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Thrombin generation in patients with severe thermal injury☆

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ABSTRACT

Background: Severe burns can induce a hypercoagulable state which is not depicted in conventional coagulation assays. The thrombin generation assay allows global assessment of coagulation and can identify hypercoagulability. We report changes in thrombin generation in patients after severe burn injury.

Methods: We measured TGA, rotational thrombelastometry and conventional assays in 20 consecutive patients with a total body surface area burned of >20% over a 2-week period: the day after burn trauma (A), the morning after surgical excision of burn wounds (B) and on post-admission days 7 (C) and 14 (D).

Results: Thrombin generation assay showed a procoagulatory state: there was an increase in the velocity of thrombin generation (increase in time to peak of +13%, increase in velocity index of +22%), and peak amount of thrombin (+25%) between days A and B. All parameters reached their highest levels on day C and returned towards normal on day D. Rotational thrombelastometry showed a hypercoagulable state with an increase in clot firmness and alpha angle. Conventional coagulation tests remained within reference values.

Conclusions: In the first two weeks following burn, both the thrombin generation assay and rotational thrombelastometry show a hypercoagulable state, while conventional coagulation tests remain normal.

Abbreviations: aPTT, activated partial thromboplastin time; AUC, area under the curve; CFT, clot formation time; CO_2, carbon dioxide; CT, clotting time; ETP, endogenous thrombin potential; ICU, intensive care unit; MCF, maximum clot firmness; PRBCs, packed red blood cells; PT, prothrombin time; ROTEM, rotational thrombelastometry; TGA, thrombin generation assay.

☆ The study was performed at the Department of Anaesthesia, Critical Care and Pain Medicine of the Medical University of Vienna.

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1. Introduction

In patients with severe burn, changes in hemostasis result from trauma itself (e.g. tissue hypoperfusion, hypothermia, altered platelet function, systemic inflammatory response) and from therapeutic interventions (e.g. hemodilution, blood loss due to wound excision) [1–4]. Results of conventional coagulation assays measured in daily clinical practice [e.g. prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT), low platelet count] are often interpreted as coagulopathy implying a bleeding tendency [5]. But this does not correlate with clinical findings. Bleeding is more often due to surgical interventions rather than coagulopathy. Instead, thromboembolic events are frequent. The incidence of venous thromboembolism ranges from 0.2 to 25% in patients with burn injury [6,7].

Therefore, a more global assessment of hemostasis would be desirable. Viscoelastic coagulation assays such as rotational thrombelastometry (ROTEM) and thrombelastography (TEG) have been described in patients with thermal injury where they depict a hypercoagulable state [5,7–10]. However, evidence regarding the value of viscoelastic coagulation assays to diagnose hypercoagulability is limited [11,13]. Moreover – although there are cut off values to detect hypocoagulability measured viscoelastic coagulation assays – there is no consensus regarding hypercoagulability [5,14,15].

The thrombin generation assay (TGA) is a well-established method to ascertain a hypercoagulable state. It assesses an individual's potential to generate thrombin [13,16–20]. Altered levels of endogenous thrombin potential (ETP) have been reported in context of thromboembolic events, trauma, hepatic dysfunction but also under physiological conditions such as pregnancy [16–18,21–23]. Thrombin generation results in patients with severe thermal injury have not yet been reported.

The objective of this study was to prospectively describe changes in thrombin generation in patients after severe thermal injury.

2. Materials and methods

This prospective observational cohort study was performed at the intensive care unit (ICU) for burn trauma patients at the General Hospital of Vienna, Austria from November 2012 to May 2014. Twenty consecutive patients with a total body surface area burned of greater than or equal to 20% and burn-depth IIb-III were included in the study. Exclusion criteria were age <18 years and pre-existing bleeding disorders.

All patients were directly admitted via ambulance to the ICU for initial management. Escharotomy/fasciotomy was done immediately after admission if necessary. Necrosectomy was performed on day three after burn injury given hemodynamic stability and diminishing of edema. Fluid therapy was guided based on hemodynamic monitoring by transpulmonary thermodilution (PiCCO® Pulsion, Munich, Germany) using a balanced crystalloid (Elomel isoton®, Fresenius Kabi, Graz, Austria) and 20% albumin (Human Albumin Baxter® Vienna, Austria) from first day after trauma whenever albumin levels fell below 20 g/l. Administration of fresh frozen plasma, fibrinogen- and prothrombin complex concentrate was guided by conventional coagulation tests and clinical bleeding at the discretion of the treating intensivists. Patients received subcutaneous low molecular weight heparin (Enoxaparin, 40 mg Sanofi, Vienna Austria) twice daily during the first week after trauma.

2.1. Blood sampling and preparation

Blood samples were drawn from indwelling arterial or central venous catheters on four days during the study period. Day A: the first day after burn trauma and admission to the ICU; day B: the day after surgical excision of burn wounds; day C and D: one and two weeks after admission, respectively. All blood samples were drawn at the trough level of low molecular weight heparin. Samples for the Thrombin Generation Assay (TGA) were drawn in citrate-theophylline-adenine-dipyridamol test tubes (Vacuette™ Greiner, Kremsmünster, Austria) and centrifuged at 3500 rounds per minute for 15 min and stored at −80 °C for subsequent testing. Samples for conventional coagulation assays and ROTEM were drawn in tubes containing trisodium citrate 3.8% (Vacuette™ Greiner, Kremsmünster, Austria; 9:1 v/v). Samples for complete blood counts were drawn into EDTA Vacuette™ tubes (Greiner, Kremsmünster, Austria). Except for TGA all samples were analyzed immediately.

2.2. Thrombin generation assay (TGA)

Thrombin generation was determined using the automated coagulation analyzer Ceveron® alpha TGA (Technoclone, Vienna, Austria). Coagulation is initiated by adding calcium chloride, the reagent C high (TGA RC high; Technoclone, Vienna, Austria) and phospholipids to plasma. The amount of thrombin generated correlates to the fluorescence emitted by the cleavage of a fluorogenic substrate by thrombin (TGA substrate, Technoclone, Vienna, Austria). The concentration of thrombin is calculated using a calibration curve and plotted against time, resulting in a thrombin generation curve: after an initial lag phase (lag time, given in minutes), thrombin is formed in the production phase, reaching a peak level (peak thrombin, given in nM thrombin and time to peak, given in minutes), after which the concentration of thrombin decreases. The velocity index of thrombin generation is defined as peak thrombin/(time to peak − lag time) [18]. The area under the thrombin generation curve is defined as the endogenous thrombin potential (AUC, given in nM thrombin) (Fig. 1).

2.3. Rotational thrombelastometry (ROTEM)

ROTEM tests (TEM Innovations, Munich, Germany) were performed in citrated whole blood in the ICU point of care laboratory according to the manufacturer's instructions. For EXTEM, 300 μl of citrated whole blood were mixed with 20 μl of the EXTEM reagent and coagulation was started with 20 μl $CaCl_2$ 0.2 mmol/l. All samples were prepared with the automated pipette integrated in the ROTEM, which allows accurate dosage of volumes between 20 and 300 μl. ROTEM

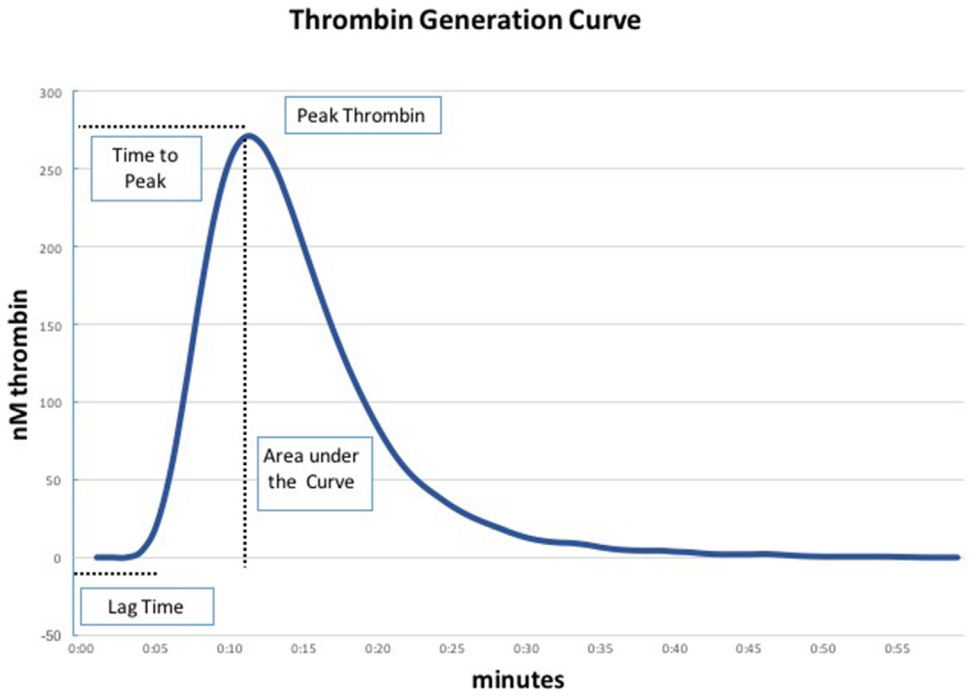

Fig. 1 – Thrombin generation curve.

quality control measurements were performed once weekly as recommended by the manufacturer. The main endpoints were clot formation time (CFT), clotting time (CT), maximum clot firmness (MCF), and the α-angle.

2.4. *Conventional coagulation assays*

The following coagulation tests were assessed from citrated plasma: prothrombin time (PT, given as percent of normal, reference value 75–140%), activated partial thromboplastin time (aPTT, given in seconds, reference value 27–41 s), fibrinogen level (Clauss method, given in mg/dl, reference value 180–390 mg/dl), antithrombin activity (AT, given in percent of normal, reference value 70–120%). All tests were performed using the STA-R Evolution® coagulometer (Diagnostica Stago S.A.S., Asnières sur Seine, France). Complete blood counts were assessed with a Sysmex XE- 2100 cell counter (Sysmex, Kobe, Japan).

2.5. *Patient data*

On admission, demographic data, total body surface area burned and the abbreviated burn severity index were recorded. For each study day, heart rate, arterial blood pressure, cardiac index (measured by PiCCO Pulsion, Munich, Germany) and the dose of norepinephrine and dobutamine were recorded. The administration of packed red blood cells (PRBCs), fresh-frozen plasma, and platelet concentrates as well as the administration of fibrinogen- and prothrombin complex concentrate and antithrombin since admission or the previous study day were also documented.

2.6. *Control group*

Given that no reference ranges for TGA exist so far, 19 patients scheduled for elective surgery served as a control group. The age of the control patients was matched to the age of the thermally injured patients. After informed consent was obtained, blood samples for routine coagulation testing, ROTEM and TGA tests were drawn from the cubital vein without stasis using a 21-gauge butterfly needle at time of presentation to the preoperative clinic. Exclusion criteria were American Society of Anesthesiologists physical status classification III or above and anticoagulant therapy.

2.7. *Statistical methods*

Normally distributed data is reported as mean (standard deviation, SD); non-normal data are reported as median [interquartile range (IQR)]. A significance level of $p<0.05$ was considered significant. Time series are plotted for variables of TGA [lag time, time to peak thrombin, peak thrombin, area under the curve (pursuant to the endogenous thrombin potential, ETP)] and velocity index, and ROTEM (CFT, CT, MCF and α-angle).

To model initial changes of variables and subsequent return to primary values, a quadratic regression on time was performed, and linear and quadratic regression coefficients are given together with unadjusted p-values. One way ANOVAS with post-hoc Dunnett tests were performed to compare measurements in patients to the control group.

Due to the explorative character of the study no adjustment for simultaneous testing of multiple hypotheses was

performed. Data was analyzed using Microsoft Excel for Mac, IBM SPSS Statistics for Mac, Version 22, R 3.3.2 and R Studio 1.0.143.

2.8. Ethics approval and consent to participate

The study has been approved by the appropriate institutional ethics committee [EK 1216/2012] and registered at the German Clinical Trials Register (DRKS00004369). Informed consent was obtained from all individual participants included in the study. All procedures have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

3. Results

3.1. Patient characteristics

Out of 22 eligible patients, 20 patients (13 male, 7 female) were included in the study (Fig. 2). Two patients were excluded: one patient participated in another clinical trial and one patient was treated with an extracorporeal veno-venous CO_2 removal system requiring systemic anticoagulation. The mean age was 52.5 years (SD±19.1), the median of burned total body surface area was 33% (IQR: 20–48) and the mean abbreviated burn severity index score was 8 (SD±2). Two patients died during the 14-day observation period. The 30-day mortality was 20%.

3.2. Thrombin generation assay

Time to peak, peak thrombin, and velocity index changed over time: The time to peak decreased by 13% between study day A and B and returned to 94% of the day A value on day D. Regression on time revealed a linear decrease (estimate=−0.34, p=0.013) and a quadratic increase (estimate=0.02, p=0.012). Peak thrombin and velocity index increased from study day A to B by 25% and 22% respectively. Both reached a peak on study day C and decreased on day D to 120% of their day A value. A linear increase (peak thrombin: estimate=35.5, p=0.003, velocity index: estimate=10.4, p=0.021) and quadratic decrease (peak thrombin: estimate=−2.0, p=0.08, velocity index: estimate=−0.6, p=0.031) were observed. No changes in lag time or area under the curve were observed ($p>0.05$). The time course for the individual TGA parameters is given in Fig. 3.

Compared to the control group, time to peak was lower ($p<0.001$ for each day in the patient group vs. control group), and peak thrombin (p=0.001 for days B, C, D), velocity index (p=0.004 for days B, C, D) and the area under the curve (p=0.024 for days C, D) were higher in the patient group. There was no difference in lag time between patients and the control group (p=0.304).

3.3. Rotational thrombelastometry

Clot formation time decreased by up to 50% between study days A and D, (estimate=−7.8, $p<0.001$) with a positive quadratic (estimate=0.3, p=0.016) regression coefficient on time. There was no change in CT during the observation period ($p>0.05$). Maximum clot firmness increased by up to 20% between study days A and D, with a positive linear (estimate=3.0, $p<0.001$) and a negative quadratic (estimate=−0.1, $p<0.001$) regression coefficient on time. Alpha angle increased by up to 12% between study days A and D, with a positive linear (estimate=1.7, $p<0.001$) and a negative quadratic (estimate=−0.1, $p<0.001$) regression coefficient on time. The time course of EXTEM parameters is given in Fig. 4.

When compared to the control group, CFT, MCF and α-angle were lower in the patient group on study days C and D ($p<0.001$, $p<0.001$ and $p<0.001$). There was no difference in CT between the patient and control group (p=0.13).

3.4. Conventional coagulation tests

Prothrombin time, fibrinogen level and AT changed over time: all 3 parameters reached their peak levels on study day C. On study day C, PT, fibrinogen levels and AT had increased by 64%, 140% and 50% respectively when compared to study day A. On study day D, both PT and fibrinogen levels decreased towards normal levels (15% and 100% increase when compared to day A, respectively) while there was decrease in AT between days C and D. (Positive linear regression coefficients (PT: estimate=3.7, p=0.015, fibrinogen: estimate=130, $p<0.001$ and

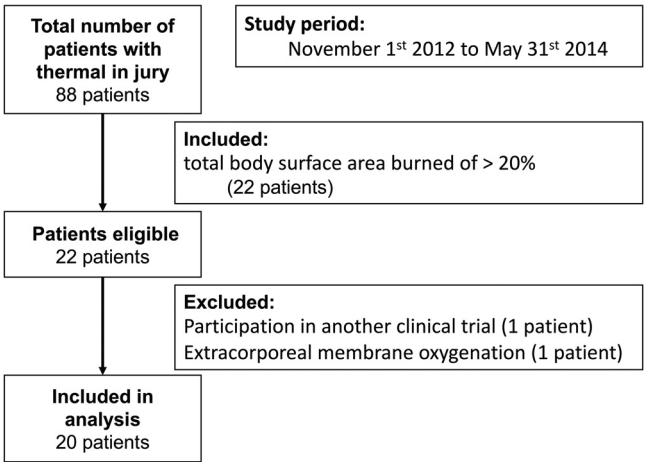

Fig. 2 – Strengthening reporting of observational studies in epidemiology — flow diagram of patients included and excluded from the study.

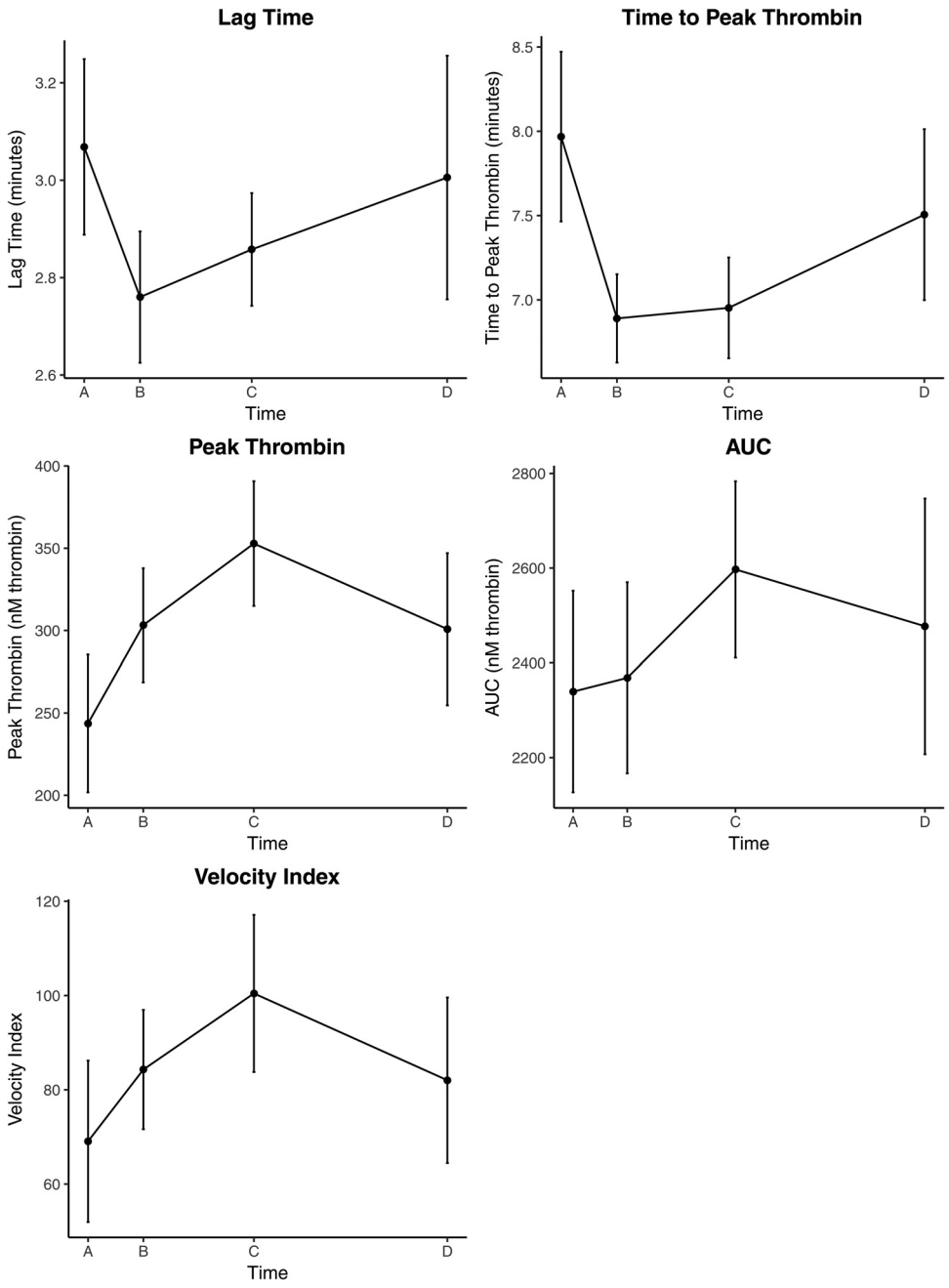

Fig. 3 – Time course of thrombin generation parameters during the 14-day study period (A) the first day after burn trauma and admission to the ICU; (B) the day after surgical excision of burn wounds; (C) one week after admission; (D) two weeks after admission.

AT: estimate=9.0, $p<0.001$) and negative regression coefficients (PT: estimate=−0.179, p=0.06, fibrinogen: estimate=−7.0, $p<0.001$ and AT: estimate=−0.4, $p<0.001$)).

No changes in aPTT were over time observed. Results of the conventional coagulation assays remained within the reference ranges (Table 1).

3.5. *Administration of blood products and factor concentrates*

Six patients received fresh frozen plasma on one or more days during the study period (Table 1). Fresh frozen plasma and PRBCs were always administered simultaneously with a mean ratio of 0.7 units of fresh frozen plasma per unit of PRBC.

One patient received prothrombin complex concentrate on two separate days: on the day of admission (PT: 45%, no relevant bleeding) and on the day of necrosectomy (PT of 21% with concomitant transfusion of PRBCs).

4. Discussion

We report changes in thrombin generation in a prospective cohort of 20 patients after severe thermal injury.

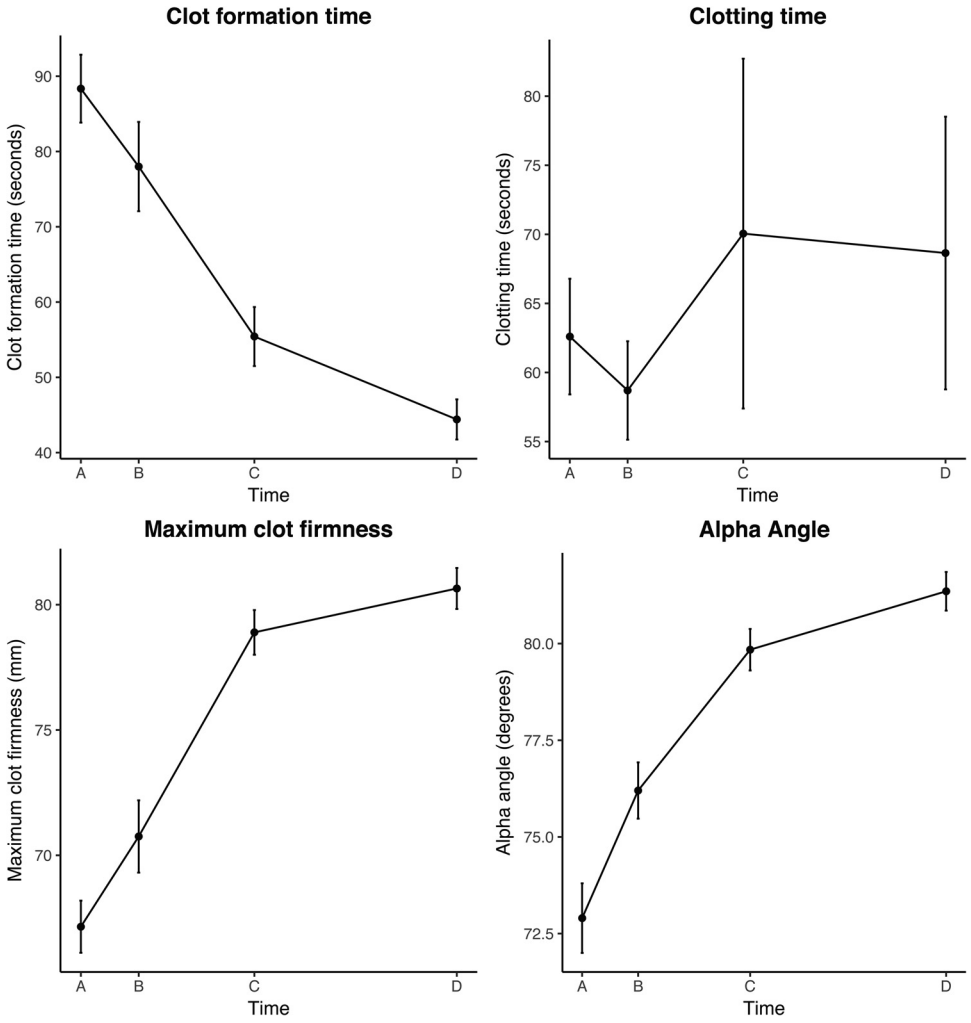

Fig. 4 – Time course of ROTEM EXTEM parameters during the 14-day study period (A) the first day after burn trauma and admission to the ICU; (B) the day after surgical excision of burn wounds; (C) one week after admission; (D) two weeks after admission.

In summary, thrombin generation parameters showed a change towards a procoagulatory state during the first two weeks after severe burn injury: the velocity of thrombin generation (measured by time to peak and velocity index) and the peak amount of thrombin increased over time. Time to peak as well as peak thrombin reached their highest levels one week after burn injury. Two weeks after the trauma, the values returned towards normal. Compared to the control group, TGA parameters showed increased thrombin generation (shorter time to peak and higher peak thrombin, velocity index and AUC). These results confirm the previously described prothrombotic shift in the first week after burn injury [1,10,24].

Thrombelastometry results showed a similar shift towards a procoagulatory state. Both the increase in α-angle and MCF met the criteria of hypercoagulability as defined by Kaufmann et al. [5,14].

Prothrombin time, aPTT and platelet count remained within their reference ranges. These conventional coagulation assays therefore were unable to identify a hypercoagulable state. Fibrinogen levels were significantly increased when compared to the control group throughout the observation period. This has been described previously and corresponds to increased FIBTEM-MCF in ROTEM [8]. Our findings are in line with the current literature, and confirm the limited diagnostic value of PT and aPTT to detect hypercoagulopathy [1,3,9,11,25,26]. In contrast to TGA, conventional coagulation assays evaluate the initial phase of thrombin generation. Noteworthy, 95% of thrombin is generated after PT and aPTT times are registered. This thrombin effect remains undetected by conventional coagulation assays [26].

However, despite growing evidence of their important limitations, conventional coagulation assays are still used in daily clinical practice to evaluate hemostasis [9,19]. A recent published survey pointed out that the majority of specialists worldwide still use conventional coagulation tests to assess coagulopathy in burn patients [27].

Clinically relevant differences between conventional coagulation assays and results of TGA have been shown more than 10 years ago in patients with chronic liver disease [28]. By definition of conventional coagulation assays, patients with liver disease might be viewed as “autoanticoagulated”. But TGA showed a balanced- or an even procoagulatory state in

Table 1 – results of routine coagulation tests, consumption of blood products and coagulation factors.

Study day	Day A	Day B	Day C	Day D
Patients observed (n)	20	20	19	18
Days after trauma	1 (1–1)	4 (3–5)	8 (8–9)	15 (14–17)
Laboratory tests				
PT (%)	78.3±18.9	80.6±16.9	91.7±16.0	89.6±22.8
aPTT (seconds)	34.9 (30.8–38.6)	38.6±4.9	38.3±5.3	40.2±9.0
Fibrinogen (mg/dl)	340.0 (247.0–401.0)	561.6±208.1	780.1±184.4	668.9±246.5
Hemoglobin (g/dl)	9.6 (8.7–11.1)	9.0 (7.9–9.5)	11.5 (8.4–14.4)	10.1 (8.7–12.5)
Platelets (G/l)	247.8±179.4	311.8±214.0	252.7±182.5	213.6±115.3
Antithrombin (%)	66.9±17.2	72.7±11.7	101.2±11.5	100.1±12.9
Hemodynamics				
Heart rate (beats per minute)	92.4±13.5	88.7±14.2	86.7±15.2	88.3±19.7
Mean arterial blood pressure (mmHg)	79.5±11.8	81.8±17.3	80.6±15.6	79.1±15.1
Cardiac index (l/min/m^2)	4.1±1.3	4.3±1.0	3.8±1.7	3.5±0.9
Norepinephrine infusion (percent)	30%	40%	37%	33%
Dobutamine infusion (percent)	15%	5%	21%	6%
Blood products & coagulation factors				
PRBCs transfused (units) 0/1–2/>2	19/1/0	9/6/5	11/5/3	9/2/7
Fresh-frozen plasma (units) 0/1–2/>2	20/0/0	15/2/3	17/2/3	15/2/1
Platelet concentrates (units) 0/1–2/>2	20/0/0	20/0/0	18/1/0	18/0/0
Fibrinogen concentrate[a]	1 (5%)	2 (10%)	0 (0%)	1 (6%)
Prothrombin complex[a]	1 (5%)	1 (5%)	0 (0%)	0 (0%)
Antithrombin[a]	1 (5%)	2 (10%)	0 (0%)	0 (0%)

Data are presented as mean±SD, median (IQR) or count (%); PT, prothrombin time; aPTT, activated partial thromboplastin time; PRBCs, packed red blood cells.
[a] Patients receiving at least one dose.

patients with chronic as well as acute liver failure [29,30]. Consequently, procoagulatory therapy, such as the administration of fresh frozen plasma or coagulation factor concentrates should be restricted to only treat clinically relevant bleeding, rather than for the sole purpose of correcting of laboratory findings [22]. Moreover, it has been shown that patients even benefit from anticoagulation. Villa et al. pointed out the importance of pharmacological anticoagulation [31]. They demonstrated the effectiveness and safety of enoxaparin in preventing portal vein thrombosis in patients with acute liver failure. The administration of the low molecular weight heparin appeared to delay the occurrence of hepatic decompensation and to improve survival.

Recently, Schöchl et al. used TGA in trauma patients and showed an increase in endogenous thrombin potential for several days in patients who received prothrombin complex concentrate concentrates [25]. Again, this change was not reflected by conventional coagulation parameters.

Nevertheless, this knowledge has not been implemented in everyday clinical practice yet. Transfusion of fresh frozen plasma occurs in 41% of patients admitted to the intensive care unit without any bleeding event [32]. Administration of fresh frozen plasma and fibrinogen concentrate due to abnormal conventional coagulation assays has also been reported in burn patients [8]. In contrast, the implementation of a perioperative, ROTEM based treatment algorithm could reduce the use of allogeneic blood products during surgical excision of burn wounds [33].

Prophylactic administration of blood products and coagulation concentrates as well as withholding of anticoagulation can lead to life-threatening thromboembolic events. Therefore, a goal-directed approach to substitution of coagulation concentrates guided by viscoelastic tests (ROTEM, TEG) is reasonable [30,34,35].

In our study, the increased procoagulatory potential seen in TGA was also reflected in ROTEM. This differs from previous reports in patients with liver disease, where viscoelastic tests showed conflicting results. In patients with acute liver failure, TEG results were normal or hypercoagulable in the majority of patients but showed a hypocoagulable state in 20% of patients [36]. Park et al. reported a hypercoagulable state regarding results of TEG analysis in burn and non-burn trauma patients [11].

To date, there are only a few studies investigating viscoelastic tests in the context of burn injury [5,10]. We could previously show that fibrinogen levels increase over time in burned patients and contribute to a hypercoagulable state [8]. These results are in line with another study by Van Haren et al. which confirms the trend towards hypercoagulability in the first week after burn trauma [10].

Both TGA and viscoelastic tests allow a more comprehensive assessment of hemostasis. But although TGA is of great interest in the context of academic research, it has not yet been implemented in daily clinical practice. This is mostly due to the limited availability and long turnaround times of TGA assays. Until TGA becomes more widely available, viscoelastic tests (ROTEM, TEG) could serve as a substitute to detect hypercoagulability at the point-of-care [11–13].

Some limitations of this study need to be considered: first, the small sample size makes it difficult to conclude on clinical

outcomes. However, the patient number is within the range of comparable studies on hemostasis in burn patients [1,8,10]. Second, although all samples were drawn at the through level of low molecular weight heparin, we cannot rule out a residual effect of the anticoagulant on thrombin generation test result. Third, we did not rule out subclinical thrombosis. Although compression ultrasound of the lower extremities to detect potential thromboembolic events was part of our initial study protocol, this was not feasible due to burned skin area, wound dressings and indwelling catheters

5. Conclusions

In summary, results of thrombin generation assay (TGA) demonstrate a procoagulatory shift in patients with severe burn injury in the first two weeks following trauma. Further studies correlating TGA results to the incidence of clinical events are warranted to individualize pro- and anticoagulant therapy in this high-risk patient population.

Declaration of interests

DA has received a speaker's fee for lecturing from CSL Behring.

MW has received travel reimbursement and speaker's fees for lecturing from Boehringer Ingelheim and CSL Behring.

There is no conflict of interest of any of the other authors.

The authors did not receive any financial compensation.

Author's contributions

DA: patient recruitment, data collection, data analysis, prepared the final manuscript.

MW: patient recruitment, data collection, data analysis, prepared the final manuscript.

CK: data analysis.

DB: patient recruitment, data collection and prepared the final manuscript.

ES: patient recruitment, data collection, data analysis, prepared the final manuscript.

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