharge. The SOFA score was developed to quantify the severity of illness based on the degree of organ dysfunction [28].

Blood sampling

Blood samples were taken from a radial arterial line after 10 mL of blood had been discarded. Blood for thromboelastometry was drawn into a 5 mL syringe and immediately anticoagulated with 0.5 mL of trisodium citrate 0.106 mol/L (Sarstedt, Nümbrecht, Germany).

Routine coagulation screening, including platelet count, INR, aPTT, levels of coagulation factors II, V, VII, X, and fibrinogen, were measured daily for the first three days and upon discharge. Routine coagulation laboratory measurements and analysis of coagulation factor activity were performed with the BCS® Analyzer and corresponding reagents (Siemens Health Care Diagnostics, Düdingen, Switzerland).

Thromboelastometry

Thromboelastometry was performed with a ROTEM® analyzer (Pentapharm GmbH, Munich, Germany) every 12 hours for 48 hours and at ICU discharge. The method, technique and variables of thromboelastometry have been described previously [29]. Briefly, ROTEM® measures viscoelastic properties of clot formation and fibrinolysis. Due to the use of a ball-bearing system for power transduction, it is less susceptible to movement and vibration.

Tests were performed using ROTEM cups and pins. The ROTEM® device was tested regularly for correct function using quality control serum (ROTROL®, Pentapharm GmbH, Munich,Germany).

We performed intrinsically and extrinsically activated tests (INTEM and EXTEM) according to the manufacturer's recommendations (INTEM test: 20 µL $CaCL_2$ 0.2 M, 20 µL thromboplastin-phospholipid, 300 µL blood; EXTEM test: 20 µL $CaCL_2$ 0.2 M, 20 µL tissue factor, 300 µL blood). In order to assess a possible effect of low-dose heparin administration to our patients, we also analyzed the addition of heparinase to the blood samples (HEPTEM test) in comparison to the results of the INTEM test. The influence of thrombocytes on the clot firmness was estimated with a platelet-inactivating test (FIBTEM test: 20 µL $CaCL_2$ 0.2 M, cytochalasin D, 20 µL tissue factor, 300 µL blood). Chemicals and reagents were purchased from Pentapharm GmbH (Munich, Germany).
The variables that were measured by ROTEM® thromboelastometry are the following: The clotting time (CT), the equivalent to the reaction time (r time) of conventional TEG®, represents the initiation of coagulation. The propagation of clot formation, reflecting thrombin generation and early fibrin polymerization, is characterized by clot formation time (CFT) comparable to the TEG® clotting time (k time) and the alpha angle. CFT is thereby defined as the time necessary to attain a clot firmness of 20 mm. The maximal clot firmness (MCF), corresponding to the maximal amplitude of conventional TEG®, describing the final strength of the clot, is influenced by the fibrinogen concentration and the platelet count.

Statistical analysis

SigmaStat version 3.5 (Systat Software, Inc., Chicago, IL) was used for statistical analysis. After testing for normal distribution (Kolmogorov-Smirnov Test), data were analyzed using analysis of variance for repeated measurements (ANOVA) and the Student-Newman-Keuls test for post hoc comparisons or, where appropriate, Friedman analysis and the Dunn test. In order to compare coagulation profiles in groups of patients with different organ failure severity, patients were divided into two groups using median SOFA score. Differences between the two groups were analyzed using analysis of variance for repeated measurements with one grouping factor (high vs. low SOFA score) and one within-subject factor (time). The correlation between routine coagulation and ROTEM® variables with the severity of organ dysfunction defined by the SOFA score was studied using the nonparametric Spearman Correlation (r) with pooled data from the continuous time points. Comparison of the clotting times between the INTEM and HEPTEM assays was

analyzed with a two-sided t-test. For all statistical tests, significance was assumed at $P < 0.05$. Values are expressed as mean ± SD or median (IQR) where appropriate.

Results

Patients

The characteristics of the included patients are shown in Table 1. Thirteen of the patients in the study suffered from severe sepsis, and seventeen patients from septic shock with hypotension not reversed by volume resuscitation [26]. Median SOFA score at ICU admission was 10. There were no thrombosis and bleeding diathesis reported during the study period.

Routine laboratory results

Coagulation factor levels, routine coagulation tests, hemoglobin, platelets and white blood cell count are shown in Table 2. The means of aPTT and INR on admission were increased beyond the normal range (Table 2). Relevant factor activities were initially at the low end of the normal range or below it, and increased during the ICU stay. Only factor V remained close to the midpoint of reference values. The platelet counts remained within the normal range, but also increased over time. Fibrinogen levels and white blood count were raised above normal during the entire stay in the critical care unit. Spearman rank correlation between disease severity assessed by the SOFA score and the routine coagulation tests INR and aPTT revealed rather low coefficients of r = 0.39 and 0.51, respectively ($P<0.001$ each).

Thromboelastometry

Variables of thromboelastometry of the entire study cohort are presented in Table 3. Average clotting time (CT), clot formation time (CFT) and maximal clot firmness (MCF) remained within the normal reference values established in a multicenter study [30] (Table 3). However, during the time course of critical illness we noted a decrease of CT and CFT and an increase of MCF in the tissue factor activated tests (EXTEM) compared to admission values (Table 3).

In patients with higher scores of organ failure, maximal clot firmness differed from the low SOFA group during the first 48 hours. This difference had resolved by the time of

discharge (Fig. 1). Clotting time did not differ between groups (Fig. 2). Clot propagation characterized by clot formation time and alpha angle was significantly slowed in the group with the higher organ dysfunction scores compared to the low SOFA group during the first 48 hours (Figs. 3 and 4). However, after 48 h the values in the two groups converged.

Platelet inhibition with cytochalasin revealed stronger impairment of maximal clot firmness in the high SOFA group (Fig. 5). The relative contribution of the fibrin clot (after inhibition of the platelet contribution with cytochalasin) remained stable during the ICU stay. No differences in the clotting time were noted between the HEPTEM and the INTEM assay results.

The variables of ROTEM® thromboelastometry clot formation time, alpha angle and maximal clot firmness correlated moderately with disease severity defined by the SOFA score (Table 3).

Discussion

The coagulation system is commonly activated during sepsis as a result of cross reactions with the inflammatory system [5, 31]. In the present cohort study we assessed coagulation abnormalities in septic patients during the critical phase of the illness using ROTEM® thromboelastometry. In order to further evaluate the relationship between perturbation of the coagulation system and organ dysfunction, we divided the cohort at the median of the initial SOFA score and compared these two groups.

Improvements in SOFA scores of these patients were associated with shortened time to initiation of coagulation, accelerated clot formation, and improved firmness of the final clot at ICU discharge when compared to values on admission. Therefore, we hypothesize that the coagulation system has recovered towards the patients' individual baselines upon discharge from the ICU. Nevertheless, key variables of thromboelastometry of the patients in our study remained within the range of normal reference values reported by a multicenter trial [30]. In this context no signs of overt DIC with adverse bleeding events were noticed. Of note, compared to the increase of the clot firmness the rise of the platelet count during the course of critical illness was

unproportional high whereas fibrinogen levels remained constant. This underlines the observation that fibrinogen levels seem to have a much higher impact on maximal clot firmness than do changes in platelet count [32, 33].
The results of ROTEM® thromboelastometry in the present study did not suggest hypercoagulability in terms of reduced clotting time, reduced clot formation time or increased maximal clot firmness compared to established normal ranges [30, 34-37]. The mean of the MCF in the FIBTEM assay of the patients with a SOFA score less than 10 was slightly higher than the reported normal range. However, since the FIBTEM assay measures only an isolated component of the overall clot firmness it may probably not be used as a reliable parameter for hypercoagulability. In this respect, our results differ from the thromboelastographical data reported in a study by Gonano et al. [38], who described hypercoagulability based on their results of decreased *r* and *k* values measured by TEG® (Haemoscope, Skokie, IL, USA), with TEG values corresponding to those of ROTEM®. Two other studies employing sepsis models also came to the conclusion that lipopolysaccharide-induced hypercoagulability may be detected by thromboelastography [22, 23]. Spiel et al. administered a bolus injection of endotoxin to healthy volunteers and demonstrated a transient decrease of the coagulation time, limited to the first 24 hours after injection, accompanied by increased markers of coagulation activation, i.e., increased levels of prothrombin fragments (F_{1+2}) [22]. Incubation of whole blood of healthy volunteers with endotoxin also produced a decrease in clotting time [23]. Clearly, the two latter investigations measured the effect of LPS on hemostasis in highly standardized models reflecting the very early phase after induction of inflammation. However, the clinical scenario of critically ill patients admitted to an intensive care unit differs from these situations.

In critically ill patients with severe sepsis and septic shock, the disease has reached a more advanced stage - with advanced inflammatory response and sustained exposure to the infectious pathogen - by the time they present to the ICU. In the clinical setting, assessment of activation of coagulation by thromboelastography may thus not present with shortening of clotting time and clot formation time but rather with a development in the opposite direction, where coagulation factors are consumed. A concomitant decrease in the production of clotting factors due to hepatic dysfunction in sepsis, however, may also play a role [39].

Our data are supported by an analysis of the placebo group in the PROWESS study, demonstrating that increased severity of sepsis is accompanied by prolonged coagulation times, suggesting decreased activity of coagulation factors as a result of increased consumption [40].

Activation of coagulation is a well-known pathophysiological process in sepsis [5, 14-17, 31]. The fact that accelerated clot formation and increased clot strength were not present in our study, by no means excludes activation of coagulation in sepsis. The more likely explanation for our findings is that inappropriate activation of coagulation may be depicted by thromboelastography only at an early and possibly preclinical phase. However, once coagulation factors are depleted, direct thromboelastographic signs of hypercoagulation may be absent.

There is concern that the administration of colloids may have affected the coagulation system. Although the doses of fluid resuscitation with 6% hydroxethyl starch 130/0.4 differed between the two groups, the administered volumes have reportedly only minor effects on parameters of thromboelastography [41, 42].

A low-dose regimen of unfractionated heparin was administered to the patients in the present study in order to prevent thromboembolic complications. This could have influenced thromboelastography analysis. However, in a heparinase assay (ROTEM®-Heptem) we analyzed clotting time as the variable of interest in this context and excluded such an effect [43]. These results suggest that the low dose of heparin used for thromboprophylaxis may not be detected by TEM.

A limitation of our study is that the reported changes of ROTEM® parameters had to be interpreted on the basis of external reference values. Future investigations in a controlled study may unveil clearer relationships between organ dysfunction and the coagulation system assessed by thromboelastography.

Conclusions

Key variables of ROTEM® remained within the reference ranges during the phase of critical illness in this cohort of patients with severe sepsis and septic shock without

bleeding complications. Although average thromboelastometry variables did not provide additional information to standard coagulation tests, certain dynamics of ROTEM® variables were noted within the reference ranges: Improved organ dysfunction upon discharge from the ICU was associated with shortened coagulation time, accelerated clot formation and increased firmness of the formed blood clot when compared to values on admission. With increased severity of illness, changes of ROTEM® variables were more pronounced.
Thromboelastography performed in patients with severe sepsis cannot reliably detect activation of coagulation in the sense of a hypercoagulable state. Further studies in patients with sepsis are warranted to investigate the role of thromboelastography in relation to bleeding and thromboembolic complications as endpoints.

Key messages

- Key variables of thromboelastometry remained within reference ranges during the course of critically illness in patients with sepsis without adverse bleeding events.
- After resolution of the critical illness in patients with severe sepsis/septic shock variables of thromboelastometry showed shortened coagulation time, accelerated clot formation and increased firmness of the formed blood clot when compared to values on admission. With increased severity of illness, these changes were more pronounced.
- Thromboelastometry, when performed in patients with established severe sepsis and septic shock cannot reliably detect activation of coagulation in the sense of a hypercoagulable state as suggested by in vitro or experimental studies.

Abbreviations

TEM: Thromboelastometry

CT: Clotting time

CFT: Clot formation time

MCF: Maximal clot firmness

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FD made substantial contributions to the design of the study, analysis and interpretation of the data and was involved in drafting the manuscript. UK, HF and JSL made substantial contributions to the thromboelastometric measurements, to the acquisition of the data and critically revised the manuscript for important intellectual content. JT critically revised the manuscript for important intellectual content. SMJ made substantial contributions to the concept and design of the study, to the analysis and interpretation of the data and was involved in drafting the manuscript. All authors read and approved the final manuscript.

Acknowledgments

We thank Natalie Araya, Judith Kaufmann and Alice Zosso for their assistance with data collection and technical help, and Jeannie Wurz for careful editing of the manuscript.

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Figure legends

Figure 1:Maximal clot firmness. *P<0.05, differences between groups. #P<0.05, difference from baseline value in the high SOFA group. Black circles, high SOFA group; white circles, low SOFA group.

Figure 2: Clotting time. Black circles, high SOFA group; white circles, low SOFA group.

Figure 3: Clot formation time. *P<0.05, differences between groups. #P<0.05, difference from baseline value in the high SOFA group. Black circles, high SOFA group; white circles, low SOFA group.

Figure 4: Alpha angle. *P<0.05, differences between groups. #P<0.05, difference from baseline value in the high SOFA group. Black circles, high SOFA group; white circles, low SOFA group.

Figure 5: Maximal clot firmness after platelet inhibition with cytochalasin D (FIBTEM), normal range 9-25 mm [30]. *P<0.05, differences between groups. Black circles, high SOFA group; white circles, low SOFA group.

Table 1: Characteristics of the study population

N	30
Age (years)	65 ± 13
Male sex	19
Source of infection	
Lungs	9
Catheter related	3
Endocarditis	1
Abdominal	6
Soft tissue/bone	6
CNS	3
Unknown/other	2
SAPS II	52.3 ± 14.6
APACHE II	29.0 ± 7.7
SOFA day 1	9.1± 4.0
SOFA day 2	8.3 ± 3.8
SOFA day 3	8.3 ± 4.2
SOFA discharge	6.5 ± 3.7
ICU Length of stay (days)	6 (3; 11)
ICU mortality (n; %)	6 (20)
28 day survival rate (n; %)	22 (73)

Characteristics of the study population. Number of patients (N), age (years), severity of illness scores, outcome, source of infection and length of stay (days) are depicted. Length of stay shown as median (25%/75% percentiles).

Table 2: Routine coagulation tests, clotting factor levels, hemoglobin, hematocrit, white blood count and CRP.

Mean/SD	Normal ranges	Day 1	Day 2	Day 3	Discharge
Platelets	140-380	191 ± 117	198 ± 135	196 ± 135	364 ± 201[a]
INR	0.9-1.15	1.18 ± 0.16	1.16 ± 0.18	1.12 ± 0.15	1.09 ± 0.14
aPTT	25-36	48.1 ± 11.4	48.8 ± 13.0	44.7 ± 11.2	39.8 ± 11.6[a]
Fibrinogen	1.3-3.6	6.2 ± 2.7	5.8 ± 1.6	5.5 ± 1.6	6.0 ± 4.4
Factor II	81-134	61 ± 22	64 ± 25	69 ± 23	76 ± 28[a]
Factor V	78-153	103 ± 45	110 ± 45	114 ± 43[a]	116 ± 33[a]
Factor VII	70-139	66 ± 29	71 ± 29	88 ± 30[a]	79 ± 29
Factor X	68-145	79 ± 29	81 ± 30	90 ± 22	84 ± 21
Hb	121-154	100.4 ± 14.3	98.8 ± 14.7	98.9 ± 11.6	102.2 ± 12.2
Hct	0.36-0.44	29.1 ± 4.4	29.1 ± 4.8	29.2 ± 3.8	30.4 ± 3.7
WBC	3.5-10.5	16.1 ± 7.5	17.4 ± 7.3	17.0 ± 6.4	16.6 ± 6.9
CRP	< 5	201 ± 87	202 ± 83	176 ± 94	113 ± 76[a]
Creatinine	59-104	172 ± 120	159 ± 113	171 ± 115	151 ± 126
Bilirubin	3-26	33.3 ± 34.3	37.0 ± 40.5	40.0 ± 46.2	32.8 ± 43.9

[a] $P<0.05$, difference from values at baseline. Platelets (G/L); INR, International Normalized Ratio; aPTT, activated partial thromboplastin time (sec); fibrinogen (g/L); coagulation factors (%); Hb, hemoglobin (g/L); Hct, hematocrit (%); WBC, white blood count (G/L); CRP, C-reactive protein (mg/L); creatinine (µmol/L); bilirubin (µmol/L).

Table 3: Results of thromboelastometry

	Normal ranges	0 h	12 h	24 h	36 h	48 h	discharge	Spearman r	Spearman *P*
CT-EXTEM	42-74	64.9 ± 7.6	66.0 ± 10.6	61.7 ± 9.6	63.7 ± 7.2	61.9 ± 7.3	57.4 ± 4.6[a]	0.09	0.36
CT-INTEM	137-246	195.9 ± 25.8	201.0 ± 31.4	199.8 ± 36.7	213.2 ± 41.1	216.3 ± 43.8	206.7 ± 40.6		
CT-HEPTEM	137-246	197.1 ± 39.3	217.2 ± 58.1	212.2 ± 47.7	212.4 ± 40.0	205.8 ± 29.6	192.8 ± 34.2		
CFT-EXTEM	46-148	97.0 ± 62.6	103.6 ± 79.2	99.3 ± 61.0	100.4 ± 64.8	94.5 ± 54.9	62.6 ± 30.7[a]	0.64	0.001
Alpha-EXTEM	63-81	73.9 ± 7.8	74.3 ± 7.1	74.0 ± 6.9	73.7 ± 6.8	74.5 ± 5.9	78.3 ± 4.1	-0.58	0.001
MCF-EXTEM	49-71	62.1 ± 11.2	62.1 ± 12.6	62.1 ± 12.1	62.1 ± 12.4	62.7 ± 11.6	67.4 ± 9.0[a]	-0.63	0.001
MCF-INTEM	52-72	63.7 ± 8.0	63.2 ± 7.6	61.9 ± 8.3	64.1 ± 7.7	64.6 ± 8.1	66.9 ± 8.4		
MCF-HEPTEM	52-72	61.0 ± 8.9	59.6 ± 8.0	59.1 ± 9.4	60.6 ± 9.2	61.5 ± 8.4	64.1 ± 7.8[a]		

EXTEM: Activation of coagulation with tissue factor, INTEM: Activation of coagulation with thromboplastin, HEPTEM: Inhibition of heparin with heparinase. CT, clotting time (sec); CFT, clot formation time (sec); alpha angle (°); MCF, maximal clot firmness (mm). [a]$P<0.05$, difference from values at baseline. Spearman rank correlation (r) is between the continuous values of SOFA score with TEM variables.

Figure 1. Maximal clot firmness

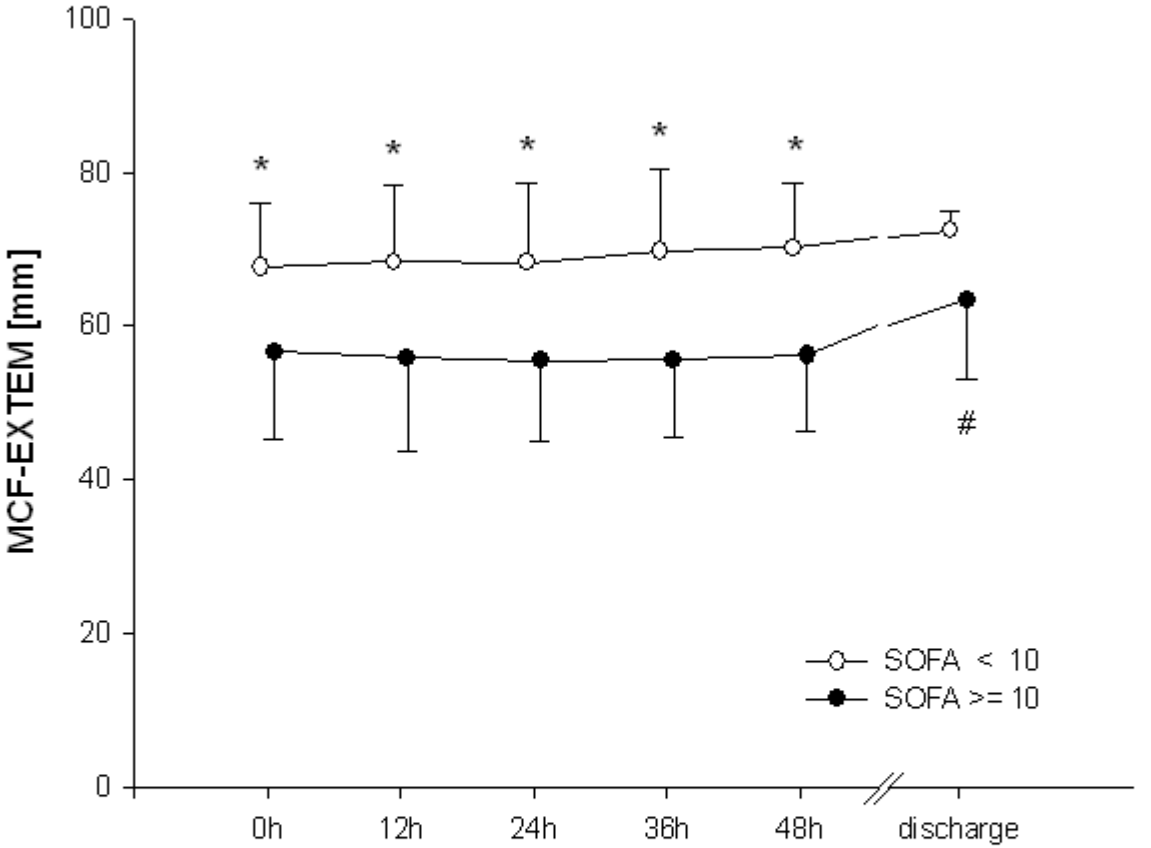

Figure 1

Figure 2. Coagulation time

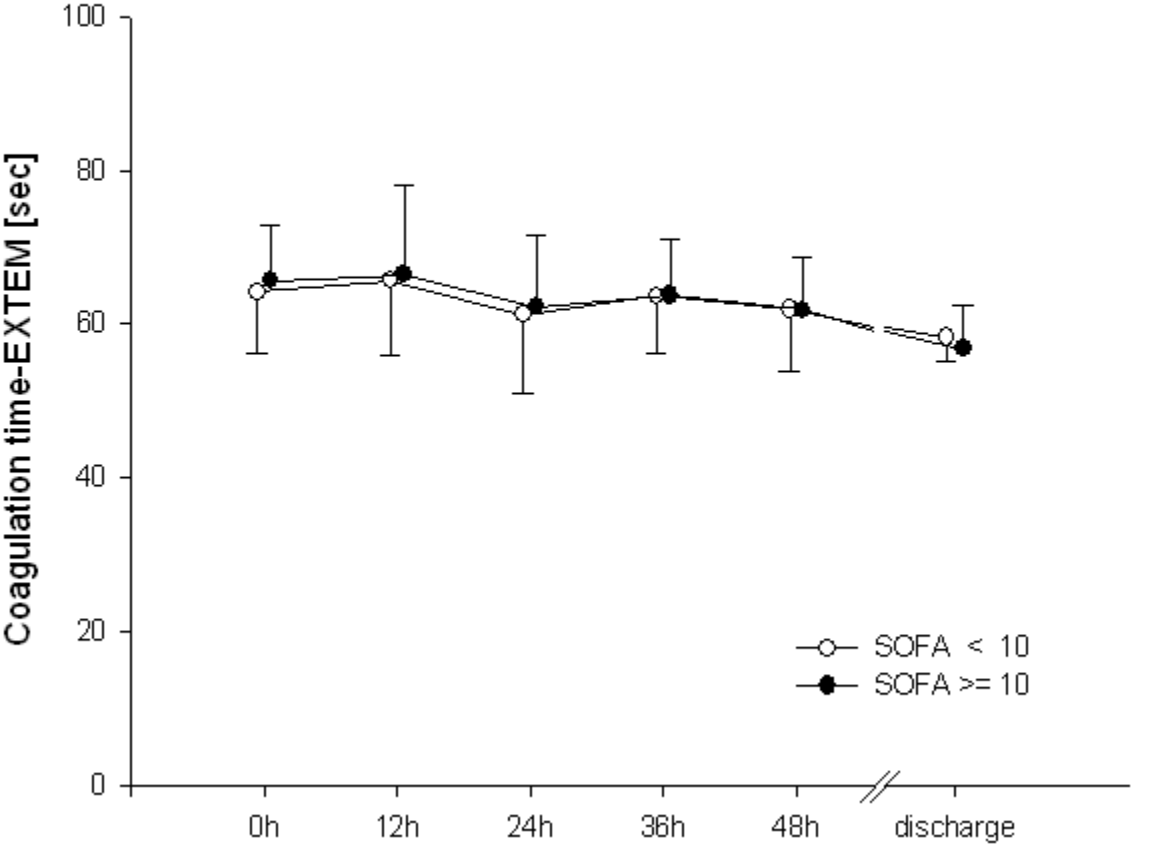

Figure 2

Figure 3. Clot formation time

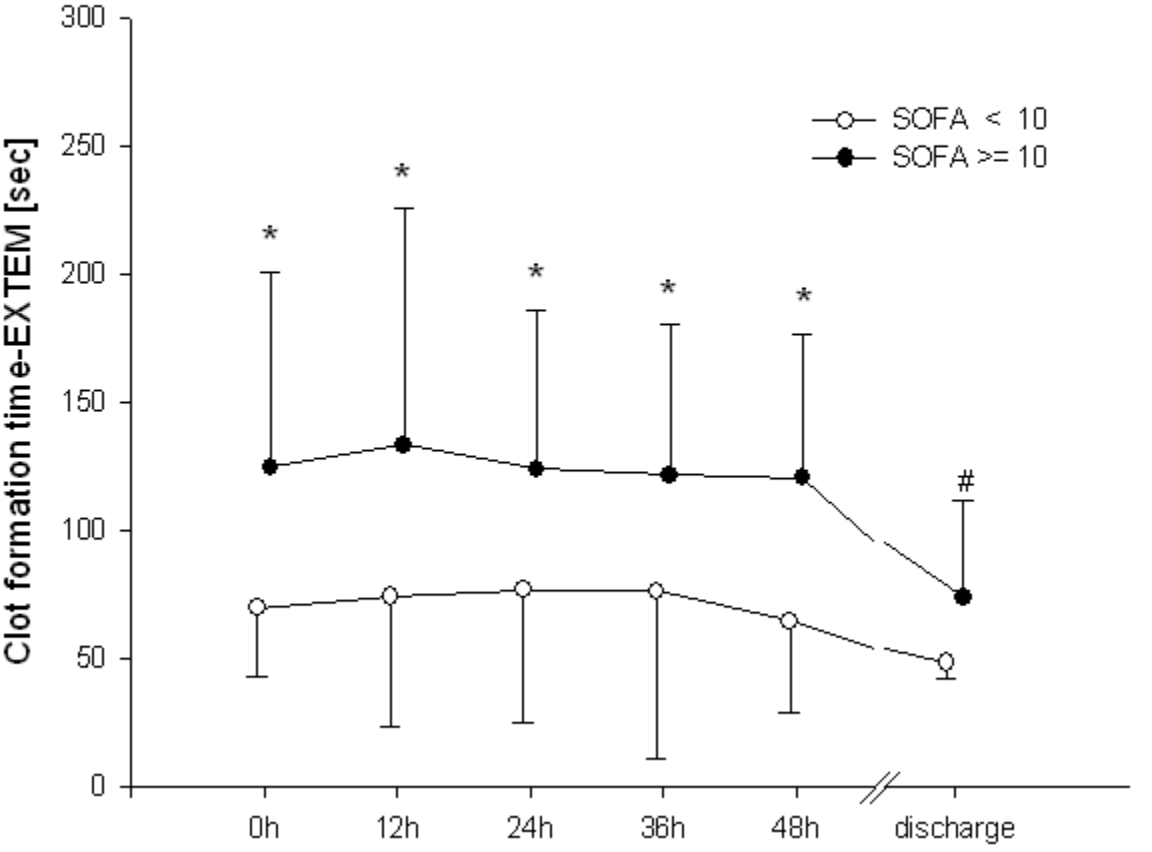

Figure 3

Figure 4. Alpha angle

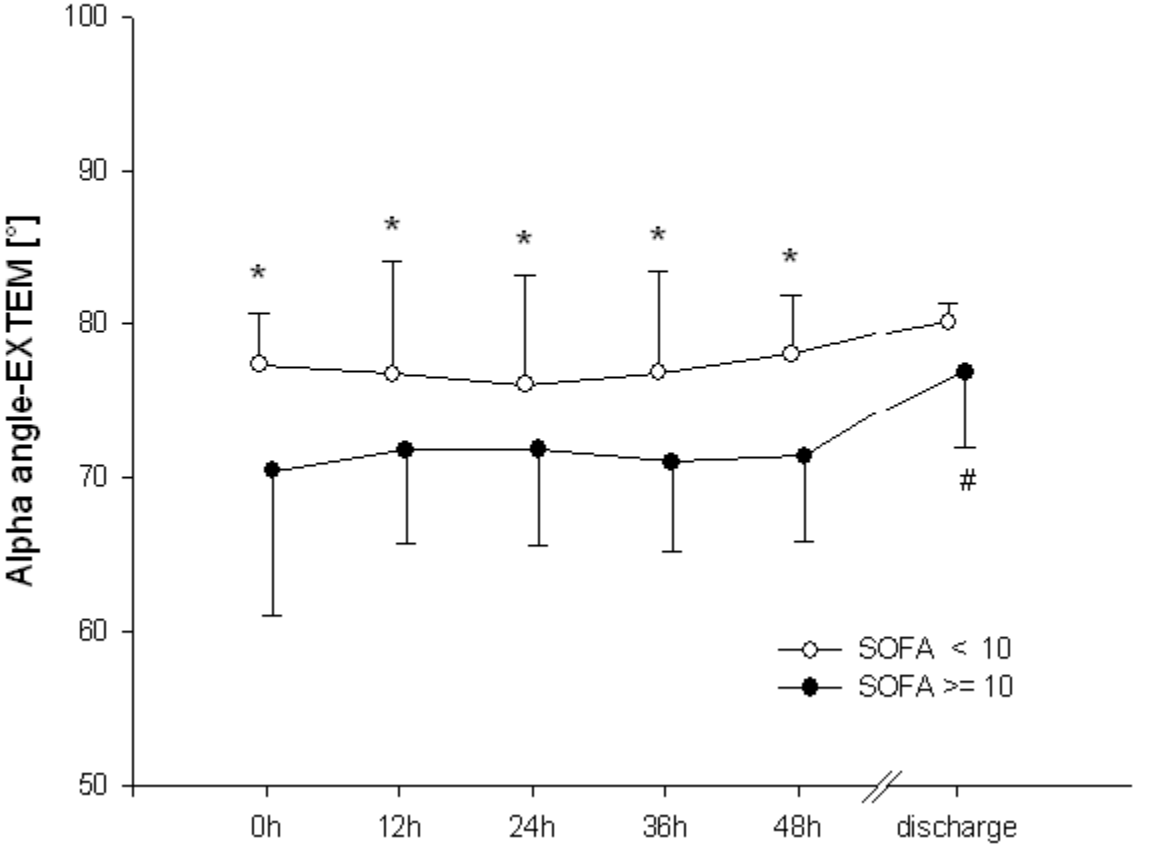

Figure 4

Figure 5. Maximal clot firmness after platelet inhibition

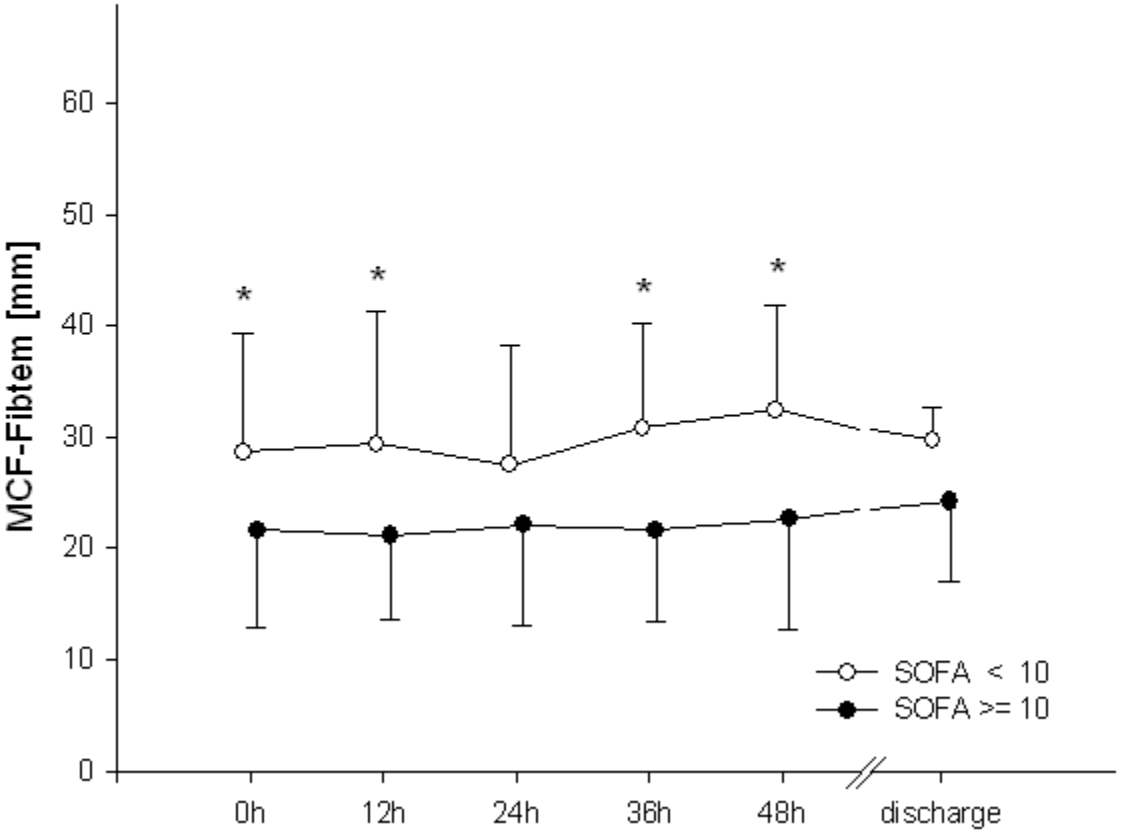

Figure 5