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Cellular microparticle and thrombogram phenotypes in the Prospective Observational Multicenter Major Trauma Transfusion (PROMMTT) Study: correlation with coagulopathy

Nena Matijevic^a, Yao-Wei W. Wang^a, Charles E. Wade^a, John B. Holcomb^a, Bryan A. Cotton^a, Martin A. Schreiber^b, Peter Muskat^c, Erin E. Fox^a, Deborah J. del Junco^a, Jessica C. Cardenas^a, Mohammad H. Rahbar^d, and Mitchell Jay Cohen^e on behalf of the PROMMTT Study Group

Yao-Wei W. Wang: Yao-Wei.W.Wang@uth.tmc.edu; Charles E. Wade: Charles.E.Wade@uth.tmc.edu; John B. Holcomb: John.Holcomb@uth.tmc.edu; Bryan A. Cotton: Bryan.A.Cotton@uth.tmc.edu; Martin A. Schreiber: schreibm@ohsu.edu; Peter Muskat: muskatp@ucmail.uc.edu; Erin E. Fox: Erin.E.Fox@uth.tmc.edu; Deborah J. del Junco: Deborah.J.deljunco@uth.tmc.edu; Jessica C. Cardenas: Jessica.C.Cardenas@uth.tmc.edu; Mohammad H. Rahbar: Mohammad.H.Rahbar@uth.tmc.edu; Mitchell Jay Cohen: mcohen@sfghsurg.ucsf.edu

^aCenter for Translational Injury Research and Division of Acute Care Surgery, Department of Surgery, Medical School, University of Texas Health Science Center at Houston, 6431 Fannin St. MSB 5.240, Houston, TX 77030, USA

^bDivision of Trauma, Critical Care and Acute Care Surgery, School of Medicine, Oregon Health & Science University, 3181 Sam Jackson Rd SW, Mail Code L-611, Portland, OR 97035, USA

^cDivision of Trauma/Critical Care, Department of Surgery, College of Medicine, University of Cincinnati, 222 Piedmont Ave., Suite 7000, Cincinnati, OH 45219, USA

^dDivision of Clinical and Translational Sciences, Department of Internal Medicine, Medical School, Biostatistics/Epidemiology/Research Design Core, Center for Clinical and Translational Sciences and Division of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, University of Texas Health Science Center at Houston, 1100 Fannin St. UPB 1100.21, Houston, TX 77030, USA

^eDivision of General Surgery, Department of Surgery, School of Medicine, University of California San Francisco, 1001 Potrero Ave. Room 3C-38, San Francisco, CA 94110, USA

Abstract

Background—Trauma-induced coagulopathy following severe injury is associated with increased bleeding and mortality. Injury may result in alteration of cellular phenotypes and release of cell-derived microparticles (MP). Circulating MPs are procoagulant and support thrombin

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Corresponding author: Nena Matijevic, PhD, Professor of Surgery, 6431 Fannin St. MSB 5.240, University of Texas Health Science Center at Houston, Houston, TX 77030, Phone: (713) 500-6807, Fax: (713) 500-0683, nena.matijevic@uth.tmc.edu.

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generation (TG) and clotting. We evaluated MP and TG phenotypes in severely injured patients at admission, in relation to coagulopathy and bleeding.

Methods—As part of the Prospective Observational Multicenter Major Trauma Transfusion (PROMMTT) study, research blood samples were obtained from 180 trauma patients requiring transfusions at 5 participating centers. Twenty five healthy controls and 40 minimally injured patients were analyzed for comparisons. Laboratory criteria for coagulopathy was activated partial thromboplastin time (APTT) ≥ 35 sec. Samples were analyzed by Calibrated Automated Thrombogram to assess TG, and by flow cytometry for MP phenotypes [platelet (PMP), erythrocyte (RMP), leukocyte (LMP), endothelial (EMP), tissue factor (TFMP), and Annexin V positive (AVMP)].

Results—21.7% of patients were coagulopathic with the median (IQR) APTT of 44 sec (37, 53), and an Injury Severity Score of 26 (17, 35). Compared to controls, patients had elevated EMP, RMP, LMP, and TFMP (all $p < 0.001$), and enhanced TG ($p < 0.0001$). However, coagulopathic PROMMTT patients had significantly lower PMP, TFMP, and TG, higher substantial bleeding, and higher mortality compared to non-coagulopathic patients (all $p < 0.001$).

Conclusions—Cellular activation and enhanced TG are predominant after trauma and independent of injury severity. Coagulopathy was associated with lower thrombin peak and rate compared to non-coagulopathic patients, while lower levels of TF-bearing PMPs were associated with substantial bleeding.

Keywords

blood coagulation; cell-derived microparticles; hemorrhage; thrombin; trauma

Introduction

Traumatic injury remains the leading cause of death for younger populations (< 44 year) worldwide and the third leading cause of death in the US [1, 2]. A large number of trauma patients on admission do not need transfusions. However, about one quarter of all severe trauma patients experience acute, excessive, and uncontrolled hemorrhage, requiring administration of transfusion products. Trauma induced coagulopathy (TIC) develops early after injury and results from the combination of severe injury and hemorrhagic shock, including tissue damage, hypoperfusion, hemodilution, hypothermia, acidosis, inflammation, and with alterations of blood hemostasis system [3, 4]. Despite recent advances in trauma research, the pathophysiology and mechanisms of hemostatic and cellular alterations in early response to trauma are not well characterized. The PROMMTT study is the first multisite study to capture real time prospective data on trauma patients requiring transfusion [5, 6], offering a unique opportunity to explore cellular and hemostatic phenotypes in severely injured trauma patients.

Early coagulopathy has specifically been associated with the need for transfusions and increased risk of trauma-associated mortality [7-9]. Circulating platelets play a fundamental role in the hemostatic response to trauma by providing the surface for key reactions involved in thrombin generation (TG), and by generating highly procoagulant platelet microparticles

(PMP) [10]. In addition to platelet-generated, MP can be released by various cell types including endothelial and circulating blood cells [11]. Better understanding of the body's early response to trauma at a cellular level may contribute to generating new and timely therapeutic approaches by identifying patients at increased risk of developing complications.

We have previously demonstrated that elevated procoagulant PMPs directly contributed to enhanced TG in healthy donors [12], and that various MPs were elevated in shock patients at admission [13] compared to healthy controls. In the PROMMTT study, our aim was to characterize MP phenotypes and TG in severely injured trauma patients at admission, and to relate any significant changes to coagulopathy (defined by traditional clotting test APTT) and bleeding. We hypothesized that PROMMTT patients would have different MP and TG phenotypes compared to a sample of healthy controls, and that these differences would be greater in PROMMTT patients with coagulopathy.

Materials and Methods

PROMMTT was conducted at ten major level 1 trauma centers in the US where 12,560 patients were screened. The PROMMTT study design and findings have been extensively published [5, 6, 14, 15]. Briefly, all trauma patients meeting criteria for the highest level trauma activation were enrolled in the study from July 2009 to October 2010. Patients were excluded if they were pregnant, prisoners, or under 16 years old. Patients were additionally excluded if they were transferred from outside hospitals, had more than 5 minutes of cardiopulmonary resuscitation prior to or during the first 30 minutes of hospitalization, or did not receive at least one unit of red blood cells (RBC) within 6h after admission, resulting in 1,245 enrolled patients. The Institutional Review Boards from each of the 10 centers and the United States Army Human Research Protections Office approved the protocol.

In addition to collecting real time prospective data and routine clinical labs, the PROMMTT study offered a unique opportunity to collect 209 research blood samples at 5 of the participating centers. Blood specimens were collected at admission, typically within the first 30 min, centrifuged 15 min at 2,500g at room temperature, plasma was separated, frozen @-80C, and batch-shipped on dry ice for analyses to the central lab in Houston, TX. For MP analysis plasma samples were further centrifuged for 3 min at 13,000g. Research blood samples were not available from all centers due to site-specific IRB restrictions. Due to either inadequate blood draws (short or clotted samples, n=13), or missing admission data (n=16), 29 subjects were excluded, resulting in the final number of 180 subjects. Twenty five healthy controls and 40 trauma patients with minimal injuries (median injury severity score, ISS=2 [1, 5]) were used for comparisons. Research blood samples were collected for flow cytometric measurements of MP phenotypes and for evaluation of plasma hemostatic potential by measuring TG. Additional study details were published previously [5, 6, 14, 15].

Microparticle phenotypes

Blood samples for MP phenotypes were collected in 3.2% Na-citrate vacutainer tubes and processed as previously described [12], as recommended by the International Society on Thrombosis and Haemostasis SSC Collaborative workshop [16], in which our laboratory

also participated. Briefly, cellular MP counts and phenotypes were analyzed by multicolor flow cytometry (Gallios, Beckman Coulter, Inc., Miami, FL) with a protocol optimized by using fluorescent beads (0.5, 0.9, and 3 μm) (Megamix, BioCytex, Marseille, France). MPs were defined as particles less than 1 μm in size. MP phenotypes included platelet (PMP), red blood cell (RMP), leukocyte (LMP), endothelial MP (EMP), phosphatidyl-serine positive (AVMP), and tissue factor-bearing MPs (TFMP), which were identified using the following monoclonal antibodies: CD41, CD235a, CD45, CD51/CD144, annexin V, and CD142, respectively. Specifically, PMP were identified by their dual positivity/staining for CD41 and annexin V, RMP by their dual positivity for CD235a and annexin V, LMP by dual positivity for CD45 and annexin V, and EMP for positive staining for CD144 and annexin V or CD144, CD51 and annexin V, respectively. TF-positive MPs were identified as MPs stained with antibody to CD142. Flowcount beads (Flow-Count, Beckman Coulter) with known concentration were used to calculate the absolute MP counts/ μL of plasma.

Thrombogram phenotypes

Plasma hemostatic potential was evaluated from citrated plasma by measuring TG, using the Calibrated Automated Thrombogram (CAT; Thrombinoscope, Maastricht, Netherlands) [17, 18]. The reaction was triggered with platelet poor plasma-low reagent, (PPP-Low, 1pM TF, 4 $\mu\text{mol/L}$ phospholipids). Thrombogram (thrombin generation curve), was characterized by 6 parameters: the lag time (representing the time until initial thrombin had formed [min]), thrombin peak time (TPT, min), thrombin peak height (TPH, nM thrombin), the endogenous thrombin potential (ETP) (nM thrombin*time, reflects the area under the curve, representing total amount of thrombin formed over time), the rate of thrombin generation (TGR, slope of the thrombin curve = $\text{TPH}/[\text{TPT} - \text{lag time}]$), and the termination time ratio, representing the time to bring TG to baseline (TTR, termination time rate = $(\text{start tail} - \text{TPT})/(\text{TPT} - \text{lag time})$).

Statistical analysis

Descriptive statistics are expressed as the mean and standard deviation (SD) or median and interquartile range (IQR: Q1-Q3 where Q1 and Q3 are the first and third quartiles) for continuous variables and proportions (%) for categorical variables. In order to evaluate whether the subset of 180 subjects was representative of the whole cohort in clinical characteristics, our study sample of PROMMTT patients with cellular and thrombogram results ($n=180$) was compared with the entire PROMMTT cohort ($n=1245$), using chi square analysis (categorical variables) and analysis of variance or its nonparametric counterpart (e.g., Mann-Whitney-U tests) for continuous variables depending on whether the underlying assumptions of the distribution were met (i.e., normal distributions with unequal variance). P values of 0.05 or less were considered as a crude indicator of potential clinical significance, without adjustment for multiple comparisons. No significant differences were noted between the groups, demonstrating the subset was representative of the study cohort (data not shown).

For this PROMMTT study population, coagulopathy was previously defined by either the INR cut-point of 1.3 or by the APTT cut-point of 35 sec; although the INR has been commonly used as trauma-related indicator of coagulopathy, in this exploratory analysis we

selected the APTT criteria for coagulopathy instead of INR, for two reasons: first, the APTT reflects the activities of the majority of the coagulation factors in the intrinsic and in the common procoagulant pathway, and second, INR was designed and intended only for patients on oral anticoagulant therapy for monitoring oral anticoagulant therapy across different labs, and not for screening of coagulation disorders.. The resulting two subgroups were compared on clinical characteristics including other coagulation test results [14]. The same statistical approaches were also applied in comparing the PROMMTT study sample with our samples of minimally injured patients and healthy controls on their respective MP and TG profiles.

In addition to coagulopathy, we sought to identify *substantial bleeding* among the PROMMTT patients, i.e., those at highest risk of hemorrhagic death or in need of the Blood Bank's Massive Transfusion protocol [5]. We classified substantially bleeding patients as those who, within 4 hours of admission, either received ≥ 5 RBC units < 2 hours apart or died < 2 hours after the last RBC unit, or within 24 hours of admission, either received 10 or more RBC units (conventional definition of massive transfusion) or died of hemorrhage (exsanguination) as the primary cause of death.

Univariate analysis identified multiple MP and TG parameters associated with coagulopathy or substantial bleeding at an unadjusted 0.05 level of significance. In exploratory logistic regression modeling, we applied the Holm-Sidak p value adjustment to help identify candidate associations worthy of further research [19, 20]. All analyses were conducted with SAS version 9.2 [21] and STATA version 12 [22].

Results

Patient Characteristics

Patient characteristics are presented in Table 1. These patients represent a severely injured (median ISS, 26) relatively young cohort (median age, 34) with a mortality rate of 26%. Using a priori criteria of APTT ≥ 35 sec, 21.7% of patients were coagulopathic (TIC+) on admission with a median APTT of 44 sec (37, 53). When compared to noncoagulopathic (TIC-), TIC+ patients were more severely injured [34 (25, 44) vs. 25 (16, 34), $p < 0.001$], had more severe shock (systolic blood pressure 101.5 ± 33.8 mmHg vs. 110.4 ± 29.9 mmHg, $p < 0.001$), higher substantial bleeding (69.2 vs. 27%, $p < 0.0001$), higher preponderance of multiorgan failure (3.7% vs. 1.0, $p < 0.01$), and a significantly higher in-hospital mortality rate (52.3% vs. 12.4%, $p < 0.001$). Details of clinical predictors of TIC were previously published [14].

Microparticle phenotypes

MP phenotypes in controls and patients are presented in Figure 1. While differences in PMP counts did not reach statistical significance, most likely due to high variance, RMP, LMP, EMP counts were significantly higher in patients compared to controls. Specifically, EMP counts were 20-fold higher in PROMMTT patients' plasma compared to controls. In addition, relative distributions of MP phenotypes were different between trauma patients and controls (Fig. 2). There were no statistically significant differences in MP phenotypes

between PROMMTT trauma patients and trauma patients with minimal injuries (data not shown). However, when MP phenotypes were compared between TIC+ and TIC-, there were significantly lower counts of circulating PMP, AVMP, and TFMP in TIC+ patients (Table 2).

Thrombogram phenotypes

Comparison of TG results in controls and patients are presented in Table 3, showing significant differences between the two groups. Trauma patients had enhanced TG after injury, demonstrated by significantly higher ETP, TPH, TGR and TTR. Representative thrombogram tracings for control, TIC-, and TIC+ patients are presented in Figure 3. All PROMMTT trauma patients, coagulopathic and noncoagulopathic, as well as trauma patients with minimal injuries (median ISS=2) were hypercoagulable compared to healthy control (Fig. 4). However, TIC+ had significantly lower TPH and TGR, and longer lagtime and TPT compared to TIC- (Table 3). Finally, we correlated TG with levels of circulating MPs, and found that PMP correlated positively ($r=0.362$) and EMP correlated inversely with TPH ($r=-0.325$).

Univariate analysis identified several MP or TG parameters to be potential candidates for significant association with clinically important bleeding. After adjusting for Glasgow Coma Scale, ISS, heart rate, and systolic blood pressure, the following parameters were identified: TPH, TPT, and TGR were significantly associated with APTT-coagulopathy, while PMP counts were significantly associated with substantial bleeding. Finally, a multivariable analysis, adjusting for age, gender, race, injury severity, and shock severity, revealed that both TPH [OR 0.99 (95%CI 0.984-0.996, $p=0.002$), and TPT [OR 1.70 (95%CI 1.076-2.684, $p=0.023$) remained significantly associated with APTT-defined coagulopathy. Importantly, multivariable analysis further identified PMP bearing TF (CD41+CD142+) to be independently associated with substantial bleeding [OR 0.892 (95%CI 0.973-0.990, $p<0.0001$). When analyzed by outcome, patients who died within 24h of admission had significantly lower TPH ($p<0.01$), and TGR ($p<0.02$), as well as PMP, RMP, and TFMP compared to survivors (all $p<0.01$). Patients who died within 12h had the lowest TPH and PMP (data not shown).

Discussion

While TIC has been widely reported to be associated with poor prognosis, the pathophysiology remains poorly understood. In this exploratory analysis, we examined microparticle and thrombogram phenotypes in severely injured trauma patients upon admission, and their relationship to coagulopathy and substantial bleeding. We documented increased platelet, RBC, leukocyte, and endothelial activation, identified by increased presence of specific cellular microparticles. We observed an enhanced TG after trauma, compared to healthy controls, and it was independent of injury severity. However coagulopathic patients, had significantly lower levels of PMP and lower thrombin peak and rate compared to non-coagulopathic patients. ETP was higher in trauma patients compared to controls, and in TIC-compared to controls, but it was similar between TIC+ and controls.

There are limited reports on cellular activation after trauma, the role of which is not fully elucidated [23, 24].

Activated platelets secrete procoagulant microparticles, small cellular membrane vesicles ($<1\ \mu\text{m}$). They are considered essential for hemostasis, as they expose phospholipids, adhesion receptors, and TF on their surface, providing binding sites for activated clotting factors and thrombin generation [12, 25, 26]. It has been reported that PMPs have 100-fold higher procoagulant activity than platelets [27]. We and others have documented that PMP are the most abundant MP in the healthy population [11, 12]. A total or differential increase in circulating MPs has been reported in a variety of disease conditions [28, 29]. However, the level, source, or pathophysiological significance of circulating MPs after severe trauma has not been systematically studied. In the PROMMTT study, trauma patients had lower relative percentages of PMP compared to healthy donors (64% vs. 87%), contrary to previous reports in patients with blunt trauma [30]. Furthermore, circulating PMPs were significantly lower in TIC+ PROMMTT patients compared to TIC-. Since TIC+ patients had lower admission platelet counts compared to TIC- ($p<0.01$) it could have potentially contributed to observed lower PMP numbers in TIC+. Importantly, PMPs, which expresses TF, were identified as a predictor of substantial bleeding (as defined in the Statistical Analysis section of the Methods). To our knowledge, this is the first clinical report of the association of TFMP with substantial bleeding early after acute trauma. This is consistent with data showing that MPs expressing both TF and phosphatidyl serine have the highest procoagulant activity and would potentially contribute most to clot generation [31]. However, according to some reports, the presence of TF on PMPs is controversial and it may in fact represent fused MP from different cell types. There are several studies documenting clinical relevance of MPs in supporting coagulation [32-38]. We and others have previously shown that PMPs, generated during extended blood storage, contribute to functional hemostasis in stored plasma and correlate with enhanced thrombin generation [12, 39]. However, since MPs are heterogeneous and contain many proteins with opposing activities (pro- and anti-coagulants, proteases and their inhibitors), more detailed studies are needed to elucidate their specific roles in severe trauma with coagulopathy.

In normal human blood, beside PMPs, low levels of other circulating MPs are detectable, including RMP, LMP and EMP. PROMMTT trauma patients had higher numbers of EMP, RMP and LMP. In healthy donors EMPs represent only 1% of total MPs, while in severely injured trauma patients they contributed 14% and were 20-fold higher in number than in controls. EMPs were high in both TIC+ and TIC- patients, and even in trauma patients with minor injuries. This suggests that elevated EMPs represent endothelial response to injury, likely independent of injury severity. Surprisingly, Park et al. detected EMPs in only one of 59 patients with blunt trauma [30]. It has been reported that EMPs may participate in coagulation, inflammation, and in a variety of other biological processes [40, 41], by supporting TG, anticoagulation, or fibrinolysis [40-44]. Interestingly, in PROMMTT patients PMPs were correlated positively with TG, while EMPs had an inverse correlation with TG. This possibly reflects the activity of thrombomodulin-bearing EMPs in plasma. Of note, the ratio of PMP:EMP in healthy donors was 100:1 and in trauma patients only 6:1, indicating a major shift in MP-dependent hemostatic balance and endothelial damage, which requires further characterization. There is great variability and often contradictory reports on

cellular activation and MP significance in clinical studies [45-49], resulting from different methodologies used, the standardization of which is currently the subject of intensive efforts by the International Society on Thrombosis and Haemostasis SSC Collaborative Workshop working group [50].

Finally, our results show PROMMTT trauma patients at admission had significantly enhanced thrombin production compared to healthy controls, even trauma patients with only minor injuries, suggesting that TG reflects systemic coagulation response that was independent of injury severity. Hypercoagulability after trauma has been reported previously [50-52]. Many factors potentially contributed to enhanced TG, including activated platelets and their release products, elevated MPs, especially TF-bearing MPs and possibly histones released from damaged cells [53]. Despite overall enhanced TG in comparison to controls, TIC+ patients had significantly lower levels of produced thrombin, lower thrombin generation rates, and longer lagtimes and times to thrombin peak, than TIC-. There are few studies reporting TG in trauma, with conflicting results. Park et al. found no difference in the amount of native TG in 52 blunt trauma patients compared to 19 controls [30], while Dunbar et al. [54] reported excessive native TG in trauma patients, which was in line with an early study by Gando et al. [55] that reported increased levels of TF in trauma patients which correlated with increased levels of TG *in vivo*. Schols and colleagues [56] recently reported that low plasma thrombin peak was associated with ongoing bleeding in patients during major surgery and dilutional coagulopathy. Our results, combined with some of the previous reports, suggest that early enhanced TG is a hallmark of traumatic injury, regardless of how substantial the injuries or presence of coagulopathy. The physiologic response to injury is to upregulate plasma procoagulant activity, reflecting the sensitivity of the hemostasis system to provocation, and protection against blood loss. However, there may be some threshold of coagulation activation that must be achieved to prevent coagulopathy and substantial bleeding. It is likely that at some point we may want to consider redefining the “normal ranges” for hemostatic variables for this particular patient population.

Several limitations are important to interpret our exploratory analysis of the relationship between admission lab values and outcomes. First, the arbitrary APTT cutoff used to define TIC may not be the most appropriate for severe bleeding or coagulopathy. Second, obtaining blood specimens from severely injured patients is sometimes difficult which might potentially affect the assay results. Third, although we employed the International Society on Thrombosis and Haemostasis recommended MP standardized protocol, flow cytometry still presents some limitations such as threshold for MP size detection. Consequently, MPs of smaller sizes, undetectable in this study, may have different phenotypes and/or significances. These issues have been resolved in the just completed Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) study conducted at 12 trauma centers in the US and Canada, with research blood sampling at multiple time points, and use of new generation, high sensitivity flow cytometers, and comprehensive functional hemostatic, coagulation, cellular, and inflammatory measures to characterize complex pathways after trauma. These data are forthcoming.

In conclusion, this study reported that severe trauma results in cellular activation and enhanced TG, independent of ISS. Coagulopathy and substantial bleeding were associated with lower TG and lower levels of TF-bearing PMPs, respectively.

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PROspective Observational Multicenter Major Trauma Transfusion (PROMMTT) Study Group:

Data Coordinating Center: Mohammad H. Rahbar, PhD (principal investigator); John B. Holcomb, MD (co-investigator); Erin E. Fox, PhD (co-investigator and study coordinator); Deborah J. del Junco, PhD (co-investigator); Bryan A. Cotton, MD, MPH (co-investigator); Charles E. Wade, PhD (co-investigator); Jiajie Zhang, PhD (co-investigator); Nena Matijevic, PhD (co-investigator); Yu Bai, MD, PhD (co-investigator); Weiwei Wang, PhD (co-investigator); Jeanette Podbielski, RN (study coordinator); Sarah J. Duran, MSCIS (data manager); Ruby Benjamin-Garner, PhD (data manager); Robert J. Reynolds, MPH (data manager); Xuan Zhang, MS (data analyst); Aisha Dickerson, MSPH (graduate assistant); Elizabeth S. Camp, MSPH (data analyst).

PROMMTT Clinical Sites:

Brooke Army Medical Center: Christopher E. White, MD (principal investigator); Kimberly L. Franzen, MD (co-investigator); Elsa C. Coates, MS, RN (study coordinator).

Medical College of Wisconsin: Karen J. Brasel, MD, MPH (principal investigator); Pamela Walsh (study coordinator).

Oregon Health and Sciences University: Martin A. Schreiber, MD (principal investigator); Samantha J. Underwood, MS (study coordinator); Jodie Curren (study coordinator).

University of California, San Francisco: Mitchell J. Cohen, MD (principal investigator); M. Margaret Knudson, MD (co-investigator); Mary Nelson, RN, MPA (study coordinator); Mariah S. Call, BS (study coordinator).

University of Cincinnati: Peter Muskat, MD (principal investigator); Jay A. Johannigman, MD (co-investigator); Bryce RH Robinson, MD (co-investigator); Richard Branson (co-investigator); Dina Gomaa, BS, RRT (study coordinator); Cendi Dahl (study coordinator).

University of Pittsburgh Medical Center: Louis H. Alarcon, MD (principal investigator); Andrew B. Peitzman, MD (co-investigator); Stacy D. Stull, MS, CCRC (study coordinator); Mitch Kampmeyer MPAS, CCRC, PA-C, (study coordinator); Barbara J. Early, RN, BSN, CCRC (study coordinator); Helen L. Shnol, BS, CRC (study coordinator); Samuel J. Zolin, BS (research associate); Sarah B. Sears, BS (research associate).

University of Texas Health Science Center at Houston: John B. Holcomb, MD (co-principal investigator); Bryan A. Cotton, MD, MPH (co-principal investigator); Marily Elope, RN (study coordinator); Quinton M. Hatch, MD (research associate); Michelle Scerbo (research associate); Zerremi Caga-Anan, MD (research associate).

University of Texas Health Science Center at San Antonio: John G. Myers, MD (co-principal investigator); Ronald M. Stewart, MD (co-principal investigator); Rick L. Sambucini, RN, BS (study coordinator); Marianne Gildea, RN, BSN, MS (study coordinator); Mark DeRosa CRT (study coordinator); Rachelle Jonas, RN, BSN (study coordinator); Janet McCarthy, RN (study coordinator).

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Highlights

- Trauma-induced coagulopathy is associated with increased bleeding and mortality.
- We evaluate thrombin generation and microparticles in trauma patients.
- Enhanced thrombin generation at admission is independent of injury severity.
- Elevated cell-derived microparticles indicate increased cellular activation.
- Coagulopathy correlates with lower thrombin and lower platelet microparticles.

Figure 1. Microparticle Phenotypes in Controls and PROMMTT Trauma Patients

PMP=platelet microparticles; EMP=endothelial microparticles; RMP=red blood cell microparticles; LMP=leukocyte microparticles. *p<0.02, **p<0.001, ***p<0.00001.

Figure 2. Microparticle Phenotypes Distribution in Controls and PROMMTT Trauma Patients
PMP=platelet microparticles; RMP=red blood cell microparticles; LMP=leukocyte microparticles; EMP=endothelial microparticles

Figure 3. Thrombogram Phenotypes in Controls and PROMMTT Trauma Patients
TIC-=noncoagulopathic; TIC+=coagulopathic patients.

Figure 4. Thrombin Peak Height (TPH) and Thrombin Generation Rate (TGR) in Controls and Trauma Patients

TIC-=noncoagulopathic; TIC+=coagulopathic; a=significantly different from healthy controls; b=significantly different from minor trauma; c=significantly different from TIC-; ISS=injury severity score.

Table 1
PROMMTT Patients Demographics and Characteristics by APTT-defined Coagulopathy, 35sec (median, IQR)

	PROMMTT patients (n=180)	TIC- (n=141)	TIC+ (n=39)	P TIC+ vs. TIC-
Age, yrs	34 (23, 51)	34 (23, 51)	33 (21, 47)	> 0.10
Male, %	69.4	75.9	65.5	> 0.10
Penetrating, %	38.8	41.8	30.9	> 0.10
ISS	26 (17, 34.7)	25 (16, 34)	34 (25, 44)	<0.001
Heart rate	106.0 (85.25, 128.5)	105 ± 26.2	110.5 ± 34	<0.05
SBP, mmHg	105.0 (82.0, 125.0)	110.4 ± 29.9	101.5 ± 33.8	<0.001
PLT, ×10 ⁹ /L	237 (184, 279)	248 (195, 283)	197 (166, 245)	<0.01
Substantial bleeding, %	36.1	27	69.2	<0.0001
MOF, %	2.0	1.0	3.7	<0.01
Mortality, %	26.0	12.4	52.3	<0.001
INR	1.25 (1.12, 1.5)	1.2 (1.1, 1.3)	1.7 (1.4, 2.1)	<0.001
APTT, sec	27.8 (24.5, 33.1)	27 (23, 29)	44 (37, 53)	<0.001

PROMMTT, Prospective Observational Multicenter Major Trauma Transfusion Study; APTT, activated partial thromboplastin time; TIC-, noncoagulopathic; TIC+, coagulopathic; ISS, injury severity score; SBP, systolic blood pressure; PLT, platelets; MOF, multi-organ failure; INR, international normalized ratio

Table 2
Microparticle Phenotypes in PROMMTT Patients, TIC- and TIC+ (median, IQR)

MP/ μ L	TIC- (N=141)	TIC+ (N=39)	p
PMP	2234 (1391, 3442)	1999 (1254, 2624)	<0.001
RMP	186 (110, 406)	215 (92, 301)	>0.10
LMP	338 (216, 544)	453 (339, 628)	>0.10
EMP	468 (305, 613)	524 (399, 686)	>0.10
TFMP	206 (43, 270)	60 (33, 195)	<0.01
AVMP	2465 (1388, 3863)	1990 (906, 3079)	<0.05

PROMMTT, PROspective Observational Multicenter Massive Trauma Transfusion study; TIC-, noncoagulopathic; TIC+, coagulopathic; MP, microparticles; PMP, platelet microparticles; RMP, red blood cell microparticles; LMP, leukocyte microparticles; EMP, endothelial microparticles; TFMP, tissue-factor-bearing microparticles; AVMP, phosphatidyl-serine positive microparticles.

Table 3
Thrombogram Parameters in Controls and PROMMTT Trauma Patients, TIC- and TIC+, median (IQR)

CAT	Controls (N=25)	PROMMTT (N=180)	P	TIC- (N=141)	TIC+ (N=39)	P TIC- vs. TIC+	P TIC- vs. CTRL	P TIC+ vs. CTRL
Lagtime	4.2 (3.4, 5.4)	3.8 (3.3, 4.3)	>0.10	3.7 (3.3, 4.3)	4.3 (3.8, 4.9)	<0.01	0.05	>0.10
ETP	1331 (1246, 1474)	1539 (1292, 1758)	<0.0001	1556 (1321, 1764)	1397 (1192, 1846)	>0.10	<0.0001	>0.10
TPT	11 (9.6, 13.4)	5.4 (4.7, 5.9)	<0.0001	5.3 (4.7, 5.8)	6.0 (5.4, 7.3)	<0.002	<0.0001	<0.0001
TPH	96 (87, 137)	358 (297, 411)	<0.0001	373 (316, 429)	292 (207, 331)	<0.0001	<0.0001	<0.0001
TGR	15 (10, 21)	260 (199, 318)	<0.0001	263 (202, 323)	166 (116, 213)	<0.0001	<0.0001	<0.0001
TTR	3.6 (3.3, 3.8)	10.7 (8.8, 12.8)	<0.0001	10.9 (8.9, 12.8)	10.2 (8.3, 13)	>0.10	<0.0001	<0.0001

PROMMTT, Prospective Observational Multicenter Major Trauma Transfusion study; TIC-, noncoagulopathic; TIC+, coagulopathic; CAT, calibrated automated thrombogram; CTRL, healthy controls; ETP, Endogenous Thrombin Potential; TPT, Thrombin Peak Time; TPH, Thrombin Peak Height; TGR Thrombin Generation Rate; TTR, Termination Time Ratio.