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Analysis of factor XIa, factor IXa and tissue factor activity in burn patients

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Abstract

Introduction—An elevated procoagulant activity observed in trauma patients is, in part, related to tissue factor (TF) located on blood cells and microparticles. However, analysis of trauma patient plasma indicates that there are other contributor(s) to the procoagulant activity. We hypothesize that factor (F)XIa and FIXa are responsible for an additional procoagulant activity in burn patients.

Methods—Multiple time-point plasma samples from 56 burn patients (total number of samples was 471; up to 20 time-points/patient collected in 3 weeks following admission) were evaluated in a thrombin generation assay using inhibitory antibodies to TF, FIXa and FXIa.

Results—Due to the limited volume of some samples, not all were analyzed for all three proteins. At admission, 10 of 53 patients (19%) had active TF, 53 of 55 (96%) had FXIa and 48 of 55 (87%) had FIXa in their plasma. 34 patients of 56 enrolled (61%) showed TF activity at one or more time-points. All patients had FXIa and 96% had FIXa at one or more time-points. Overall, TF was observed in 99 of 455 samples analyzed (22%), FXIa in 424 of 471 (90%) and FIXa in 244 of 471 (52%). The concentration of TF was relatively low and varied between 0 and 2.1 pM, whereas that of FXIa was higher, exceeding 100 pM in some samples. The majority of samples with FIXa had it at sub-nanomolar concentrations. No TF, FXIa and FIXa activity was detected in plasma from healthy individuals.

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Authors' contributions

JWS took care of patients, provided patient plasma samples and clinical data; SMP participated in analyzing plasma samples, in data analysis and in writing the manuscript; DYJ and LTM participated in collecting patient samples and obtaining clinical characteristics; KGM participated in conceiving the study and in editing the manuscript; SB conceived the study, participated in sample and data analysis and in writing and editing the manuscript.

Competing interests

K. G. Mann is a consultant for Baxalta and is the Chairman of the Board of Haematologic Technologies Inc. He reports fees from Bayer, Biogen IDEC, CSL Behring, Merck, Pfizer, Stago, The Medicines Company, Vascular Solutions and XO1, outside the submitted work. Other authors declare no competing interests.

Conclusions—For the first time reported, the majority of plasma samples from burn patients have active FXIa and FIXa, with a significant fraction of them having active TF. The concentration of all three proteins varies in a wide range.

Keywords

Burn trauma; Factor XIa; Factor IXa; Tissue factor; Thrombin generation

1. Introduction

One of every 11 deaths worldwide occur as a result of injury, leading to more than 5 million deaths per year. That is almost 1.7 times greater than combined fatalities from HIV/AIDS, tuberculosis and malaria [1]. Approximately 5% or more than 300,000 of these injury-related deaths are caused by fire-related burns, although the vast majority of burns are not fatal. It has been estimated that in the US there are approximately 0.5 million people treated for thermal injuries every year, with 40,000 of them being hospitalized [2]. In addition to direct damage to the body surface caused by the burn, there several other risk factors increasing morbidity and mortality of patients with thermal injuries. One of such factors is inhalation of smoke or products of combustion, which often cause a long-lasting respiratory injury. An incidence risk of this injury type increases with an increase in total body surface area damaged by a burn [3]. Two other risk factors are related age and gender, with elderly males showing the highest mortality rate [4, 5].

It has been also observed in multiple studies that burns are, in general, hypercoagulable. An elevated procoagulant activity could be related to a partial activation of coagulation proteins [6] do to the tissue factor (TF) exposure upon the vasculature and tissue injury. Additionally, a depletion of natural anticoagulants in trauma patients [7] together with increased levels of fibrinolysis inhibitors [8] would further exaggerate the procoagulant response and would increase clot stability [9].

In our previous studies we observed the presence of factor (F)XIa, FIXa and TF in plasma of patients with cardiovascular and inflammatory diseases [10–13]. The concentration of these proteins correlate with the severity of the disease and potential outcomes for the patients [10, 14]. We also observed that the frequencies of these 3 proteins correlate with trauma severity and shock in patients with blunt and penetrating injuries [15]. Based on these data, we hypothesized that FIXa, FXIa and TF could be present in burn patient plasma and that their presence/concentrations could correlate with burn severity. To test the hypothesis, we quantitated these 3 proteins in 471 plasma samples from burn patients.

2. Methods

2.1. Study subjects

Fifty six burn patients (43 male and 13 female were recruited at the Burns/Trauma Section, Department of Surgery, MedStar Washington Hospital Center and their informed consent was obtained. The age of patients varied between 18 and 77 years (median 37 years). Total

body burn surface varied between 0.2 and 97%. Forty eight patients survived and 8 deceased.

2.2. Materials

Citrate platelet-poor plasma (PPP) was prepared in-house using 10 healthy donors. Trypsin inhibitor from corn (CTI; prevents contact pathway initiation of coagulation) was prepared as previously described [16]. Phospholipid vesicles (PCPS) composed of 25% dioleoyl-*sn*-glycero-3-phospho-L-serine and 75% of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (both from Avanti Polar Lipids, Inc; Alabaster, AL) were prepared as described previously [17]. Inhibitory monoclonal anti-TF (αTF-5; prevents binding of TF to FVIIa), anti-FXIa (αFXI-2; inhibits FIX activation by FXIa) and anti-FIXa (αFIX-91; inhibits FX activation by FIXa) antibodies were produced and characterized in-house [18, 19]. The fluorogenic substrate used was benzyloxycarbonyl-Gly-Gly-Arg-7-amido-4methylcoumarin• HCl (Z-GGR-AMC) (Bachem, Torrance, CA, USA). TF, FIXa and FXIa activity in plasma was calculated from calibration curves developed with human FIXa, human FXIa or relipidated $TF_{1–263}$ (all from Haematologic Technologies, Inc., Essex Junction, VT). Human thrombin was produced in-house. Buffer was prepared using N-[2-hydroxyethyl]piperazine-NN-[2-ethanesulfonic acid] (HEPES) and NaCl (Fisher Scientific) (HBS; 20 mM HEPES, 0.15 M NaCl, pH 7.4). $CaCl_2$ used was from Fisher Scientific. The assay was performed in untreated, polystyrene 96-well plates (Costar, Lowell, MA, USA). The plate reader used was the BioTek Synergy 4 and analysis was performed using the Gen5 plate reader software (BioTek, Winooski, VT).

2.3. Blood Sample Collection and Citrate Plasma Preparation

Multiple time-point blood samples were collected into citrate tubes (9:1, 3.2% citrate) at 0, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 336 and 504 hours of admission (up to 20 samples per patient). All samples were taken from an arterial or venous line inserted for standard clinical care. Citrate blood-containing tubes were centrifuged immediately at 3,000g for 15 min at room temperature. Citrate plasma was frozen and stored at −80°C until assayed.

2.4. Thrombin generation assay (TGA) for FXIa, FIXa and TF

The assay is based on the response of thrombin generation in contact pathway-inhibited (CTI) plasma to the addition of inhibitory monoclonal antibodies to FXIa, FIXa and TF. Citrate plasma samples were thawed at 37°C for 3 min and 5 mg/mL CTI was immediately added to achieve a 0.1 mg/mL final concentration. 80 μL of each plasma sample was added to a 96-well plate and inhibitory monoclonal antibodies (anti-TF, anti-XIa and anti-IXa) were added (when desired) to achieve a 0.1 mg/mL final concentration. 20 μL of 2.5 mM Z-GGR-AMC substrate/90 mM $CaCl_2$ solution was added to plasma samples to achieve a 417 μM/15 mM final concentrations and subsequently incubated at 37°C for 3 min to allow recalcification of the plasma. 20 μL of PCPS solution was then added to achieve a 20 μM final concentration, thus initiating thrombin generation and fluorescence readings began immediately. Hydrolysis of the AMC substrate was followed over a 3600 s period and converted to thrombin concentration using a calibration curve built by sequential dilutions of human thrombin. Concentrations of TF, FXIa and FIXa were calculated from corresponding

calibration curves constructed by titrating purified proteins into multi-donor pooled plasma from healthy individuals.

3. Results

3.1. Patients

Median time for burn wound excisions in this cohort was 50 hours. Eight patients in the cohort developed sepsis and the average number of grafting procedures in this group was 1.6 surgeries. 10 patients received transfusion of blood products and the average transfusion amount for this subgroup was 23 units. Only 3 patients in this cohort developed a VTE and it is not correlative with factor levels. Fifteen of 56 patients enrolled into study had an inhalation injury in addition to the burn. Eight of those patients deceased (7 male and 1 female). No mortality occurred among patients without the inhalation injury. Patients with an inhalation injury had a substantially higher TBSA (58.9% ± 33.3%) than those without such injury (7.3% ± 6.9%). There also was a substantial difference in TBSA between deceased patients with inhalation (79.1%) and those who survived (35.9%). An analysis of Baux score for the entire study population showed that for deceased subjects this score was on average 125.9, whereas for survivors it was 49.8. The major contributor leading to this difference was the TBSA, because the median age of survivors and deceased was similar (37 and 44 years, respectively). Even more pronounced difference between deceased and survivors was observed when a more complex FLAMES PD score was compared. For the survivors, this score was 3.7%, whereas for deceased patients it was almost 20-fold higher (71.4%).

3.2. Quantitation of endogenous FXIa, FIXa and TF activity in TGA

With no exogenous activator of blood coagulation added, recalcified CTI-containing citrate plasma from healthy individuals (multi-donor or from individual donors) does not generate any detectable concentrations of thrombin in this assay over a period of 60 min, i.e. during the duration of the assay (Fig. 1). In contrast, the vast majority of CTI citrate plasma samples from burn patients produce relatively high concentrations of thrombin at identical conditions. For example, in plasma sample from patient A depicted in Fig. 1, thrombin generation in the absence of any exogenous activator starts at 709 s, proceeds at a high rate of 6.2 nM/s and the concentration of active thrombin reaches as high as 478 nM. For comparison, thrombin generation in normal plasma triggered with 5 pM relipidated TF occurs at a 1.6 nM/s rate after a lag phase of 501 s and the maximum concentration of active thrombin reaches only 83 nM. For the 55 samples taken at admission, there was a median lag phase of 735 s (ranging from 254 s to >3600 s) and a median peak thrombin value of 320 nM (ranging from 0 nM to 662 nM). Similarly, for the entire 471 samples analyzed, there was a median lag phase of 995 s (137 s to >3600 s) and a median peak thrombin value of 422 nM (0 nM to 984 nM).

The quantitation of all 3 proteins in plasma was based on the prolongation of the lag phase of thrombin generation to monoclonal inhibitory antibodies against each protein. Titration of purified FXIa or FIXa into multidonor plasma showed that the lag phase shows a linear response (on a log-log scale) to the exogenous protein added. For the relipidated TF added, a

linear response was observed on a semi-log scale. An addition of αTF-5 to burn patient A plasma presented in Fig. 1 prolongs the lag phase from 709 s to 930 s. Using the calibration curves, this shift in the lag phase corresponds to an active TF concentration of 2 pM. Similarly, an addition of αFXI-2 prolongs the lag phase from 709 s to 2126 s. Based on the calibration curve, this prolongation corresponds to 13.4 pM endogenous FXIa in this plasma sample. Furthermore, an addition of αFIX-91 completely abolishes thrombin generation, indicating (from the FIXa calibration curve) that this plasma sample contains 70 pM FIXa.

3.3. Frequency of FXIa, FIXa and TF in burn patients

Plasma samples from 53 burn patients were collected at admission and analyzed for the presence of TF. Of those samples obtained at admission, 10 (19%) had quantifiable TF (Table 1). Overall, 455 samples from all 56 patients enrolled were analyzed for this protein and plasma samples from 34 patients (61%) showed TF activity at one or more time-points. The frequency of TF in all samples analyzed was 22% (99 of 455) and no distinguishable pattern over time was observed.

For FXIa, 53 of 55 patients (96%) with admission plasma samples had active FXIa at the 0 hr. time-point and all 100% (56 of 56) of patients had it at one or more time-points. For all 471 samples analyzed, the frequency of FXIa was 90% (424 of 471), suggesting a slightly higher frequency at admission than at later time-points.

The frequency of FIXa was lower than that of FXIa but substantially higher than that of TF. With 55 admission samples analyzed, 48 of them (87%) had quantifiable FIXa and 96% of patients (54 of 56) had FIXa at one or more time-points. For all samples analyzed, 244 of 471 (52%) had quantifiable FIXa, indicating that the frequency of FIXa at admission is substantially higher than at later time-points.

When patients were grouped by TBSA and divided into quartiles (IQR = 23.5), admission samples were analyzed for frequencies and concentrations of TF, FXIa and FIXa. For TF, the lowest frequency was observed in the 3rd quartile (7%) and the highest in the 1st (29%), while the median across all quartiles was 0 pM and the widest range observed was in the 4th from 0 to 1.5 pM. The majority of admission samples had FXIa and the lowest frequency was observed in the 4th quartile at 92% despite having the highest median of 15.1 pM and also the largest range of concentrations (0 to 60.2 pM). Similarly, the majority of admission samples also had detectable FIXa, with the highest frequency (100%) observed in the 1st quartile and the lowest (71%) in the 2nd. Although medians for FIXa varied between 115 pM and 185 pM, the highest median was observed in the 4th quartile (185 pM) along with the widest range (0 to 960 pM).

3.4. Variability in TF, FXIa and FIXa concentration in patient plasma

There was a pronounced inter- and intra-patient variability with respect to the occurrence and concentration of all 3 proteins. For some samples, analysis was not done for all 3 proteins due to limited sample volume. Overall, TF was relatively low and varied between 0 and 2.1 pM with an average value of 0.14 pM ± 0.33 pM (Table 1). FXIa concentration was substantially higher and varied from 0 to >100 pM, but was between 5 and 20 pM for the majority of plasma samples (average concentration 9.16 pM ± 15.02 pM). Even higher

concentrations were quantitated for FIXa, in several cases exceeding 2,000 pM. For the majority of FIXa-containing plasma samples, however, this concentration was between 100 and 200 pM, with an average value of 113.09 pM ± 243.68 pM.

The presence and contribution of all three proteins to the endogenous procoagulant activity over time was quite significant. Fig. 2 shows thrombin generation profiles in plasma from patient B at two adjacent time-points (36 and 48 hours). In a 36 hr. plasma sample (panel A) thrombin generation enters the propagation phase in the absence of any inhibitory antibody after 540 s of the lag phase and thrombin generation proceeds at a 9.5 nM/s maximum rate reaching 550 nM concentration of active thrombin. An addition of αTF-5 has no effect on thrombin generation profile indicating that this plasma samples contains no active TF. An addition of αFXI-2 prolongs the lag phase to 1021 s, suppresses the maximum rate of thrombin generation to 1.2 nM and maximum thrombin concentration to 181 nM indicating a presence of 27 pM FXIa in this sample. An addition of αFIX-91 abolishes thrombin generation leading to the quantitation of FIXa at 280 pM concentration.

Remarkable changes in thrombin generation and in the contribution of these 3 proteins to it could be observed at a 48 hr. time-point (Fig. 2, panel B). In the absence of antibodies the lag phase of thrombin generation increased from 540 s (36 hr.) to 1086 s (48 hr.) indicating a significant decrease in the endogenous procoagulant activity. The role of contributors to this activity also underwent pronounced changes. In this plasma sample αTF-5 prolonged the lag phase to 1281 s indicating the presence of active TF at 0.7 pM concentration. Concentration of FXIa dropped to 4.3 pM and no thrombin generation was observed when αFXI-2 was added, indicating that this plasma sample contains no detectable FIXa.

3.5. Correlation between TF, FXIa and FIXa occurrence and concentration

There is no distinctive pattern in the concentration of TF, FXIa and FIXa during the entire observation period. TF, for example, was detected in plasma from only 10 patients at admission, whereas 34 patients in total had TF in their plasma at later time-points. Occurrence of TF and its concentration at various time-points does not appear to follow any time-dependent pattern (Figs. 3A and 4A).

Similarly, it is hard to explain an underlying cause for changes in FXIa concentration over time. For example, patient C had a relatively high FXIa concentration of 53 pM at admission (Fig. 3B), which decreased to 13 pM after 2 hr., was only 3.3 pM at the 8 hr. blood draw, then all of a sudden jumped to 86 pM at a 24 hr. time-point and dropped again to 1.1 pM at the next blood draw (36 hr.). In contrast, patient D (Fig. 4B) had a relatively low FXIa concentration at admission (8.1 pM), which jumped to 50 pM after 2 hr., decreased to 6.3 pM at the 4 hr. time-point and stayed relatively low through the entire observation period of 504 hr.

An occurrence and concentration of FIXa mimicked, in general, those of FXIa (Figs. 3C and 4C). Further analysis showed that there was good correlation between the concentrations of these two proteins with $R^2 = 0.68$ at the admission (n = 55; one patient was without a 0 hr. sample) (Fig. 5A) and with $R^2 = 0.58$ for all plasma samples analyzed (n = 471) (Fig. 5B), suggesting that FIXa is generated largely (perhaps exceptionally) by FXIa. The ratios of

FIXa/FXIa concentrations were 12.6 and 12.3, respectively. No correlations between TF and FXIa or TF and FIXa were observed.

Of the total 56 patients, 8 (14%) had a 3-week duration in the hospital in which 17, on average, blood draws occurred during the first week, 1 occurred at the end of two weeks and 1 again at the end of three weeks. A downward trend in averaged normalized values (based on maximum) was observed on a weekly basis for FXIa and FIXa and for TF from week 1 to week 3. For TF, levels went from 0.12 pM during week 1 to 0.29 pM at the end of week 2 to 0.05 pM at the end of week 3. For FXIa, levels went from 0.29 pM to 0.14 pM to 0.0 pM, respectively, and for FIXa levels went from 0.16 pM during week 1 to 0.0 pM at the end of weeks 2 and 3.

4. Discussion

This study illustrates the prevalence and distribution of 3 factors of coagulation, tissue factor (TF), factor (F)XIa and FIXa, in plasma from a population of 56 burn patients during the course of their stay at the hospital (ranging from <2 hours to 21 days). Coagulopathy is often observed in patients with severe burns and the occurrence of which is associated with an increase in thromboembolic complications and ultimately an increase in mortality [20, 21].

TF is a transmembrane glycoprotein that is located on subendothelium of blood vessels and is essential for normal hemostasis [22]. It is not exposed to blood under normal conditions but following damage to the vasculature and consequent intravascular, and thus TF, exposure to blood it initiates the extrinsic pathway of coagulation. Initiation occurs *via* TF complexing with circulating FVIIa and activating FIX and FX, eventually resulting in thrombin generation and solid clot formation [23]. Published studies have also shown TF activity to be expressed on blood cells, primarily monocytes [24–26] and neutrophils [27], although the latter is debated [28], along with circulating microparticles [29] shed from blood cells. More recently, TF presence in platelet α granules has been suggested [30], although the subject of platelet TF remains controversial [31, 32]. In blood from healthy individuals, studies have shown low levels of TF (if any) not exceeding 20 fM could be present [25, 33]. However, patients suffering from hematologic disorders [14, 34] and disease states associated with tissue damage such as trauma [35], irritable bowel syndrome [11] and cardiovascular disease [12, 36] have shown elevated levels of circulating TF activity. Consistent with elevated TF activity in patients with tissue damage are studies showing that TF becomes enhanced by mechanisms related to tissue disruption, endotoxin stimulation, inflammation and also as an immunologic response [25, 26].

In the current study, 22% of plasma samples from burn patients had detectable TF. A correlation between the extent of coagulopathy and burn severity has been observed, in which the exposure of TF may contribute to overall systemic coagulopathy [7, 37]. This correlation is most likely due to endothelial damage and systemic inflammatory and immunologic responses [37], all of which are known to contribute to TF expression [25, 26]. Similarly, it has been hypothesized that burn severity correlates with thromboembolic risk [38], which could occur as a result of the hypercoagulable state caused, in part, by TF activity. In the current study, there were no distinct correlations observed between burn

severity (as measured by %TBSA burned, Baux, APACHE II, and FLAMES scores) and levels or frequency of TF. These data, however, do not eliminate the role of the extrinsic pathway in burn coagulopathy. It is quite possible that TF, being a membrane protein, has a different distribution on cells *in vivo* than on shed microparticles in plasma, the latter being a subject of the analysis.

In contrast to the relatively low levels and frequency of TF measured in this patient population, FXIa was detected in 90% of samples at concentrations significantly higher than those observed for TF, with no correlation between TF and FXIa observed. It has been commonly accepted that in normal hemostasis FXI activation occurs *via* TF-initiated feedback by thrombin. However, under pathological conditions it can also occur *via* the intrinsic pathway when FXIa is generated by FXIIa [39]. The specific conditions for which FXIa can be generated by FXIIa include general activation of the contact pathway *via* exposure of blood to an artificial surface of any charge [40] along with both organic and inorganic polyphosphate release from dying cells (DNA/RNA) [41] and platelets [42], respectively. FXIa, not detectable in the plasma of healthy individuals, has been detected in a number of cardiovascular diseases [12] along with chronic obstructive pulmonary disease [13], irritable bowel syndrome [11] and ischemic cerebrovascular disease, in which its presence was shown to correspond with a worse prognosis [14, 43]. Furthermore, increased FXIa levels have been shown to correlate with an increased risk of venous and arterial thrombotic events [44], potentiating the use of FXIa as a novel thrombotic marker for patients at such a risk and/or as a prime target for antithrombotic strategies [45, 46].

Following severe burns, there is a progressive death of the surrounding tissues that was shown to occur *via* necrosis [47], resulting in the spilling out of cellular contents such as nucleic acids and the subsequent activation of FXI by FXIIa [41]. Activation of FXI due to severe burn-induced necrosis could be responsible, in part, for the coagulopathy often observed in patients with severe burns [20]. Also to consider are elevated levels of thrombopoietin previously observed in the plasma of burn patients that was shown to enhance platelet activation [48]. This enhanced platelet activation and subsequent polyphosphate release may also, in part, be responsible for the observed levels of FXIa in this population of burn patients due to the activation of FXI by FXIIa [42]. The much higher frequency of FXIa as compared to TF in this patient population suggests that the observed coagulopathy could be largely driven by the intrinsic pathway of coagulation. Furthermore, FIXa, immediately downstream of FXIa in the coagulation cascade, was observed in 52% of samples and maintained a relatively strong correlation with FXIa overall ($R^2 = 0.58$) while no correlation with TF was observed. These data once again suggest that the primary pathway driving burn coagulopathy in this patient population could be the intrinsic pathway, although the TF pathway should be considered as well.

FIX can be activated by both the extrinsic FXase complex (TF:FVIIa) and FXIa to subsequently form the intrinsic tenase complex with FVIIIa and activate FX, ultimately leading to thrombin generation. Studies have shown FX activation to be approximately 50-fold more efficient when driven by the intrinsic FXase complex as compared to the TF:FVIIa complex [23], establishing FIXa as a potential marker for hypercoagulability. This is further supported by observations that increased FIXa levels have been detected in patients

with acute coronary syndromes [49] whereas hemophilia B, deficiency of FIX, leads to bleeding events in which the severity correlates with the level of FIX [50].

Limitations of this study include; 1) The sample size for those patients who stayed for over 2 weeks in the hospital was relatively small to be conclusive about the levels of TF, FXIa and/or FIXa over an extended period of time. 2) Data reported do not necessarily infer a causal relationship between burn injury and burn-induced coagulopathy. 3) The mechanism(s) of FXIa and FIXa generation in trauma patient blood is not clear.

5. Conclusions

In conclusion, TF and, for the first time reported, FXIa and FIXa were detected and quantified in the plasma of burn patients at a variety of concentrations and durations. The frequencies and correlations (or lack of) between TF, FXIa and FIXa suggest that the observed burn-induced coagulopathy is largely driven by the contact (intrinsic) pathway of coagulation although the extrinsic pathway should not be ignored. In a subset of 8 patients who remained in the hospital for a duration of 3 weeks, a downward trend in all 3 protein levels was observed, although a larger sample size is needed to be conclusive. This last observation is supported by a previous study indicating that hypercoagulable states seen in burn patients often stabilize within a week following injury [7].

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Highlights

- TF, FXIa and FXIa are responsible for the procoagulant activity in burn patients.
- Burn induced coagulopathy is driven mostly by the contact pathway.
- A prolonged hospital stay drives levels of all 3 proteins down.

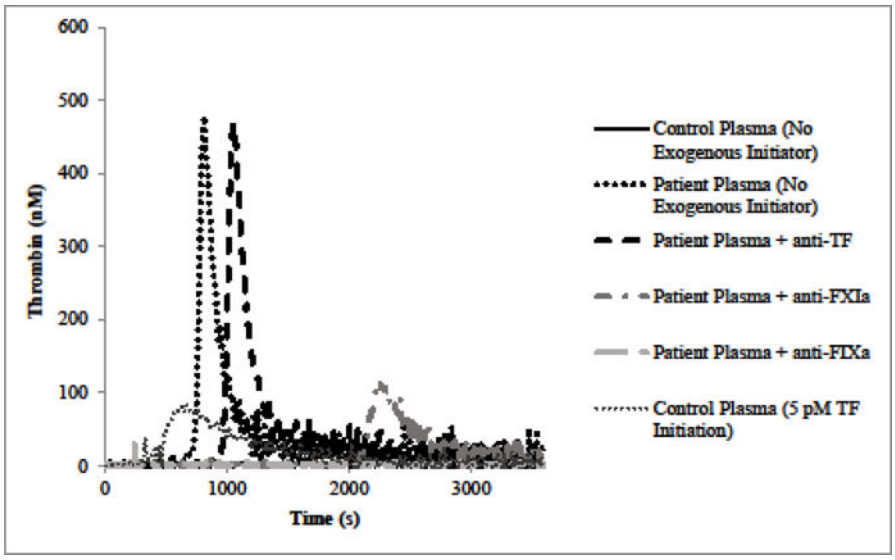

Figure 1.

Thrombin generation over time in re-calcified citrate plasma from a burn patient at the 12 hour time-point and in pooled plasma from healthy individuals. No exogenous activator was added to burn patient plasma and contact pathway was prevented by CTI with either no inhibitory antibodies added (•••••) or in the presence of inhibitory antibodies against TF (▬ ▬ ▬), FXIa (<img>), and FIXa (▬ ▬). As a control, pooled plasma from healthy individuals was evaluated in the absence of an exogenous initiator (<img>) or in the presence of 5 pM relipidated TF (■ ■ ■ ■ ■ ■).

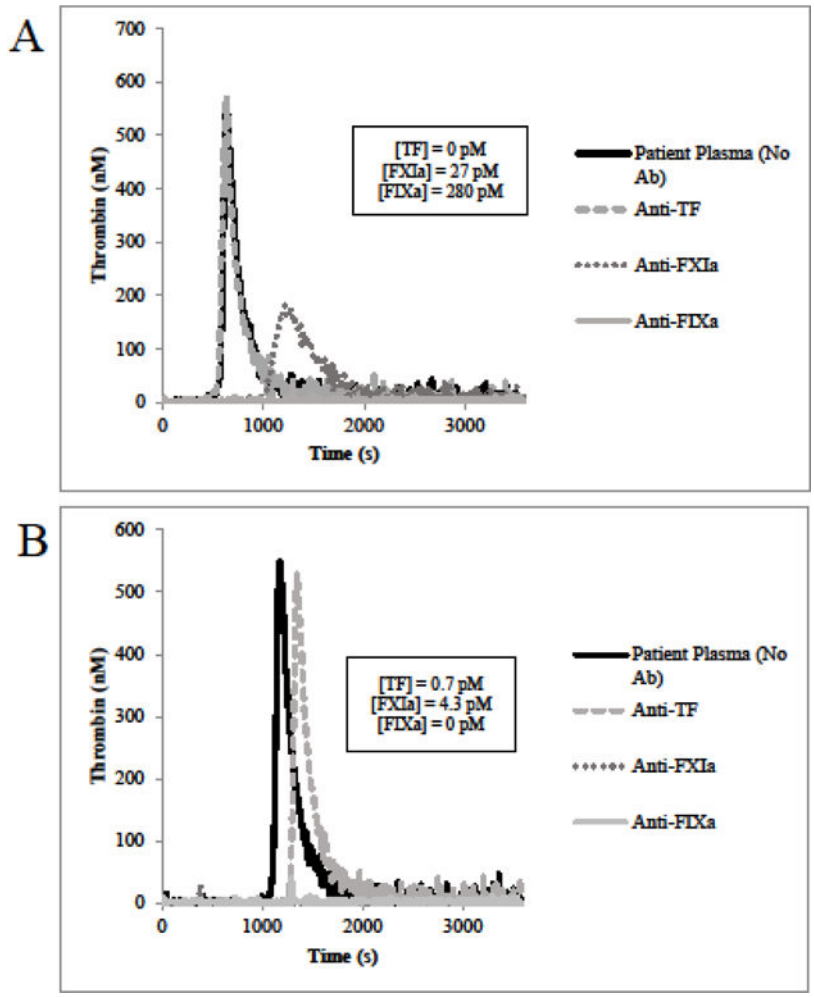

Figure 2.
Thrombin generation in Patient B at the 36 hour time-point (**A**) and 48 hour time-point (**B**).

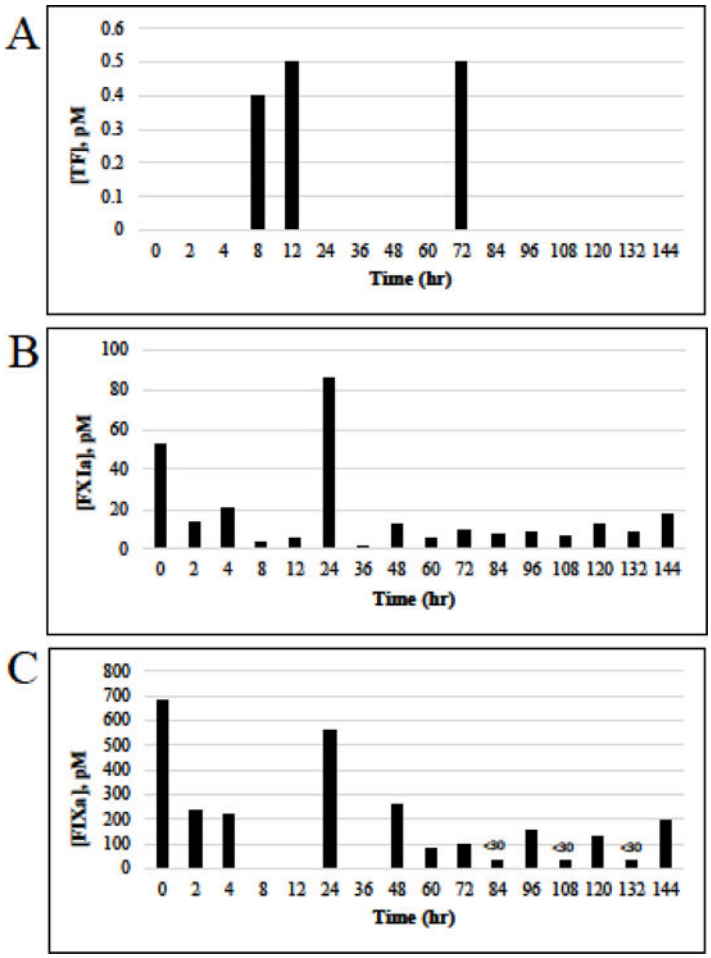

Figure 3.

Changes in detectable TF (**A**), FXIa (**B**) and FIXa (**C**) over time in Patient C.

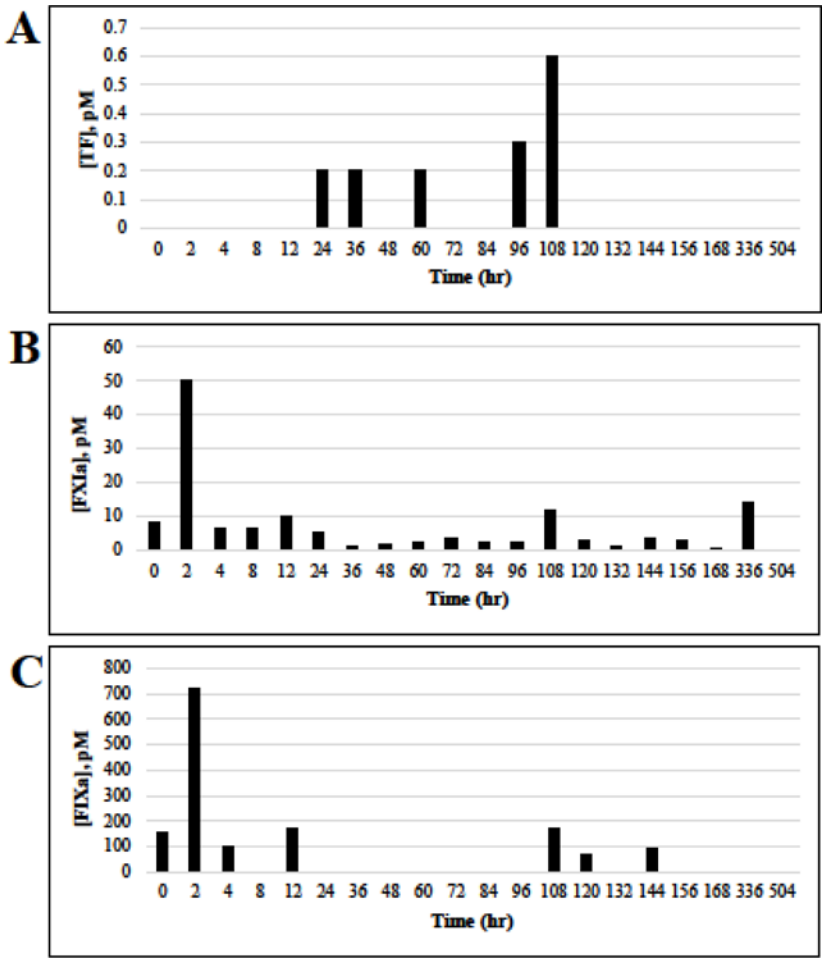

Figure 4.
Changes in TF (**A**), FXIa (**B**) and FIXa (**C**) over time in Patient D.

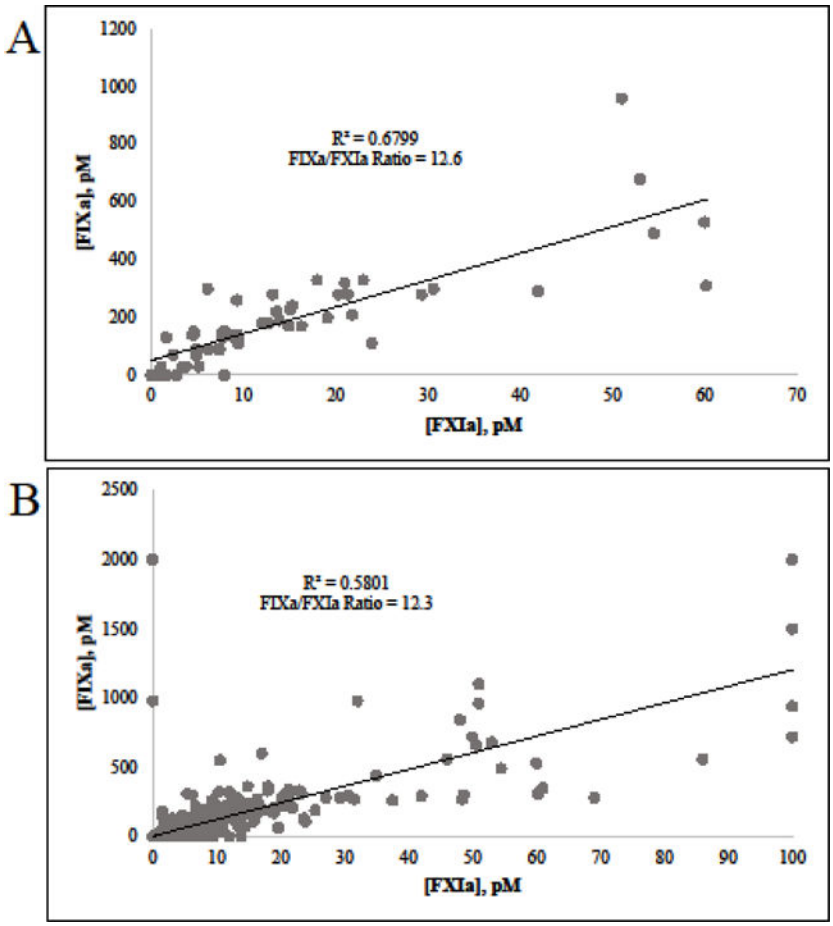

Figure 5.

Correlations between FXIa and FIXa at 0 hour time-points (n = 55; one patient did not have a 0 hour time-point) (**A**) and overall (n = 470; one sample was not processed for both FXIa and FIXa due to limited volume) (**B**). For samples that had detectable but not quantifiable amounts of FXIa and FIXa the detectability limits were used (<0.5 pM/>100 pM and <30 pM/>2000 pM, respectively).

Table 1

Protein frequencies and concentrations in patients at admission versus in patients over the overall course of their stay. Also shown are protein frequencies and concentrations in all samples analyzed.

	TF			FXIa			FIXa		
	Frequency	**Range**	**Mean**	**Frequency**	**Range**	**Mean**	**Frequency**	**Range**	**Mean**
Admission	10 of 53 (19 %)	0 – 1.5 pM	0.12 ± 0.32 pM	53 of 55 (96%)	0 – 60.2 pM	15.09 ± 15.69 pM	48 of 55 (87%)	0 – 960 pM	189.82 ± 175.75 pM
Overall	34 of 56 (61%)	0 – 2.1 pM	0.14 ± 0.33 pM	56 of 56 (100%)	0 – >100 pM	9.16 ± 15.02 pM	54 of 56 (96%)	0 – >2000 pM	113.09 ± 243.68 pM
All Samples Analyzed	99 of 455 (22%)	0 – 2.1 pM	0.14 ± 0.33 pM	424 of 471 (90%)	0 – >100 pM	9.16 ± 15.02 pM	244 of 471 (52%)	0 – >2000 pM	113.09 ± 243.68 pM