Published in final edited form as:

J Trauma Acute Care Surg. 2015 November ; 79(5): 726–731. doi:10.1097/TA.0000000000000839.

THROMBIN GENERATION AND PROCOAGULANT MICROPARTICLE (MP) PROFILES AFTER ACUTE TRAUMA: A PROSPECTIVE COHORT STUDY

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Abstract

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Conflicts of Interest: No conflicts of interest to disclose

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Objective—The two sides of Trauma Induced Coagulopathy (TIC), the hypo- and the hyper-coagulable states, are poorly understood. To identify potential mechanisms for venous thromboembolism and bleeding after acute trauma, we estimated changes in circulating procoagulant MPs and thrombin activity during hospitalization for trauma.

Methods—Whole blood was collected by venipuncture into 3.2% trisodium citrate at 0, 6, 12, 24 and 72 hours after injury, and discharge. Platelet poor plasma was harvested and stored at -80°C until analysis. Thrombin generation was determined using the calibrated automated thrombogram (CAT), reported as lagtime (minutes), peak height (nM thrombin) and time to reach peak height (ttPeak - minutes). The concentration of total procoagulant MPs (number/uL) was measured by flow cytometry. Data are presented as median [interquartile range].

Results—Among 443 trauma patients (1734 samples; ISS=13.0 [6.0, 22.0], hospital LOS=4.0 [2.0, 10.0] days, age=48 [28, 65] years, 70.7% male, 95% with blunt mechanism, mortality 3.2%), no discernable patterns in thrombin generation or MP concentration were observed over time. The peak height and MPs were significantly different from healthy volunteers and were 337 [285, 395] nM and 400 [211, 772] per uL plasma, respectively. Extreme (defined as highest or lowest 5%) values reflecting a possible “hypercoagulable state” (lagtime 1.98, peak height 486.2, ttPeak 3.61, and total procoagulant MP 2278) were reached within 12 hours after acute trauma, while extreme values representing a possible “hypocoagulable state” (lagtime 18.6, peak height 17.8 and ttPeak 29.45) were not reached until 1-3 days.

Conclusion—Although there was no predictable pattern of coagulopathy observed in each patient after trauma, those who reached extreme values did so relatively early after injury. These findings should be taken into account when designing risk model tools involving coagulation laboratory parameters.

Keywords

Trauma; Thrombin; Microparticle; Prospective; Cohort

Introduction

Trauma Induced Coagulopathy (TIC) detected early after injury reflects injury severity and is prognostic for blood transfusion requirement and death.¹⁻² Maintenance of hemostasis, often with blood products aimed at limiting hemorrhage, comes with the price of increased risk of venous thromboembolism (VTE).³⁻⁵ The two sides of TIC, the hypo- and the hyper-coagulable states, are poorly understood. Existing therapies to treat TIC are based on limited understanding of their mechanisms. Hence, assays with enhanced sensitivity and specificity are needed in order to understand the basis for TIC. In a previous study, we noted that the concentration of plasma procoagulant microparticles (MPs) and peak thrombin generation in patients with blunt trauma correlated with injury severity while the standard clotting assays (prothrombin time and activated partial thromboplastin time) were within the normal range.⁶ Our long-term goal is to identify potential mechanisms for VTE and bleeding after acute trauma. In a prospective cohort study, we estimated serial changes in plasma procoagulant MP concentration and thrombin generation potential over time among patients hospitalized for acute trauma. We hypothesized that TIC occurs early after injury and it is quantifiable.

Methods

In a prospective cohort study, all trauma patients transported to the Mayo Clinic Emergency Department (ED) by ambulance or air transport from February 2011 to June 2014 were considered for inclusion. Exclusion criteria were age < 18 years, anticoagulation (e.g., heparin, warfarin) or antithrombotic therapy (excluding aspirin or non-steroidal anti-inflammatory drugs), preexisting coagulopathy, more than 12 hours from time of injury, transfusion of blood products prior to blood sample collection, active cancer, sepsis, renal failure, burn injuries or declined consent by the patient or legal guardian. The time of injury (TOI) was determined by the pre-hospital medical providers based on information at the injury scene. If the time of injury was unclear, the pre-hospital medical providers estimated the time and relayed this information to the emergency communication center. A trauma alert page was then sent to the hospital and laboratory staff as to the TOI. We collected demographic and baseline clinical characteristics, including injury severity score (ISS), patient age and sex, body mass index (BMI), hospital length of stay (LOS), all-cause mortality, and start and stop of anticoagulant-based thromboprophylaxis and other medications affecting coagulation. Transfusion therapy was mainly based on Mayo Clinic Trauma Center transfusion guidelines but also at the discretion of the medical provider. Blood samples also were collected for reference (control) analysis from 89 volunteers with no history of thrombosis (i.e., stroke, myocardial infarction or venous thromboembolism) or recent antithrombotic (thienopyridine; including aspirin or non-steroidal anti-inflammatory drugs) or anticoagulant (heparin, low-molecular-weight heparin, warfarin) therapy who were recruited by advertisement within the Mayo Clinic Employee Portal. This study was approved by the Mayo Clinic Institutional Review Board.

Sample Collection and Processing

Blood samples were collected at baseline and at 2, 6, and 12 hours, and 1 and 3 days after injury, and at hospital discharge. When patients were unable to provide consent at the time of the trauma, consent was obtained from the patient or legal guardian within 30 days of hospital discharge; samples were discarded when consent could not be obtained. A total of 18mL of whole blood was collected by antecubital venipuncture or via existing indwelling catheters into 4.5 mL citrated Vacutainer tubes (0.105M buffered sodium citrate, 3.2% Becton Dickinson, Plymouth, UK) and processed to platelet free plasma by double centrifugation (3000g, 15 minutes) as recommended by the ISTH vascular biology SSC Collaborative Workshop⁷, and stored in multiple aliquots at -80 degrees Celsius until analysis. All samples were processed within 1 hour of collection.

Calibrated Automated Thrombogram (CAT) Analyses

Thrombin generation was measured with the Calibrated Automated Thrombogram (CAT, Thrombinoscope BV, Maastricht, The Netherlands), utilizing a Fluoroskan Ascent plate reader (390 nm excitation, 460 nm emission, Thermo Electron Corp, Vantaa, Finland), as previously described by Hemker et al⁸⁻⁹ and Owen et al.¹⁰ Assays of trauma patient samples were performed in triplicate. Corn trypsin inhibitor (25mg/mL) 25uL (50ug/mL final concentration) was added to each plasma sample prior to CAT analyses. Thrombin generation was initiated using two different reagents: addition of 20uL of either PPP (5 pM

tissue factor and 4 μ M phospholipid, Stago, US) or PRP (1 μ M tissue factor, Stago, US) reagent. Then, 80 μ L of citrated platelet poor plasma was added to each well of U bottom 96-well microtiter plates (Nunc, Thermo Fischer Scientific, Rochester, NY) using a single channel pipette. After an incubation period (10 minutes at 37 °C), 20 μ L of warmed FLUCA reagent (Fluca kit, TS50, Thrombinoscope, BV), which contains the fluorogenic substrate and CaCl_2 was added to each well via an automated dispenser. Thrombin generation curves were recorded continuously for 90 minutes at a rate of 3 readings per minute. Separate wells containing the thrombin calibrator, which corrects for inner filter effects and quenching variation among individual plasmas were also measured in parallel.⁹ A dedicated program, Thrombinoscope, was used to calculate thrombin activity over time. The parameters derived were: lagtime (LT), time to peak (ttPeak), and peak height(PH). We did not analyze samples collected after anticoagulant-based (mainly heparin) prophylaxis; we have found that prophylactic dose of heparin completely attenuates thrombin generation in CAT analyses. When unfractionated heparin was added, at varying concentrations from 0.1 to 0.4 units per mL, to platelet poor plasma (Cryocheck), we noted a significant decrease in PH values (85% decrease) and prolongation of LT (200% increase) even at the lowest dose.

Microparticle (MP) Analyses

The flow cytometric assay to measure plasma MPs, without ultracentrifugation, was adapted from the method of Ayers et. al.¹¹ The MPs were analyzed by FACSCanto II flow cytometer (BD Biosciences, San Jose CA) and using BD Diva Software v6.0. Samples were run at high flow rate of 120 μ L per minute. To note, every morning prior to patient sample analyses, filtered HBS buffers with and without antibodies were analyzed to be sure the reagents were free of contaminants. Fluoribrite beads of 0.2 to 2.0 μ m in 1:10,000 dilution of HBS buffers were analyzed to ensure the instrument was optimized for gating on MPs, which were defined in this study as events < 1 μ m in diameter and positive for Annexin-V (procoagulant) and cell-specific markers.

To establish procoagulant properties of the MP, a dual labelling procedure was used. For each sample analysis, 100 μ L of test plasma samples were diluted with 890 μ L of filtered hepes buffered saline (HBS; pH 7.4) and 10 μ L Hirudin (to achieve final concentration of 1 μ M) was added to prevent clot formation. Then, all the samples were stained with 4 μ L fluorochrome-labelled Annexin-V-FITC (BD Pharmingen, 556420) monoclonal antibody, which binds to procoagulant phosphatidylserine (Annexin-V) expressed on MP and either 4 μ L of R-phycoerythrin (PE) labelled monoclonal antibody to CD42a which binds to single chain integral membrane glycoprotein, GPIIb/IIIa, on platelet derived MPs (BD Pharmingen, 558819 or 561853) or 4 μ L of IgG PE (isotype control). Following 30 minute incubation of samples with the antibodies, 800 μ L of filtered HBS-Ca was added as well as 100 μ L Trucount® beads (BD Biosciences, San Jose CA). Each tube was run for a minimum of 300 seconds or until 1500 events were collected in the Trucount bead gate. Trucount® beads facilitated the accurate calculation of MP absolute numbers, using the following formula:

Absolute count of cell population=

$$\frac{\text{Number of events in quadrant containing cell population}}{\text{Number of events in absolute count bead region}} \times \frac{\text{Total number of beads per test*}}{\text{Test volume}}$$

A MP gate on a flow cytometry plot of forward scatter (FSc) vs. side scatter (SSc) was used to distinguish MP from small platelets, as previously published.¹²⁻¹³ All buffers were filtered twice through Millex-GP 33mm filters in preparation of daily sample analyses. All antibodies were also filtered twice through 0.2 µm membrane filters. Unfiltered buffers and antibodies contain interfering numbers of chemical microparticles (data not shown). Additionally, we used a commercially available reference plasma, Cryocheck (Precision Biologic, Dartmouth N.S.), which was analyzed with every carousel of patient samples to ensure that our technique for MP analysis was consistent. The overall mean of AnnV pos MP counts in Cryocheck was calculated to be 3046 ± 574 per uL plasma. Between two experienced research technologists, the range of coefficient of variation (CV) using this reference plasma has consistently been 15 – 23%. Using the compensation set up feature of DIVA software (V6, BD Biosciences, San Jose CA), compensation was determined by running unstained and single color positive control samples following the manufacturer's recommendations.

Statistical Analyses

Data analysis was performed using SAS, version 9.3 (SAS Institute Inc, Cary, North Carolina) and R version 3.0.2. Descriptive statistics are presented as median values (interquartile range). A comparison of continuous variables between trauma patients and volunteers (controls) was performed using analysis of variance, the dependent variable being the ranked lab value and including age and sex in the model as additional independent variables. “Extreme” values were defined as the greatest or lowest 5% of values, regardless of patient or the time of sample acquisition relative to time of injury. For each lab parameter, the most “hypercoagulable” value within the first 24 hours for each patient was identified; this was defined as the lowest 5% lag time and ttPeak, the greatest 5% peak height and MP counts. The most “hypocoagulable” value was defined as values at the other end of extreme 5%. The alpha-level was set at 0.05 for statistical significance.

Results

From Feb 2011 through April 2014, we screened 2106 patients and 1418 met inclusion criteria. Of these eligible patients, 288 declined to participate and 332 patients did not return mail-in consents and their study samples were discarded. Of the 798 enrolled, we randomly analyzed blood samples of 443 patients; to assess for existence of discernible pattern in thrombin generation and MP counts after injury. The number of patient samples analyzed at each timepoint were: 1) 228 baseline, 2) 368 at 6 hours, 3) 391 at 12 hours, 4) 310 at 24 hours, 5) 210 on day 3, and 6) 155 on the day of discharge. Demographic data are displayed in **Table 1**. Of the 443 patients sampled, 90 were transfused within the first 24 hours after arrival to our Trauma Center. The median [IQR] amongst the patients who received each type of blood product within this time frame were: packed red blood cells (**RBC**) 4 [2,7], frozen or thawed plasma 5 [2,6], platelets (**PLT**) 2 [1,2], and cryoprecipitate (**Cryo**) 2[1,2].

Comparison of Laboratory Values between Trauma Patients and Volunteers

The median [IQR] patient and volunteer ages were 48 [28, 65] and 40 [27, 53; $p = 0.002$] years, respectively; 71 % and 49% of trauma patients and volunteers were men, respectively

($p < 0.001$). For both PPP and PRP, thrombin peak height and ttPeak were significantly greater and shorter, respectively, as compared to volunteers, while the lagtime did not differ significantly using PPP reagent. These samples from trauma patients also had significantly greater procoagulant MP levels in peripheral blood even after adjusting for age and sex (Table 2). We also observed a wide variance of thrombin generation and MP levels as compared to healthy volunteers (Supplementary Digital Content Figures 1 and 2).

Trend-Over-Time & Time-To-Reach Extreme Values

The plots of each individual patient's thrombin activity and MP counts over time did not exhibit a discernable pattern; each patient displayed a unique pattern after injury (Supplementary Digital Content Figure 3a,b,c,d). Since no particular trends in the serial blood analyses of individual patients were observed, extreme (defined as highest or lowest 5%) values reflecting a possible “hypercoagulable state” (Table 3 - 5) and “hypocoagulable state” were calculated (Table 3 and 4). We observed that the hypercoagulable extreme values were reached within 12 hours after acute trauma, while hypocoagulable extreme values were not reached until 1-3 days.

Comparisons of CAT and MP Values with Degrees of Injury Severity and Blood Transfusions

Among the cohort of 443 patients, 261 patients presented with injury severity score (ISS) of less than 15, 96 patients with ISS 15-24 and 86 patients had severe injury with ISS ≥ 25 . We observed shortened lagtime, decreased peak height and ttPeak were found to be associated with greater injury severity (Table 6). However, there were no significant differences between these groups of patients in regards to MP counts. There were no overall significant differences in CAT and MP values between those who received any blood type transfusion and those who did not.

Discussion

In this study, we have observed that endogenous thrombin generation and circulating procoagulant MP are significantly different in trauma patients when compared to volunteers. In particular, peak height and ttpeak values were greater and lesser, respectively, in the trauma patients relative to volunteers. Hence, patients exhibit acceleration of thrombin generation, indicating that the plasma coagulome is important in generating the hypercoagulable milieu needed for thrombotic complications. As injury severity increased, a shortened lagtime and ttPeak were observed, which are consistent with accelerated rate of thrombin generation.

In our cohort study, serial blood draws, relative to time of injury, were obtained from trauma patients until their discharge. In thus doing, we did not observe any discernible pattern over time for individual patient level thrombin activity and MP counts. Curry et al. recently showed, in their pilot study of 50 patients, an increase in peak thrombin generation immediately after trauma admission as compared to the values at time of admission.¹⁴ However, the mean time from injury to admission was not specified. Similarly, Matijevic et al. describe their findings of cellular MPs and thrombogram in patients enrolled in the

Prospective Multicenter Major Trauma Transfusion (PROMMTT) Study.¹⁵ They only collected one time blood sample at the time of admission for trauma but the time at which these samples were collected relative to the time of injury is not specified. Obtaining blood relative to time of injury is important as it allows us to gain a better understanding of where, in the time continuum of injury response, a patient may reside. Regardless, this study supports the findings of these other investigators who have shown that thrombin generation and procoagulant MP counts are altered after trauma as compared to non-injured volunteers.^{7,14,15}

Thrombin Generation Assay

Calibrated Automated Thrombogram (CAT) is a functional assay of global thrombin generation. It measures the rate of thrombin generation and inhibition in citrated plasma, and has been used to quantify procoagulant activity in several diseases including VTE and coronary artery disease.¹⁶⁻¹⁸ In our previous pilot study, we performed native thrombin generation assays, as initially described by Dunbar et al¹⁹, in which no agonist such as tissue factor or phospholipid source (PCPS) were added. The role of CAT in clinical practice has yet to be defined largely due to lack of official standardization of the assay with its associated large interlaboratory variability. However, Dargaus et al were able to reduce assay variability when assays were performed using identical equipment, standardized reagents, carefully selected reference plasma for normalization of results.²⁰ We have previously demonstrated that CAT assays can be performed with reproducible results when commercially available, reference plasma is used with every patient sample runs.²¹ Additionally, the presence of lot to lot variability of PPP and PRP reagents have to be taken into account if CAT data are to be used to make clinical correlations. In brief, we plan to perform experiments which hopefully will begin to dissect the pro and anticoagulant activities accounting for the observed net thrombin generation response after trauma. The investigators of Trans-Agency Research Consortium for Trauma-Induced Coagulopathy (TACTIC) study 1-UM-1-HL120877-2 have begun the performance of comprehensive analyses of coagulation and inflammation profiles in trauma patients. Hopefully, this multicenter, multi multidisciplinary team of physicians and scientists can enhance our understanding of the mechanisms for development of coagulopathy in trauma patients after injury.

Microparticle (MP) Analyses by Flow Cytometry

MPs are found in healthy individuals. In normal plasma, MP derived from platelets are most common (>80%), followed by MPs derived from endothelial cells (<10%) and leukocytes (<10%).²² Thrombogenic potential of MPs that express surface phosphatidylserine (Annexin V+) are procoagulant in vitro.^{13, 22-23} Thus, endogenous MPs expressing membrane phosphatidylserine may not contribute intrinsically to thrombosis but may drive thrombosis in an environment where procoagulant enzymes are generated, such as would be expected in peripheral blood of patients after major trauma.⁷ In this study, MP analyses were limited to Annexin-V binding (procoagulant) MPs some of which are derived from platelets. Further evaluation needs to be performed to quantify the number and subtypes of MPs present in our bank of stored samples. Curry et al. quantified subtypes MPs in platelet poor plasma without ultracentrifugation. We adopted their technique as data variability became significantly less

when ultracentrifugation step was omitted. Curry et al. found significantly greater red blood cell, procoagulant (Annexin V+) MPs and decrease in endothelial cell derived MPs. As compared to their 41 survivors, the 9 non-survivors had significant decrease in platelet derived procoagulant MPs. As performed by Curry and others, we have employed the recommendations within the ISTH SSC guidance for standardization of MP analysis.²⁴ Similar to CAT assays, an obstacle to translating MP analyses methods into the clinical arena is the lack of standardization that has been uniformly accepted by the research community. Namely, there is a lack of consensus in regards to MP analyses performed using identical equipment, standardized reagents, carefully selected reference plasma for normalization of results. This is an area of research in dynamic flux and it remains to be determined if characterization of procoagulant MPs could find practical applications in the clinical arena.

Limitations

Our study has several limitations. First, our definitions of hyper or hypocoagulable states were arbitrary. We chose the greatest or least 5% of thrombin generation variables to define hyper and hypo- coagulable states, respectively. In regards to total procoagulant MPs, we defined plasma samples with the greatest number (in the top 5%) to be hypercoagulable. Second, the patients enrolled in this study were not consecutive admissions as our overall consent rate was approximately 60%. Lastly, we did not stratify our findings based on fluid administration and blood transfusions, which may affect our findings secondary to hemodilution or alteration of coagulation secondary to blood product administration.

Conclusion

In this prospective cohort study, we observed an increase in thrombin generation by CAT and procoagulant MPs after injury. Although there was no predictable pattern of coagulopathy observed in each patient after acute trauma, patients who reached extreme values did so relatively early after injury. These findings should inform the design of risk model tools involving coagulation laboratory parameters. Challenges remain regarding the lack of standardization and lack of reference controls to allow reproducibility of data amongst different centers. Hence, it remains to be determined if thrombin assays by CAT and characterization of procoagulant MPs could find practical applications as diagnostic indicators of post-injury TIC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

We gratefully acknowledge research coordinators Amy Headlee, L.P.N., Teron T. Cox and Debbie A. Dixon for patient recruitment and data collection; Nancy C. Hawley, R.N., C.C.T.C., Maria Nacionales and Matthew J. Hange of the Clinical Research Unit of the Center for Translational Science Activities for support in blood sample collection and processing; Robert D. Litwiller, Teresa K. Kimlinger of the Hematology Research Laboratory, Mayo Clinic, Rochester, MN for technical assistance in sample analyses; and Mayo Clinic Medical Transport and the Mayo 1 Medical Director, Scott P. Zietlow, M.D., and Kathleen S. Berns, M.S., R.N., C.N.S., for their support in patient recruitment and blood sample collection.

Disclosure of Funding: This project described was supported by Grant Number K08 GM093133-05 (MSP) from the National Institute of General Medical Sciences (NIGMS), W81XWH-10-2-2010 (MSP) from the Telemedicine and Advanced Technology Research Center (TATRC) of the Department of Defense, 1 UL1 RR024150 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), the NIH Roadmap for Medical Research and by Grant Number UL1 TR000135 from the National Center for Advancing Translational Sciences (NCATS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH, Department of Defense and NCRR.

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Table 1

Demographic and Baseline Characteristics of 443 Trauma Patients

Age at Trauma, median [IQR]; <i>years</i>	48 [28, 65]
Male sex; <i>n (%)</i>	313 (70.7%)
Blunt mechanism; <i>n (%)</i>	423 (95.5%)
ISS, median [IQR]	13.0 [6.0, 22.0]
Hospital LOS, median [IQR]; <i>days</i>	4.0 [2.0, 10.0]
Death; <i>n (%)</i>	14 (3.2%)
INR (admission), median [IQR]	1.0 [1.0, 1.1]
PTT (admission), median [IQR]; <i>seconds</i>	26.0 [24.0, 29.0]
Hemoglobin (admission), median [IQR]; <i>g/dL</i>	13.7 [12.0, 14.8]
RBC median [IQR]; units(First 24 Hours of Arrival) *	4 [2, 7]
Plasma median [IQR]; units(First 24 Hours of Arrival) *	5 [2, 6]
Platelet median [IQR]; units(First 24 Hours of Arrival) *	2 [1, 2]
Cryoprecipitate median [IQR]; units(First 24 Hours of Arrival) *	2 [1, 2]
Tranexamic Acid Administration in Pre-Hospital or ED (%)	10 (2.3%)
Bebulin (PCC) Infusion in Pre-Hospital or ED (%)	4 (1%)

* Median [IQR] amongst the patients who received each type of blood product within the first 24 hours of arrival (77 patients received RBC, 53 patients received plasma, 51 patients received platelets and 14 patients received cryoprecipitate)

Table 2

Comparison of Laboratory Values between Trauma Patients and Uninjured Screened Volunteers (Adjusted for Sex and Age)

Variable	Patient median (IQR)	Volunteer median (IQR)	P-value
<u>5 pM TF/ 4 uM PS (PPP reagent)</u>			
Lagtime (min)	2.67 (2.38, 3.27)	2.67 (2.33, 2.96)	0.320
Peak Height (nM)	337.6 (285.9, 395.6)	320.6 (287.0, 343.9)	0.0081
ttPeak (min) [#]	4.73 (4.19, 5.56)	5.11 (4.61, 5.67)	< 0.0001
<u>1pM TF (PRP reagent)</u>			
Lagtime (min)	8.34 (6.89, 10.56)	9.00 (8.00, 10.07)	< 0.001
Peak Height (nM)	49.2 (33.9, 71.4)	25.6 (19.8, 34.1)	<0.0001
ttPeak (min) [#]	17.19 (14.55, 20.22)	20.56 (18.56, 22.78)	<0.0001
Total procoagulant MPs (number per uL plasma)	401 (212, 772)	241 (146, 530)	0.0015
Platelet-derived procoagulant MPs (number per uL plasma) *	19.4 (10.6, 40.8)	31.6 (13.1, 65.8)	0.0023

[#] ttPeak – Time to peak

Table 3Time-to-Extreme Values for CAT Data using PPP^{*} reagent

Variable	N	Extreme 5% Value	Time-to-Extreme Value (Days)
<u>Hypercoagulable</u>			
Lagtime (min)	1575	1.98	0.32 (0.12, 0.50)
Peak Height (nM)	1575	486.2	0.50 (0.09, 2.97)
ttPeak (min)	1575	3.61	0.32 (0.24, 0.59)
<u>Hypocoagulable</u>			
Lagtime (min)	1575	5.00	2.95 (1.03, 7.27)
Peak Height (nM)	1575	194.6	0.97 (0.49, 3.08)
ttPeak (min)	1575	8.00	2.95 (0.93, 6.68)

* PPP – 5 pM tissue factor (TF)/ 4uM phospholipid (PS)

Table 4Time-to-Extreme Values for CAT Data Using PRP^{*} reagent

Variable	N	Extreme 5% Value	Time-to-Extreme Value (Days)
<u>Hypercoagulable</u>			
Lagtime (min)	1574	5.33	0.25 (0.07, 0.47)
Peak Height (nM)	1574	133.2	0.07 (0.05, 0.26)
ttPeak (min)	1574	10.89	0.20 (0.06, 0.45)
<u>Hypocoagulable</u>			
Lagtime (min)	1574	18.56	2.98 (0.95, 4.87)
Peak Height (nM)	1574	17.8	1.86 (0.75, 4.00)
ttPeak (min)	1574	29.45	2.95 (0.93, 5.69)

* PRP – 1pM tissue factor (TF)

Table 5

Time-to-Extreme Values for Microparticles

Variable	N	Extreme 5% Value	Time-to-Extreme Value (Days)
CD42 +, Procoagulant MPs (per uL plasma) *	1734	126	0.55 (0.25, 4.06)
Total Procoagulant MPs (per uL plasma)	1734	2278	0.25(0.05, 1.98)

* CD42+, Procoagulant MPs - Annexin V + MPs derived from platelets only

Table 6

Comparisons of CAT and MP Values with Degrees of Injury Severity

Variable	ISS < 15 (n = 261)	ISS 15-24 (n = 96)	ISS 25 (n = 86)	P-value
<u>5 pM TF/ 4 uM PS (PPP reagent)</u> Lagtime (min)	2.79 (2.38, 3.33)	2.67 (2.33, 3.11)	2.67 (2.20, 3.36)	<0.0001
Peak Height (nM)	342.6 (293.2, 396.8)	347.4 (290.2, 409.9)	319.4 (267.6, 376.2)	<0.0001
ttPeak (min) [#]	4.94 (4.33, 5.78)	4.56 (4.06, 5.14)	4.52 (3.92, 5.30)	<0.0001
<u>1pM TF (PRP reagent)</u> Lagtime (min)	8.67 (7.11, 11.00)	7.86 (6.67, 10.22)	8.22 (6.64, 10.21)	<0.0001
Peak Height (nM)	46.9 (33.2, 68.1)	49.8 (34.0, 73.4)	52.4 (35.9, 77.5)	0.0071
ttPeak (min) [#]	17.89 (15.33, 21.00)	16.70 (14.05, 19.36)	15.97 (13.56, 18.84)	<0.0001
Total Procoagulant MPs (per uL plasma)	405.6 (211.5, 786.9)	424.3 (237.9, 770.7)	372.7 (197.8, 758.3)	0.370
CD42 +, Procoagulant MPs (per uL plasma) [*]	20.6 (11.1, 42.1)	18.5 (9.9, 36.1)	19.0 (9.1, 42.9)	0.0520

[#] ttPeak – Time to peak^{*} CD42+, Procoagulant MPs – Platelet derived, procoagulant microparticles