Surgery 166 (2019) 1122–1127

Contents lists available at ScienceDirect

Surgery

journal homepage: www.elsevier.com/locate/surg

Presented at the Academic Surgical Congress 2019

Whole blood thrombin generation is distinct from plasma thrombin generation in healthy volunteers and after severe injury

Julia R. Coleman, MD, MPH[a,*], Ernest E. Moore, MD[b], Jason M. Samuels, MD[a], Joshua J. Ryon, BA[b], Jesse T. Nelson, BS[c], Alexander Olson, BS[d], Sandi Caus, BS[d], Matthew G. Bartley, MD[a], Navin G. Vigneshwar, MD[a], Mitchell J. Cohen, MD[b], Anirban Banerjee, PhD[a], Christopher C. Silliman, MD, PhD[e,f], Saulius Butenas, PhD[d]

[a] Department of Surgery, University of Colorado-Denver, Aurora, CO
[b] Department of Surgery, Ernest E Moore Shock Trauma Center at Denver Health, CO
[c] Rosalind Franklin School of Medicine, North Chicago, IL
[d] Department of Biochemistry, University of Vermont, Burlington, VT
[e] Department of Hematology, Children's Hospital of Colorado, Aurora, CO
[f] Vitalant Research Institute, Denver, CO

ARTICLE INFO

Article history:
Accepted 3 July 2019
Available online 13 September 2019

ABSTRACT

Background: Plasma thrombin generation has been used to characterize trauma-induced coagulopathy, but description of whole blood thrombin generation is lacking. This study aimed to evaluate plasma and whole blood thrombin generation in healthy volunteers and trauma patients. We hypothesized that (1) plasma and whole blood thrombin generation are distinct, (2) whole blood thrombin generation is more pronounced in trauma patients than in healthy volunteers, and (3) thrombin generation correlates with clinical coagulation assays.

Methods: Blood was collected from healthy volunteers and trauma patients at a single, level-1 trauma center. Whole blood thrombin generation was assessed with a prototype point-of-care whole blood thrombin generation device, and plasma thrombin generation was measured with a calibrated automated thrombogram analogue. Plasma and whole blood thrombin generation were compared and correlated with international normalized ratio and thrombelastography.

Results: Overall, 10 healthy volunteers (average age 30, 50% men) were included and 58 trauma patients (average age 34, 76% men, 55% blunt mechanism, and with a median new injury severity score of 17) were included. Plasma and whole blood thrombin generation differed with more robust thrombin generation in plasma. Trauma patients had a significantly increased whole blood thrombin generation compared with healthy volunteers]. Plasma thrombin generation correlated with international normalized ratio, whereas whole blood thrombin generation did not correlate with thrombelastography.

Conclusion: Plasma and whole blood thrombin generation are distinct, highlighting the need to perform standardized assays to better understand their correlation and to assess how whole blood thrombin generation confers differential outcomes in trauma.

Introduction

Trauma-induced coagulopathy (TIC) is one of the leading causes of preventable death in injured patients.[1] Although TIC includes a spectrum of phenotypes from a hypocoagulable to a hypercoagulable state, it is the former, characterized by prolonged clot formation and accelerated fibrinolysis, which poses the greatest risk of early mortality after traumatic injury.[2] Approximately 15% of trauma patients present in this hypocoagulable profile owing to a combination of shock, depletion of blood factors, platelet dysfunction, endotheliopathy, and fibrinolysis.[2–4] Despite this pathologic coagulation cascade, the majority of trauma patients paradoxically present with increased thrombin generation.[5–7] This observation has been described in plasma from severely injured

Presented at the Annual Academic Surgical Congress on February 06, 2019 in Houston, TX.

* Reprint requests: Julia Coleman, MD, MPH, University of Colorado-Denver, 12631 E. 17th Ave, C302, Mailbox: Julia Coleman, Aurora, CO 80045.
E-mail address: julia.coleman@ucdenver.edu (J.R. Coleman).

https://doi.org/10.1016/j.surg.2019.07.014
0039-6060/

patients. However, the description of whole blood thrombin generation (TG) is lacking.[6,7] Given that platelets are critical in initiating clot formation, characterizing TIC in whole blood assays is crucial. This need has been recognized in other hematologic pathologies, such as hemophilia, where point-of-care whole blood TG is being explored as a diagnostic and therapeutic tool. Although the plasma-based prothrombin time/international normalized ratio (PT/INR) and whole blood thrombelastography (TEG) have been compared, differences between plasma and whole blood TG have yet to be investigated fully.

This study aimed to compare plasma and whole blood TG in healthy volunteers and trauma patients and to examine their respective correlations to conventional plasma-based and whole blood viscoelastic assays. We hypothesize that plasma and whole blood TG are distinct, and whole blood TG is more pronounced in trauma patients than healthy volunteers. Because whole blood TG and citrated rapid TEG are both measured in whole blood in the presence of exogenous tissue factor, and plasma TG and PT/INR are both measured in plasma after addition of phospholipids (PL), we also hypothesize that plasma TG correlates to PT/INR, whereas whole blood TG correlates to TEG.

Methods

Study design

To compare plasma and whole blood TG among healthy volunteers, blood was collected from healthy adult (≥18 years) donors recruited at Denver Health. This study was approved by the Colorado Multiple Institutional Review Board (COMIRB 18-0625), and all subjects provided informed consent. We collected basic demographic data, including age, sex, and body mass index.

For our analysis of trauma patients, we conducted an analysis of prospectively collected data from our Trauma Activation Protocol (TAP), a registry which includes all trauma activation patients from 2015 to 2017 at the Ernest E. Moore Shock Trauma Center at Denver Health, an American College of Surgeons verified and Colorado state certified, academic level-1 trauma center. The TAP study was approved by the Colorado Multiple Institution Review Board (COMIRB#13-3087) and performed under waiver of consent. Clinical data were collected by trained research professional assistants and included age, sex, body mass index, mechanism of injury, new injury severity score (NISS), field and hospital arrival systolic blood pressure (SBP), Glasgow Coma Scale, base deficit, complete blood count, PT/INR, partial thromboplastin time, number of blood products transfused, clinical outcomes, and mortality.

Participants

For our healthy volunteer recruitment, volunteers were screened via a questionnaire (Supplemental Digital Content 1) to determine eligibility. Volunteers were excluded if they had any conditions known to affect coagulation as listed in the questionnaire.

Criteria for inclusion in TAP were adult patients (≥18 years old) who presented to the hospital as a trauma activation, an emergency department walk-in, or non-transfer patients who were upgraded to a trauma activation on arrival. The criteria for activation were traumatic injury with any of the following: (1) Glasgow Coma Scale < 8 with presumed thoracic, abdominal, or pelvic injury; (2) respiratory compromise with presumed thoracic, abdominal, or pelvic injury; (3) blunt trauma with SBP < 90 mm Hg; (4) mechanically unstable pelvic injury; (5) penetrating injuries to the neck or torso with SBP < 90 mm Hg or that require endotracheal intubation; (6) amputation proximal to the ankle or wrist; or (7) when the emergency medicine attending or the chief resident in surgery suspected that urgent operative intervention may be required. Exclusion criteria include any patient <18 years, patients whose initial blood sample was not collected within 1 hour of injury, infusion of blood products before collection of blood samples, consultations from external hospitals, documented chronic liver disease (total bilirubin >2.0 mg/dL or advanced cirrhosis discovered on laparotomy), known inherited defects of coagulation function (eg hemophilia or Von Willebrand's disease), subsequent downgrades from trauma activation, and patients who were pregnant or prisoners. Patients were removed from the study if any of these criteria became known after activation.

Procedures

Whole blood samples were collected in healthy volunteers and trauma activation patients at the scene by trained paramedics or on hospital presentation within 1 hour of injury in citrated vacuum tubes (3.5 mL, 3.2% sodium citrate) for TEG and plasma calibrated automated thrombogram (CAT) and sodium citrate-corn trypsin inhibitor (CTI) vacuum tubes (3.2% sodium citrate with 100 μg/mL CTI, which inhibits the contact pathway of coagulation) for whole blood TG. A team of trained research professional assistants completed whole blood TG and TEG within 1 and 2 hours of venipuncture, respectively, in the research lab adjacent to the emergency department. Citrated rapid (CR) TEG was performed with the TEG 5000 Thrombelastography Hemostasis Analyzer (Haemonetics, Niles, IL) according to the manufacturer instructions,[8] and whole blood TG was performed with the prototype point-of-care WB TG device (near patient testing thrombin generation). This latter, experimental, point-of-care prototype was created for the evaluation of TG in WB and, while not yet FDA-approved, it is currently undergoing laboratory evaluation at 3 trauma centers. The device functions by activating thrombin generation by relipidated tissue factor, after which point, the reaction is monitored continuously by means of a thrombin-specific, fluorogenic substrate. Change in the intensity of fluorescence produced by the cleavage of the fluorogenic substrate by thrombin is monitored over time and compared to an internal calibrator with known stable thrombin activity. Whole blood TG was initiated using 6.5 pM relipidated tissue factor reagent and 15 mM CaCl2 (Stago, Parsippany, NJ), and TG curves were recorded continuously for 90 min at a rate of 3 readings per min alongside an internal calibrator. Remaining blood after TEG and whole blood TG was spun down to platelet-poor plasma (1,000 g at 4°C for 15 min, then 12,600 g at 4°C for 6 min) for storage in -80°C until time of plasma TG with a BioTek plate reader (BioTek Instruments, Inc, Winooski, VT) (CAT analogue). Plasma CAT TG was initiated using 20 μM (final concentration) of artificial acidic PL (prepared inhouse) and 15 mM CaCl2, and TG curves were recorded continuously for 60 min at a rate of 3 readings per min.

CR TEG yields the following variables: activated clotting time (ACT, time elapsed from initiation of test until onset of clot formation in seconds), angle (rate of clot strength increase in degrees), maximum amplitude (MA, maximal clot strength achieved in millimeters), and percent clot lysis 30 minutes after reaching MA (lysis 30 minutes after MA in %). The measurements for TG include lag time (minutes from calcium addition to 10 nM of thrombin detected), peak thrombin (the maximum thrombin concentration [in nM] during the assay), time to peak thrombin (min), maximum rate of thrombin generation (nM/min, also known as velocity index), and endogenous thrombin potential (ETP, the total thrombin which can be generated in nM/min).

Table I
Comparison of plasma and whole blood thrombin generation measurements in healthy volunteers and trauma patients

	Plasma	Whole blood	*P* value
Healthy volunteers ($n = 10$)			
Lag time (min)	7.0 (5.8–7.9)	7.0 (6.0–8.0)	.97
Peak thrombin (nM)	123.5 (96.5–157.3)	82.5 (75.2–91.6)	.02
Time to peak thrombin (min)	13.0 (11.3–15.0)	10.5 (9.7–12.2)	.04
Maximum rate (nM/min)	62.2 (49.5–67.5)	21.3 (17.8–26.1)	.02
ETP (nM.min)	1,801 (1,627—2,025)	728.4 (634–797)	.002
Trauma patients ($n = 58$)			
Lag time (min)	5.4 (3.9–6.8)	3.8 (2.6–4.9)	< .0001
Peak thrombin (nM)	318 (234–386)	126 (84–150)	< .0001
Time to peak thrombin (min)	8.7 (7.3–10.3)	6.6 (4.9–9.2)	< .0001
Maximum rate (nM/min)	168 (122–213)	42.6 (23–64)	< .0001
ETP (nM.min)	2425 (2097–2726)	823.2 (708–972)	< .0001

Numbers presented as median with 25 and 75 interquartile range.

Statistical analysis

The outcomes of interest were the plasma and whole blood TG measurements: lag time, peak thrombin, time to peak thrombin, maximum rate of thrombin generation, and ETP. Plasma and whole blood TG measurements were compared for each individual healthy volunteer and trauma patient with the Wilcoxon matched paired signed rank test. Whole blood TG between healthy volunteers and trauma patients were compared with the Mann-Whitney test. Because TG provides a detailed description of the enzymatic cascade to form thrombin, we also compared the correlation of plasma TG to plasma-based PT/INR and WB TG to CR-TEG ACT and angle (TEG measurements representative of the enzymatic phase of coagulation) using Pearson's correlation. Statistical analyses were performed using R software.[9] Statistical significance was established at $P < .05$.

Results

Healthy volunteers: plasma and whole blood TG

Overall, 10 healthy volunteers were included in this study. Half were men and the average age was 30 years. There were significant differences between whole blood and plasma TG (Table I). Compared to whole blood TG, plasma TG reflected a greater peak thrombin (123.5 nM vs 82.5 nM, $P = .02$), greater time to peak thrombin (13.0 min vs 10.5 min, $P = .04$), greater maximum rate (62.2 vs 21.3 nM/min, $P = .02$), and greater ETP (1,801 vs 728 nM/min, $P = .002$).

Trauma patients: Plasma and whole blood TG

Overall, 58 trauma patients were included in this study (Table II). The average age was 34 years, and the majority (76%) were men. Approximately half (55%) presented after blunt trauma, and the median NISS was 17. The median presenting SBP was 119 mm Hg (92–126 interquartile range), and 21% presented in shock (SBP < 90 mm Hg). Whereas only 5% of patients required massive transfusion, 44% required red blood cell transfusion in the first 24 hours. Mortality rate was 17%.

As seen in the healthy volunteers, there were significant differences between plasma and whole blood TG measurements. Compared to whole blood, plasma lag time was greater (5.4 min vs 3.8 min), peak thrombin was greater (317.5 nM vs 125.5 nM), time to peak thrombin was greater (8.7 min vs 6.6 min), maximum rate was greater (168 nM/min vs 42.6 nM/min), and ETP was greater (2,425.0 vs 823.2 nM/min) ($P < .0001$ for all) (Table I).

Table II
Trauma patient population demographics, hematology and clinical outcomes

Demographics	
Age (y)	33.5 (25.5–46.6)
% male	44 (76%)
Injury characteristics	
% blunt mechanism	32 (55%)
Time from injury to arrival (min)	27 (21–34)
NISS	17 (9–34)
% TBI	18 (31%)
Laboratory values	
SBP (mm Hg)	119 (92–136)
Lactate (mmol/L)	3.5 (2.2–6.6)
Base deficit (meq/L)	5.8 (3.4–10.6)
PT/INR	1.1 (1.0–1.2)
PTT (s)	25.8 (23.3–27.9)
Thrombelastography	
ACT (s)	105 (97–121)
Angle (degrees)	74.4 (67.6–77.8)
MA (mm)	62.0 (57.5–65.0)
LY30 (%)	1.6 (0.5–3.0)
Whole blood thrombin generation	
Lag time (min)	3.8 (2.8–4.9)
Peak thrombin (nM)	86.4–146.7)
Time to peak thrombin (min)	6.7 (4.9–9.1)
Maximum rate (nM/min)	42.6 (22.9–61.6)
ETP (nM.min)	823 (711–966)
Clinical outcomes	
RBCs/first 6 h (units)	0 (0–2)
% massive transfusion (>10 units RBCs/first 6 h)	3 (5%)
ICU LOS (d)	3 (0–5)
Ventilator d	1 (0–3)
Hospital duration of stay (d)	4 (3–13)
% mortality	10 (17%)

Numbers presented as median with 25 and 75 interquartile range or raw number with percent total as appropriate.
ICU, intensive care unit; *LY30*, fibrinolysis 30 minutes after MA; *PTT*, activated partial prothrombin time; *TBI*, traumatic brain injury (defined as head and neck abbreviated injury score ≥3).

Compared to healthy volunteer WB TG, trauma patients had a distinct WB TG profile characterized by robust thrombin generation (Table III). Trauma patients had a lesser lag time (3.8 minutes vs 7.0 minutes, $P < .0001$), greater peak thrombin (125.5 nM vs 82.5 nM, $P = .003$), lesser time to peak thrombin (6.6 minutes vs 10.5 minutes, $P < .0001$), greater maximum rate (42.6 nM/min vs 21.3 nM/min, $P = .006$), and greater ETP (823.2 nM/min vs 728.4 nM/min, $P = .02$). A small standard error across the whole TG measurements was also noted as 0.2 minutes for lag time, 5.7 nM for peak thrombin, 0.3 minutes for time to peak thrombin, 30.2 nM/min for ETP, and 4.3 nM/min for maximum rate. When comparing the thrombin generation of trauma patients by mechanism, although there was a suggestion of a trend toward lesser lag time in

Table III
Comparison of whole blood thrombin generation in healthy volunteers and trauma patients

	Healthy Volunteers ($n = 10$)	Trauma patients ($n = 58$)	P value
Lag time (min)	7.0 (6.0–8.0)	3.8 (2.6–4.9)	< .001
Peak thrombin (nM)	82.5 (75.2–91.6)	125.5 (84.4–149.9)	.003
Time to peak thrombin (min)	10.5 (9.7–12.2)	6.6 (4.9–9.2)	< .001
Maximum rate (nM/min)	21.3 (17.8–26.1)	42.6 (22.6–63.6)	.006
ETP (nM/min)	728 (636–797)	823 (708–972)	.02

Numbers presented as median with 25 and 75 interquartile range.

patients with blunt mechanism (3.6 vs 4.1 minutes, $P = .17$), there were no detectable differences. Additionally, there were no differences in WB TG by NISS or traumatic brain injury, possibly owing to small sample size.

Plasma TG correlated moderately with plasma-based PT/INR, with lag time and time to peak thrombin correlating most strongly ($r = 0.6$, $P < .0001$ for both), such that the greater the PT/INR, the greater the lag time and time to peak thrombin (Table IV, Fig 1). Peak thrombin and ETP negatively correlated with INR ($r = -0.4$, $P = .004$ and $r = -0.5$, $P = .0006$, respectively), such that the greater the PT/INR, the lesser the peak thrombin and ETP. Whole blood TG measurements, however, did not correlate with CR-TEG ACT or angle (r ranged from 0.1–0.2, $P > .05$).

Table IV
Correlation of TG and PT/INR in trauma patients

	Pearson's r	P value
Lag time (min)	0.6	< .0001
Peak thrombin (nM)	−0.4	.004
Time to peak thrombin (min)	0.6	< .0001
Maximum rate (nM/min)	−0.2	.07
ETP (nM/min)	−0.5	.0006

Discussion

In this investigation, we sought to compare plasma and whole blood TG in healthy volunteers and trauma patients and to examine their respective correlations to conventional plasma-based and whole blood coagulation assays. We hypothesized that plasma and whole blood TG yield different profiles, that whole blood TG is more pronounced in trauma patients than in healthy volunteers, and that plasma TG correlates to PT/INR, whereas whole blood TG correlates to TEG. These data supported our hypotheses in that plasma and whole blood TG were distinct, with plasma TG yielding greater peak thrombin, time to peak thrombin, and ETP than whole blood. Whole blood TG was more pronounced in trauma patients compared with healthy volunteers, with a lesser lag time and time to peak thrombin and increased peak thrombin, maximum rate, and ETP. Plasma TG correlated with INR, such that the greater the INR, the greater the lag time and time to peak thrombin and the lesser the peak thrombin and ETP; in contrast, whole blood TG did not correlate with TEG ACT or angle.

Thrombin is the final end product of a complex coagulation cascade under the control of a myriad of pro- and anticoagulant proteins and is responsible for activating platelets and converting soluble fibrinogen to insoluble fibrin clot.[10] As such, thrombin generation is an important measure of global hemostatic balance after severe injury. The calibrated, automated thrombogram-based technology detects thrombin generation through use of a fluorogenic substrate and an internal calibrator with known concentration of thrombin activity, allowing for measurement of change in fluorescence intensity to determine thrombin concentration in a sample.[11,12] Studies examining this cascade in blood from severely injured patients have examined TG in platelet-poor plasma only.[5,13] Although this has been informative of the protein activity in plasma after severe injury, it fails to account for the cellular contributors in whole blood which may affect thrombin generation. These data indicate that plasma and whole blood TG measurements are distinct, which can be explained by the differential methodologies of these assays (use of an acidic PL additive in the plasma TG assay). Plasma TG resulted in greater ETP and peak thrombin compared to whole blood TG, likely due to the PL additive supporting more robust TG. These artificial acidic PL concentration in plasma CAT ultimately can lead to an even more robust TG than is observed with blood cells and platelets at their physiologic concentration. For example, only 1 to 2 μM PL is required to lead to TG similar to that observed with platelets at their mean physiologic concentration.[14] This uniquely robust TG created in the plasma CAT assay may explain why some studies of plasma TG in medical and surgical patients have found inconsistent clinical correlations.[6,7,15,16] Another difference in the whole blood and plasma TG assays includes the collection of blood in CTI (whole blood TG) versus citrated (plasma TG) vacutainer tubes. Vacutainer tubes with CTI were used for whole blood TG as a precaution for the prevention of the contact pathway. In the case of plasma TG, CTI is added before the TG assay is performed and thus, for both assays, citrate and CTI are present. Additionally, at 6.5 pM concentration of relipidated tissue factor, the collection of blood in citrate versus CTI tube has no effect on TG in whole blood and plasma; therefore, we expect the collection of blood in citrated versus CTI tubes does not explain the TG differences. Another difference in the plasma and whole blood TG is the concentration of proteins; however, the dilution of plasma to 67% in plasma CAT assay has a limited effect on TG because both natural procoagulant proteins and serine protease inhibitors circulating in blood are diluted. Whether the use of PL in the plasma CAT is enough to explain the lack of correlation between plasma and whole blood TG seems likely, but is unclear, and these results support future investigative work with "standardized" thrombin generation assays, where similar concentrations of PL and tissue factor are added to both plasma and whole blood before TG detection and comparison.

Although the results of plasma and whole blood TG reflect differential methodologies, these data provide insight into the cellular contributions to TG, which can be detected in whole blood TG. The central role of platelets in hemostasis was emphasized in the cell-based model proposed by Monroe and Hoffman nearly 20 years ago.[17] Platelets provide a catalytic scaffolding for prothrombinase assembly, initiating thrombin generation and further contributing to thrombin formation through release of polyphosphates and factor V-rich alpha granules, resulting in a rapid acceleration of initial thrombin generation.[12,18] In vitro investigations have elucidated that the factor V/Va, released from platelets, is functionally and structurally distinct from plasma factor V/Va and serves as a more efficient protein cofactor for prothrombinase, leading to distinct pronouncement of TG after initial plasma-based enzymatic activation.[19] Not only do platelets play a role in thrombin formation, but in vitro work has also supported the role of red blood cells

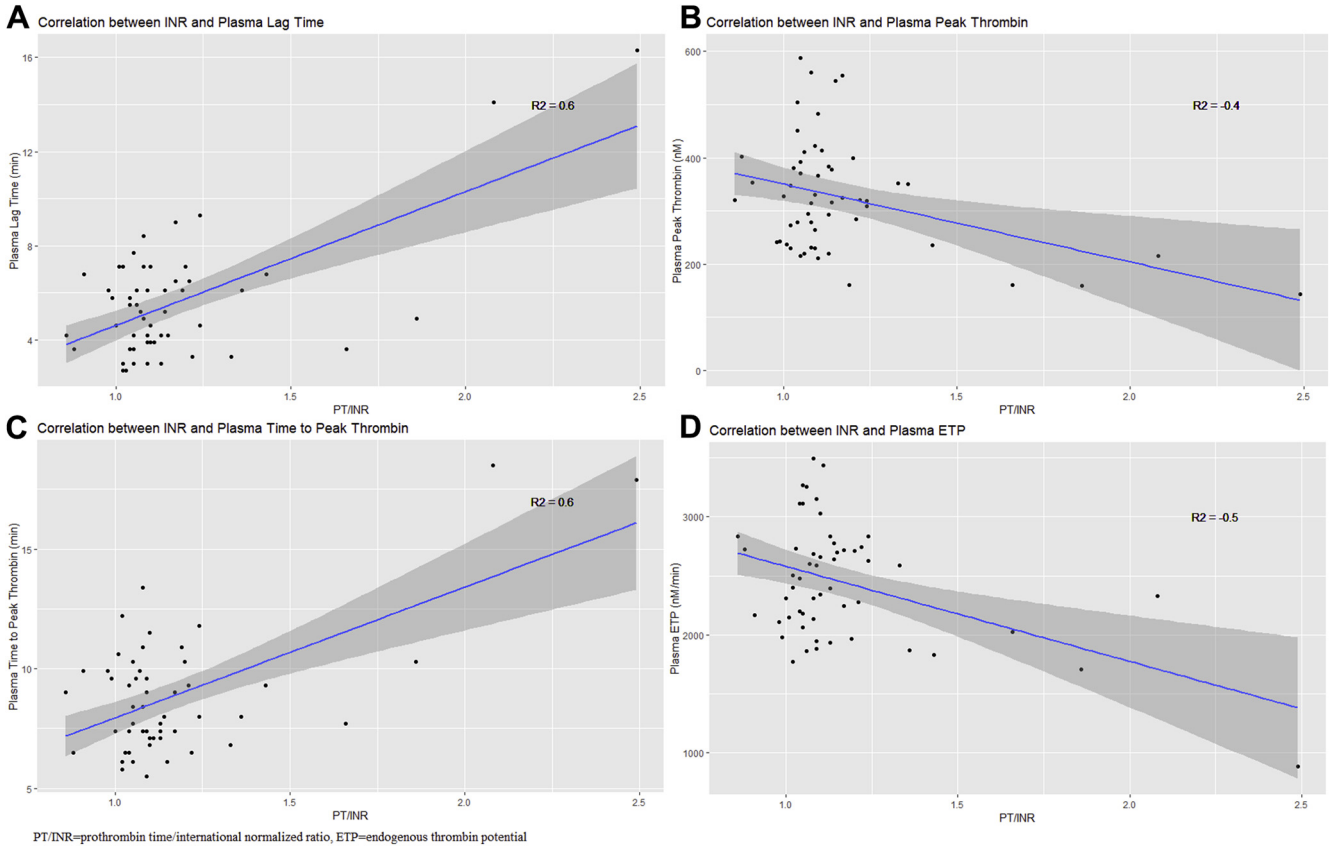

Fig 1. Correlation between plasma thrombin generation (TG) measurements and prothrombin time/international normalized ratio (PT/INR). *EPT*, endogenous thrombin potential.

(RBCs) in supporting prothrombinase and accentuating thrombin generation.[20] Whole blood exhibits a lesser lag phase than platelet-rich plasma, hypothesized to be driven by RBC expression of procoagulant PL on RBC membranes and factor Va-dependent factor Xa binding to RBCs.[21,22] These cellular-specific contributions to coagulation may explain why whole blood TG did not correlate with the enzymatic phase as detected on TEG (whereas the plasma-based TG and INR correlated well as would be expected). Ultimately, these results support further investigations into whole blood TG in trauma patients. Standardized TG assays, as mentioned previously with equivalent additive methodologies, may detail a discordance between plasma and whole blood TG that could be explained by cellular contribution.

Whole blood TG was more pronounced in trauma patients compared to healthy volunteers in these described data, with a lesser lag time and time to peak thrombin and increased peak thrombin, maximum rate, and ETP in trauma patients. These findings support previous literature examining plasma thrombin generation after severe injury in animal models and human patients.[5-7,13] In a prospective, observational study of 406 trauma activation patients compared with 29 healthy volunteers, trauma patients had a delayed lag time but increased peak thrombin, decreased time to peak thrombin, and increased maximum rate. Additionally, an inability to form this robust thrombin generation after trauma was linked to poor outcomes, such that depressed plasma peak thrombin was an independent predictor of massive transfusion and in-hospital mortality (odds ratio of 4.18 and 2.78, respectively). Other investigations of trauma patients describing increased peak thrombin in trauma patients report direct correlation to injury severity, such that the greater the NISS, the greater the peak thrombin.[16,23,24] TG's correlation with hypercoagulability and thrombotic morbidity has also been explored. In a prospective, case-cohort study of 454 trauma patients, including 83 patients who developed venous thromboembolism, initial time to peak thrombin was an independent predictor of symptomatic venous thromboembolism within 92 hours after trauma.[25] These aforementioned investigations introduced the concept of increased TG in trauma patients but have not included whole blood TG, which remains a gap in the current literature.

Limitations of this study include differential methodology of plasma and whole blood TG which limited our ability to correlate these 2 assays directly. The concentration of tissue factor added to citrated rapid TEG causes such a massive thrombin burst that it may skew our ability to correlate whole blood thrombin generation with ACT and angle. Another limitation in our analysis is that we evaluated singular TG measurements instead of combinations of measurements, which in composite, may describe a hypercoagulable or hypocoagulable profile correlative to conventional hemostatic assays. Lastly, this study is limited by a small sample of trauma patients, which limits the ability to differentiate thrombin generation by descriptors of tissue injury and hemorrhagic shock or prediction of mortality. The complexity of thrombin biology will need to be investigated further in a larger-scale study of whole blood TG and with expanded in vitro work looking at plasma and whole blood TG with analogous additives.

In conclusion, this study compared plasma and whole blood TG in healthy volunteers and trauma patients. These data support our hypotheses that plasma and whole blood TG are distinct, a difference which can be explained by differential methodologies, but which highlights the need for expansive in vitro work to elaborate the cellular contributions to thrombin generation. Whole blood TG was more pronounced in trauma patients compared to healthy

volunteers, a finding supported in previous literature on plasma TG and suggestive of unique systemic response to local injury in trauma. This study informs a novel, growing field of trauma-induced coagulopathy research on thrombin biology in the whole blood environment and highlights the need to gather additional data to further elucidate the differences between plasma and whole blood TG and how differential TG profiles may confer improved clinical outcomes.

Funding/Support

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health (T32 GM008315 and P50 GM049222), the National Heart, Lung and Blood Institute (TACTIC grant UM1 HL120877; SB) and Department of Defense (USAMRAA, W81XWH-12-2-0028). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or other sponsors of the project. This research was also supported with materials from Haemonetics.

Conflict of interest/Disclosures

Research conducted by these investigations for this manuscript and other works is supported with materials from Haemonetics and Instrumentation Laboratories.

Acknowledgements

We would like to graciously acknowledge the University of Colorado Department of Surgery for supporting the trauma research fellows, the National Institutes of Health for the T-32 grant which makes the trauma research fellows' work possible, the research assistants at Denver Health who helped to collect the data, and Denver Health for supporting ongoing data collection from patients at their site. Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health (T32 GM008315 and P50 GM049222), the National Heart, Lung and Blood Institute (TACTIC grant UM1 HL120877; SB), and the Department of Defense (USAMRAA, W81XWH-12-2-0028). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or other sponsors of the project. This research was also supported with materials from Haemonetics.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.surg.2019.07.014.

References

1. Sauaia A, Moore FA, Moore EE, et al. Epidemiology of trauma deaths: a reassessment. *J Trauma*. 1995;38:185–193.
2. Moore HB, Moore EE, Liras IN, et al. Targeting resuscitation to normalization of coagulating status: Hyper and hypocoagulability after severe injury are both associated with increased mortality. *Amer J Surg*. 2017;214:1041–1045.
3. Gando S, Hayakawa M. Pathophysiology of trauma-induced coagulopathy and management of critical bleeding requiring massive transfusion. *Semin Thromb Hemost*. 2016;42:155–165.
4. Cohen MJ, Christie SA. Coagulopathy of trauma. *Crit Care Clin*. 2017;33:101–118.
5. Cardenas JC, Rahbar E, Pommerening MJ, et al. Measuring thrombin generation as a tool for predicting hemostatic potential and transfusion requirements following trauma. *J Trauma Acute Care Surg*. 2014;77:839–845.
6. Selby R, Geerts W, Ofosu FA, et al. Hypercoagulability after trauma: hemostatic changes and relationship to venous thromboembolism. *Thromb Res*. 2009;124:281–287.
7. Voils SA, Lemon SJ, Jordan J, Riley P, Frye R. Early thrombin formation capacity in trauma patients and association with venous thromboembolism. *Thromb Res*. 2016;147:13–15.
8. Haemonetics. *TEG 5000 System User Manual. P/N 06-510-US, Manual revision: AC*. Niles, IL: Haemonetics Corporation, Haemoscope Division; 2010.
9. Wickham H, François R, Henry L, Müller K. dplyr: A grammar of data manipulation. R package, version 0.7.7. Vienna, Austria: The R Foundation; 2018. https://cran.r-project.org/package=dplyr.
10. Narayanan S. Multifunctional roles of thrombin. *Ann Clin Lab Sci*. 1999;29:275–280.
11. Ninivaggi M, Apitz-Castro R, Dargaud Y, de Laat B, Hemker HC, Lindhout T. Whole-blood thrombin generation monitored with a calibrated automated thrombogram-based assay. *Clin Chem*. 2012;58:1252–1259.
12. Prior SM, Mann KG, Freeman K, Butenas S. Continuous thrombin generation in whole blood: New applications for assessing activators and inhibitors of coagulation. *Anal Biochem*. 2018;551:19–25.
13. Cardenas JC, Cap AP, Swartz MD, et al. Plasma resuscitation promotes coagulation homeostasis following shock-induced hypercoagulability. *Shock*. 2016;45:166–173.
14. Butenas S, Branda RF, van't Veer C, Cawthern KM, Mann KG. Platelets and phospholipids in tissue factor-initiated thrombin generation. *Thromb Haemost*. 2001;86:660–667.
15. Saliba W, Zahalka W, Goldstein L, Ron G, Elias M. Padua prediction score and thrombin generation in hospitalized medical patients. *Thromb Res*. 2014;134:803–806.
16. Park MS, Xue A, Spears GM, et al. Thrombin generation and procoagulant microparticle profiles after acute trauma: A prospective cohort study. *J Trauma Acute Care Surg*. 2015;79:726–731.
17. Hoffman M, Monroe 3rd DM. A cell-based model of hemostasis. *Thromb Haemost*. 2001;85:958–965.
18. Rand MD, Lock JB, van't Veer C, Gaffney DP, Mann KG. Blood clotting in minimally altered whole blood. *Blood*. 1996;88:3432–3445.
19. Gertz JM, Bouchard BA. Mechanisms regulating acquisition of platelet-derived factor V/Va by megakaryocytes. *J Cell Biochem*. 2015;116:2121–2126.
20. Whelihan MF, Zachary V, Orfeo T, Mann KG. Prothrombin activation in blood coagulation: the erythrocyte contribution to thrombin generation. *Blood*. 2012;120:3837–3845.
21. Whelihan MF, Mann KG. The role of the red cell membrane in thrombin generation. *Thromb Res*. 2013;131:377–382.
22. Peyrou V, Lormeau JC, Herault JP, Gaich C, Pfliegger AM, Herbert JM. Contribution of erythrocytes to thrombin generation in whole blood. *Thromb Haemost*. 1999;81:400–406.
23. Park MS, Owen BA, Ballinger BA, et al. Quantification of hypercoagulable state after blunt trauma: microparticle and thrombin generation are increased relative to injury severity, while standard markers are not. *Surgery*. 2012;151:831–836.
24. Prior SM, Cohen MJ, Conroy AS, et al. Correlation between factor (F)XIa, FIXa and tissue factor and trauma severity. *J Trauma Acute Care Surg*. 2017;82:1073–1079.
25. Park MS, Spears GM, Bailey KR, et al. Thrombin generation profiles as predictors of symptomatic venous thromboembolism after trauma: A prospective cohort study. *J Trauma Acute Care Surg*. 2017;83:381–387.