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Viscoelastic measurements of platelet function, not fibrinogen function, predicts sensitivity to tissue-type plasminogen activator in trauma patients

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Summary

Background—Systemic hyperfibrinolysis is a lethal phenotype of trauma-induced coagulopathy. Its pathogenesis is poorly understood. Recent studies have support a central role of platelets in hemostasis and in fibrinolysis regulation, implying that platelet impairment is integral to the development of postinjury systemic hyperfibrinolysis.

Objective—The objective of this study was to identify if platelet function is associated with blood clot sensitivity to fibrinolysis. We hypothesize that platelet impairment of the ADP pathway correlates with fibrinolysis sensitivity in trauma patients.

Methods—A prospective observational study of patients meeting the criteria for the highest level of activation at an urban trauma center was performed. Viscoelastic parameters associated with platelet function (maximum amplitude [MA]) were measured with native thrombelastography (TEG), and TEG platelet mapping of the ADP pathway (ADP-MA). The contribution of fibrinogen to clotting was measured with TEG (angle) and the TEG functional fibrinogen (FF) assay (FF-MA). Another TEG assay containing tissue-type plasminogen activator (t-PA) (75 ng mL⁻¹) was used to assess clot sensitivity to an exogenous fibrinolytic stimulus by use of the TEG

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Disclosure of Conflict of Interests

M. P. Chapman reports receiving non-financial support from Haemonetics LLC, TEM GmbH and Barkey GmbH, outside the submitted work. In addition, M. P. Chapman has a pending patent on Methodologies and Reagents for Detecting Fibrinolysis and Hyperfibrinolysis. A. Sauaia reports receiving grants from the Department of Defense, during the conduct of the study, as well as other support from the American Association for the Surgery of Trauma outside the submitted work. E. Gonzalez and H. B. Moore have a pending patent on the Identification of Novel Disease States Using Viscoelastic Analysis in the Presence of a Thrombolytic Agent. The other authors state that they have no conflict of interest.

lysis at 30 min (LY30) variable. Multivariate linear regression was used to identify which TEG variable correlated with t-PA-LY30 (quantification of fibrinolysis sensitivity).

Results—Fifty-eight trauma patients were included in the analysis, with a median injury severity score of 17 and a base deficit of 6 mEq L⁻¹. TEG parameters that significantly predicted t-PA-LY30 were related to platelet function (ADP-MA, $P=0.001$; MA, $P<0.001$) but not to fibrinogen (FF-MA, $P=0.773$; angle, $P=0.083$). Clinical predictors of platelet ADP impairment included calcium level ($P=0.001$), base deficit ($P=0.001$), and injury severity ($P=0.001$).

Results and Conclusions—Platelet impairment of the ADP pathway is associated with increased sensitivity to t-PA. ADP pathway inhibition in platelets may be an early step in the pathogenesis of systemic hyperfibrinolysis.

Keywords

blood platelet disorder; fibrinogen; fibrinolysis; platelet function tests; trauma

Introduction

Hemorrhage is the leading cause of preventable death from trauma [1]. Eighty per cent of bleeding-related mortality occurs in the first 24 h after injury, with a median time to death of 2 h [2]. An endogenous coagulopathy induced by trauma is associated with a four-fold increase in mortality [3]. This trauma-induced coagulopathy (TIC) has been attributed to numerous defects in coagulation, including depletion of coagulation factors [4], autoheparinization resulting from endothelial glycocalyx degradation [5], fibrinogen depletion [6], platelet dysfunction [7], and systemic hyperfibrinolysis [8]. Because of these numerous potential contributors to TIC, it is not surprising that distinct phenotypes emerge. The most lethal phenotype of TIC is hyperfibrinolysis, with a mortality rate ranging from 50% to 90% [9-11].

The driver of systemic hyperfibrinolysis is tissue-type plasminogen activator (t-PA). Elevated levels of t-PA are found in patients with thrombelastography (TEG)-proven hyperfibrinolysis with concurrent depletion of its primary inhibitor, plasminogen activator inhibitor-1 (PAI-1) [12]. Apart from PAI-1, healthy plasma contains a number of proteins that regulate fibrinolysis [13], and dilution of these factors by the addition of saline to whole blood promotes sensitivity to t-PA-mediated fibrinolysis [14]. Protease inhibitors of fibrinolysis that act along with PAI-1 include α_2 -antiplasmin, α_2 -macroglobulin, α_1 -antitrypsin, plasminogen activator inhibitor-2, and C1-inhibitor [15]. Additional inhibition of fibrinolysis is accomplished by direct modification of fibrin filament by factor XIII [16] and thrombin activator fibrinolysis inhibitor [17].

Recent experimental work has indicated that the cellular components of blood may play a pivotal role in the regulation of fibrinolysis [18]. Red blood cell (RBC) lysis products have been shown to promote fibrinolysis by an as yet unknown mechanism. Conversely, small-volume replacement of whole blood with platelet lysate inhibits t-PA-mediated fibrinolysis. Affinity chromatography has identified a number of proteins from platelet lysate that directly bind t-PA and plasminogen [18], and these have previously been found to inhibit the

fibrinolytic system [13]. The role of platelets in the regulation of fibrinolysis has previously been suggested by *in vitro* work [19]. Given the *in vitro* data indicating the role of platelets in moderating fibrinolysis, we hypothesized that defects in platelet function would correlate with increased t-PA sensitivity in the blood of trauma patients.

Materials and methods

Patient population

The local institutional review board approved this study (COMIRB Protocol no. 13-3087). Adult trauma patients meeting the criteria for the highest level of activation at our level 1 trauma hospital from April 2014 to February 2015 were included in this study. Patients taking antiplatelet or anticoagulant therapy were excluded from the analysis. Patient demographics, injury mechanism, laboratory test results and transfusion requirements were prospectively collected by professional research assistants, who cover the emergency department (ED) 24 h a day, 7 days a week. The five clinical professional research assistants performed the study TEG assays, and individually have experience in performing > 1000 TEG assays.

Blood collection from trauma patients

Blood was collected in 3.5-mL tubes containing 3.2% citrate and 4 mL of heparin (19 units mL⁻¹) in the prehospital ambulance or upon arrival to the ED. Prehospital or ED healthcare workers drew study patient blood samples concurrently with the first set of blood samples used for in-hospital laboratory analysis. Professional research assistants analyzed within 2 h of blood draw and TEG platelet mapping within 30 min. The hospital's clinical laboratory performed conventional laboratory tests, including a complete blood count, International Normalized Ratio (INR)/prothrombin time ratio, partial thromboplastin time (PTT), and arterial blood gases.

Blood collection from non-trauma patients

Nursing staff collected samples from volunteers at an outpatient clinic after approval of the institutional review board (protocol no. 14-0366). The study was open to patients and hospital staff not taking any antiplatelet or anticoagulant medications. Volunteers with medical comorbidities, including diabetes, renal disease, or liver disease, were excluded. Blood samples were obtained via venous puncture, and collected in identical tubes as described for the trauma patients. TEG assays were performed by research assistants in a similar time frame as described for the trauma patients.

TEG

Whole blood was recalcified and analyzed, according to the manufacturer's instructions, on a TEG 5000 Thrombelastograph Hemostasis Analyzer (Haemonetics, Niles, IL, USA). The following indices were obtained from the tracings of the TEG: Reaction time (*R*-time) (min), angle (°), maximum amplitude (MA [mm]), and lysis 30 min after MA (LY30 [%]). A t-PA challenge was conducted on the same citrated blood tube as used for the native TEG. Five hundred microliters of whole blood was pipetted into a customized vial containing lyophilized t-PA (Molecular Innovation, Novi, MI, USA) to a final concentration of 75 ng

mL⁻¹ t-PA, and mixed by gentle inversion. A 340-IL aliquot of this mixture was transferred from the vial to a 37 °C TEG cup, preloaded with 20 µL of 0.2 mol L⁻¹ CaCl₂. The rationale for using 75 ng mL⁻¹ t-PA is based on unpublished data demonstrating that exogenous t-PA added to a TEG cup at this concentration increases LY30 as compared with native blood with no additional t-PA. When a lower concentration of t-PA (50 ng mL⁻¹) was employed in this study, no significant difference was seen. Previous authors have used a similar assay with t-PA to provoke fibrinolysis in whole blood [19]. The TEG functional fibrinogen (FF) assay (FF-MA) was performed in a similar fashion. Five hundred microliters of citrated blood was added to the designated FF vial containing a mixture of tissue factor and a monoclonal glycoprotein IIb–IIIa receptor antagonist (abciximab), gently mixed, and then assayed with TEG. The FF assay has previously been shown to have a high correlation with the standard Clauss assay in trauma patients [20].

Platelet mapping

Whole blood collected in heparin (19 U mL⁻¹) was analyzed with the TEG/platelet Mapping assay (Haemonetics), and was mixed with a solution containing reptilase and FXIIIa and then activated with 2 mM ADP. Although there are other commercially available activators of platelets, the ADP pathway was selected because ADP pathway inhibition has previously been associated with poor outcome in trauma [7,21].

Metabolomics analysis

Nine trauma patients and 10 non-trauma volunteers in this study had plasma previously analyzed via exploratory metabolomics. Metabolomics analyses were performed as previously reported [22,23]. Briefly, 20 µL of plasma was extracted in ice-cold lysis/extraction buffer (methanol/acetonitrile/water, 5 : 3 : 2). Water-soluble and methanol-soluble fractions were applied to a C18 reversed-phase column (phase A – water and 0.1% formic acid; phase B – acetonitrile and 0.1% formic acid) in an ultra-HPLC system (Ultimate 3000; Thermo Fisher, Waltham, MA, USA), coupled on line with a high-resolution quadrupole Orbitrap instrument run in both polarity modes (QExactive; Thermo Fisher). Metabolite assignment and peak integration for relative quantification were performed with MAVEN (Princeton, NJ, USA), against the KEGG pathway database and an in-house validated standard library (> 650 compounds; Sigma Aldrich; St Louis, MO, USA; IROATech). Analyses to identify targeted metabolites correlating with changes in hemorrhagic shock previously identified in rodent models [22,24] were performed.

Statistical analysis

SPSS version 22 (IBM, Armonk, NY, USA) was used for statistical analysis. The variables from the TEG were used to approximate the indices related to clot formation, based on the accepted model [25], wherein *R*-time relates to coagulation factor levels, angle correlates with fibrinogen–platelet interactions, and MA correlates with platelet–fibrinogen interactions contributing to maximal clot strength. The separate contributions of platelets and fibrin to final clot strength were further quantified with the TEG Platelet Mapping assay (ADP-MA and FF-MA). TEG parameters are presented as median values with the range between the 25th and 75th percentiles. These parameters were contrasted between volunteer non-trauma blood and trauma patient blood with a Mann–Whitney *U*-test, with significance

set to an alpha of 0.05. Hyperfibrinolytic trauma patients (defined as those with a LY30 of > 3% [26]) were contrasted with non-hyperfibrinolytic patients for assessment of differences in FF-MA, ADP-MA, and t-PA-LY30, by use of the same methodology. Impairment of the ADP-MA platelet pathway and FF-MA was classified as less than the 25th percentile of non-trauma volunteers. Increased sensitivity to fibrinolysis was classified as t-PA-LY30 greater than the 75th percentile in non-trauma volunteers.

These variables were included in a multivariate linear regression model with t-PA-LY30 as the dependent variable. t-PA-LY30 has been previously validated for the quantification of changes in sensitivity to t-PA-mediated fibrinolysis [18,27]. Spearman's rho was used to assess the correlation between blood product utilization and t-PA-LY30. The same methods were used to assess correlations between metabolites and ADP-MA for targeted metabolites, including succinate, spermidine, spermine, glycocholate, and lactate. Laboratory values were obtained at the attending physician's discretion; thus, not all studied patients had complete data on all variables. However, only two variables had significant missing data: temperature (8/58, 14%) and base deficit (17/58, 29%), with all others being complete or with minimal missing data (one or two patients had missing values). Multivariate linear regression included only patients with complete TEG data ($n = 58$, 100%). Additional investigation was performed to assess clinical variables correlating with platelet function (ADP-MA and MA) and fibrinogen function (FF-MA and angle). To account for multiple comparisons with the 13 clinical variables of interest after a Bonferroni correction, alpha was set to 0.004.

Results

Fifty-eight trauma patients were included in the analysis. The median age was 36 years (25th–75th percentile, 25–49 years), and 80% were male. Blood samples were obtained at a median of 25 min (14–35 min) after injury. These patients suffered moderate to severe injuries, with a median Injury Severity Score of 17 (9–30), and 39% sustaining penetrating injuries. Despite the high degree of injury severity, the majority of these patients had normal conventional hematologic parameters at hospital arrival, with a median hematocrit of 43 (38–46), a platelet count of $259\,000\ \mu\text{L}^{-1}$ ($206\text{--}306\ \mu\text{L}^{-1}$), and an INR of 1.10 (1.03–1.20). The majority of patients did not have evidence of sustained hemorrhagic shock on presentation to the ED, and had a median systolic blood pressure of 122 mmHg (92–140 mmHg), and a median base deficit of $6\ \text{mEq L}^{-1}$ ($3\text{--}8.5\ \text{mEq L}^{-1}$). The median core temperature was $36.5\ ^\circ\text{C}$ ($36.2\text{--}36.8\ ^\circ\text{C}$), and the median calcium concentration was $8.0\ \text{mg dL}^{-1}$ ($7.5\text{--}8.5\ \text{mg dL}^{-1}$). There were eight (14%) patients with hyperfibrinolysis defined as LY30 of > 3%. Blood products within the first 6 h were administered to 32% of patients; overall, 32% of patients received RBCs, 28% plasma, 19% platelets, and 10% cryoprecipitate. Volunteer non-trauma blood was obtained from 96 subjects. The median age was 31 years (27–39 years), and 51% were male.

Median citrated native TEG values of trauma patients were as follows: *R*-time, 6.5 min (5.0–8.1 min); angle, 66.0° ($60.4\text{--}68.7^\circ$); MA, 64.3 mm (58.8–67.5 mm); and LY30, 0.95% (0.5–1.8%). The values for healthy volunteers were as follows: *R*-time, 14.1 min (13.0–14.9 min); angle, 43.6° ($38.2\text{--}51.4^\circ$); MA, 55.5 mm (52.5–60.1 mm); and LY30, 1.3% (0.9–2.3%).

Trauma patients had shorter *R*-times ($P < 0.001$), steeper angles ($P < 0.001$) and higher MAs ($P < 0.001$) than non-trauma volunteers, and comparable LY30 ($P = 0.065$) (Fig. 1).

The median platelet ADP-MA was 35.3 mm (24–54.1 mm) in trauma patients, and was significantly lower ($P < 0.001$) than in non-trauma volunteers (58.5 mm [52.8–64.5 mm]). Trauma patients had a higher FF-MA than non-trauma volunteers (22.5 mm [20.1–25.4 mm] versus 20.5 mm [17–22.6 mm], $P < 0.001$), and increased t-PA-LY30 (13.2% [4.5–32.9%] versus 8.1% [4.5–12.1%], $P = 0.003$; Fig. 2). Six trauma patients (10%) had impairment of FF-MA, and 42 (78%) had impairment of ADP-MA. Increased sensitivity to fibrinolysis was identified in 30 (52%) trauma patients.

FF-MA in the eight hyperfibrinolytic trauma patients was lower than in non-hyperfibrinolytic trauma patients (19.3 mm [15.6–23 mm] versus 22.8 mm [21.1–27 mm], $P = 0.020$), and they continued to show a reduction in ADP-MA (14.5 mm [10.25–42.1 mm] versus 36.8 mm [26.1–54.4 mm], $P = 0.098$) and increased t-PA-LY30 (63.8% [25.9–78.3%] versus 10.3% [4.3–26.1%], $P < 0.001$). FF-MA was impaired in two hyperfibrinolytic trauma patients (25%) patients, and ADP-MA was impaired in seven hyperfibrinolytic trauma patients (88%).

Regression analyses of all TEG parameters, apart from *R*-time, were significantly associated with t-PA-LY30 (Table 1). After adjustment, regression analysis to identify variables contributing to t-PA-LY30 revealed that only MA ($P < 0.001$) and ADP-MA ($P = 0.001$) contributed significantly to the model. In a stepwise regression model that included only these two variables, the adjusted R^2 was 0.535, whereas in a forced full model including all of the TEG variables, the adjusted R^2 was 0.544, suggesting minimal contribution to predicting t-PA-LY30 of the additional TEG parameters: *R*-time, angle, and FF-MA. t-PA-LY30 showed a high correlation with all blood product use (Table 2).

Clinical parameters correlating with platelet ADP-MA, MA, FF-MA and angle are shown in Table 3. Variables that correlated with ADP-MA were calcium level ($P = 0.001$), base deficit ($P = 0.001$), and injury severity ($P = 0.001$). MA correlated with platelet count ($P < 0.001$) and PTT ($P = 0.001$). No parameters showed significant correlations with FF-MA or angle. Correlations of metabolites with ADP-MA ($n = 9$) are shown in Table 4. Succinate, spermine and spermidine showed strong negative correlations with ADP-MA. Lactate and glycocholate did not correlate with ADP-MA. All metabolites apart from glycocholate were significantly elevated in trauma patients as compared with non-trauma volunteers (Table 4).

Discussion

On evaluation of a cohort of moderate to severely injured patients, t-PA-LY30 showed a high correlation with blood product use. In a regression model, t-PA-LY30 was associated with ADP-MA and MA, but not with other TEG parameters. Although the majority of the trauma patients had native TEG parameters suggestive of hypercoagulability as compared with non-trauma volunteers, ADP-MA was significantly decreased and t-PA-LY30 was significantly increased. The clinical variables that correlated with platelet ADP-MA were related to metabolic and injury severity factors. On comparison of these injured patients' viscoelastic

clotting parameters with those of non-trauma volunteers, the physiologic response to injury appears to be a quicker-forming, stronger clot that has increased susceptibility to fibrinolysis.

The paradoxical coagulation status of hypercoagulability and platelet ADP impairment with increased sensitivity to t-PA was not expected in this study. To some extent, it should not be surprising that injury primes the systemic circulation for coagulation, but it is counterintuitive that these clots would be prone to degradation. This finding is supported by previous studies suggesting that postinjury changes in thrombin generation and fibrinolysis are not necessarily linked [28,29]. The separation between tissue injury and hemorrhagic shock in animal models may provide a partial mechanism. Recent animal work has demonstrated that polytrauma initiates early hypercoagulability, followed by hypocoagulability triggered by hemorrhagic shock [30]. Similarly, when the two components of trauma were completely separated in a rat model, tissue injury inhibited fibrinolysis, whereas shock promoted fibrinolysis [27]. When we used a more realistic representation of military trauma with significant blast injury, swine were found to be hypercoagulable after injury [31]. Schreiber *et al.* [32], using kaolin-activated TEG, found that, during the first day after injury, the majority of their trauma patients had shortened *R*-times and increased angles, which is in agreement with our study. However, these authors did not evaluate platelet function or t-PA sensitivity in their study. Comparison with a large sample of non-trauma volunteers without an activator enabled identification of this change in coagulation following injury. A large multicenter study using tissue factor to activate the viscoelastic assays showed that 10% of trauma patients had a hypercoagulable state within 24 h of injury [33]. The use of tissue factor may have masked endogenous circulating factors that promote clot formation following trauma.

Crude FF-MA and angle were associated with t-PA-LY30 but, after adjustment with other TEG parameters, they were no longer significant. Although fibrinogen levels have been reported to reach critically low levels earlier than any other coagulation protein in the course of severe bleeding after injury [6,34], fibrinogen depletion is more likely to be the result than the cause of increased systemic fibrinolysis. This is supported by our results showing that FF-MA was reduced in trauma patients with systemic hyperfibrinolysis, but did not correlate with a patient's sensitivity to t-PA. Moreover, the majority of patients in our study with hyperfibrinolysis had FF-MA levels that were comparable to those in non-trauma healthy volunteers. This could be attributable to the limitations of this assay, as Schlimp *et al.* [35] demonstrated in non-trauma patients that the TEG FF assay overestimates the fibrinogen concentration, owing to the contribution of platelets, particularly in the setting of a high platelet count. However, it would be difficult to draw this conclusion for our trauma patients, as the majority of these trauma patients had reductions in ADP-MA and normal platelet counts.

Hemorrhagic shock has been suggested to be necessary for trauma patients to develop TIC [4,36]. However, traumatic brain injury in the absence of hypotension has been associated with platelet inhibition via the ADP pathway [21]. This concept is critical to understanding the pathophysiology of TIC. Not all trauma patients will present to the hospital in hemorrhagic shock. These patients may appear to have normal hemostatic parameters, but,

in fact, harbor coagulation changes that may only become unmasked when they progress to hemorrhagic shock. There is increasing evidence that hypotension drives hyperfibrinolysis [10], and is not dependent on tissue injury [37]. This hypotension-driven hyperfibrinolysis has been consistent in human studies [37] and animal work indicating a t-PA-mediated process [27]. Therefore, quantification of a patient's sensitivity to t-PA on presentation to the hospital could help to prompt pre-emptive resuscitation efforts aimed at attenuating progression to systemic hyperfibrinolysis. This early intervention is critical for interrupting the trajectory of the actively bleeding patient towards developing hemorrhagic shock and coagulopathy. In patients with TIC, t-PA levels have been found to be $\sim 50 \text{ ng mL}^{-1}$ [4]. Importantly, this concentration of t-PA was not found to cause fibrinolysis in non-injured patients when a method for measuring fibrinolysis sensitivity was developed. This suggests that hyperfibrinolytic trauma patients not only have increases in t-PA, but are also deficient in inhibitors of fibrinolysis, and this was the reason for evaluating platelet function in these patients.

The central role of platelets in coagulation has been widely accepted following the landmark proposal of the cell-based model of hemostasis by Hoffman and Monroe [38]. Stalker *et al.* elegantly demonstrated the contribution of platelets to hemostasis through their complex networking and scaffolding [39]. Their *in vivo* model illustrates the hierarchical clustering of activated platelets forming a central, fibrin-rich core surrounded by a protective platelet shell. These complex platelet-platelet interactions create a unique microenvironment that is prothrombotic and antifibrinolytic [40,41]. Furthermore, minimal thrombus generation can potentiate platelet aggregation and promote clot formation [42], which supports our observation that platelet impairment of the ADP pathway correlates with t-PA sensitivity.

There may be a physiologic role for downregulating ADP activity in platelets during trauma. ADP has been identified as an important paracrine mediator in coordinating blood clot contraction in low flow states [43] and forming an outer protective shell of platelets during thrombin formation [39]. There are numerous agonists that can initiate platelet activation, leading to adhesion and degranulation [44]. Thrombin and collagen are potent agonists that can lead to the 'superactivated' platelet [45]. Evaluation of the hierarchy of clot formation shows that thrombin is the primary driver of a central dense core of a platelet plug, whereas ADP acts peripherally [39]. Therefore, ADP inhibition would not necessarily reduce the clot strength, particularly in the presence of potent activators, which is supported by our TEG parameters, as the clot strength is retained in the majority of these trauma patients. Increased sensitivity to fibrinolysis in a procoagulant environment during low flow states would reduce the probability of non-specific clot generation occluding uninjured vasculature. Whereas moderate ADP inhibition may have a potential physiologic role, excessive inhibition probably predisposes patients to hyperfibrinolysis, particularly in the presence of elevated systemic t-PA.

The majority of patients in this study did not have profound shock as reflected by the base deficit, so other metabolic derangements that remain unrecognized by standard laboratory testing were probably present. For example, the present study showed that platelet ADP-MA correlated with a reduction in calcium levels. This observation corresponds with the findings of our previous study [46], which identified numerous abnormalities of metabolism shortly

after injury, including increases in the levels of calcium-chelating metabolites, such as succinate. In an animal model of rapid hemorrhage that produced systemic hyperfibrinolysis, similar metabolic changes were observed within 5 min of initiation of bleeding [22]. Other non-calcium-binding metabolites, such as bile acids [47,48], can also inhibit platelets, and the levels of these have been observed to increase in animals in hemorrhagic shock [24]. Metabolic changes in the arginase pathway resulting from hemorrhagic shock increase the production of polyamines [22], which have also been shown to inhibit platelets [49,50]. In our *post hoc* analysis metabolic analysis, ADP-MA was negatively correlated with succinate and polyamines. Lactate did not correlate with these changes, but was significantly elevated in trauma patients. This has important clinical implications, as it has previously been demonstrated that correcting acidosis-induced coagulopathy with an agent such as tris-hydroxymethyl-aminomethane [51] and bicarbonate [52] does not improve clotting. Although our metabolic analysis was limited to nine trauma patients, there are compelling preliminary data showing that unmeasured metabolites contribute to early platelet inhibition following trauma, and further work is needed to confirm these data.

Injury severity was also associated with a decrease in platelet function. Cohen *et al.* found that injury severity correlated with both coagulopathy and the release of damage-associated molecular pattern (DAMP) proteins, such as high-mobility group box nuclear protein 1 (HMGB1) [53]. HMGB1 is known to bind to toll like receptor 4 (TLR4) and stimulate inflammation [54]. Activation of the TLR4 pathway has also been recently implicated in platelet impairment [55]. The early metabolic changes and release of DAMPs following injury are potential mechanisms of platelet ADP inhibition that prime the injured patient for systemic hyperfibrinolysis if they are allowed to progress to hemorrhagic shock. Unfortunately, we did not measure DAMPs in our study population, and cannot make a definitive association between danger signals and platelet dysfunction; however, the emerging role of DAMPs in driving TIC through a platelet-mediated process is a compelling hypothesis.

Over 75% of the patients evaluated in this study had impairment of the ADP pathway as compared with previously reported values of healthy controls, which is consistent with our previous evaluation of the ADP pathway in trauma patients [7]. This study is limited to viscoelastic assays of platelet impairment of the ADP pathway, without aggregometry, or the standard quantification of fibrinogen levels with a Clauss assay. Solomon *et al.* [56] previously demonstrated that platelet aggregometry detected platelet dysfunction, and viscoelastic assessment of platelet function (MA minus FF-MA with ROTEM) predicted mortality and blood product use in trauma patients. At the other end of the spectrum, platelet hyperactivity detected with thrombin receptor agonist peptide-activated aggregometry was found to be associated with death from brain injuries, and not bleeding [57]. Such data imply that there is a spectrum of fibrinolysis, in which impairment of fibrinolysis is also a risk factor for mortality resulting from organ failure [10]. A limiting factor of this study is we cannot conclude that our assays are independent of fibrinogen and platelet interactions. Numerous investigators have reported that viscoelastic parameters are attributable to both platelets and fibrinogen when clot formation and strength are evaluated [35,58,59]. However, Ostrowski *et al.* [60] demonstrated, when controlling for multiple variables, that FF-MA and angle correlate with fibrinogen levels and not MA. Clot strength can be seen to be

predominantly attributable to platelets, as seen in the non-trauma volunteer data, in which FF-MA has a median amplitude of 20.5 mm as compared with a value of 58.5 mm for ADP-MA. The synergy between platelets and fibrinogen in clot formation is critical for clot formation, and complete separation of platelets from fibrinogen cannot be definitively shown with viscoelastic assays. However, the pathogenesis of trauma-induced hyperfibrinolysis remains poorly understood, and these data support an early role of platelet dysfunction over fibrinogen function as being contributory to the process. The present study is also limited to one time point in the patient's treatment course prior to resuscitation. Expansion to additional time points encompassing the entire trajectory from injury to death or recovery will facilitate the elucidation of the proposed mechanisms for developing systemic hyperfibrinolysis.

In conclusion, trauma patients have a paradoxical coagulation profile characterized by a shorter clotting time, a higher clot strength, impairment of the ADP pathway, and increased sensitivity to t-PA-mediated fibrinolysis. This early impairment of the ADP pathway may be attributable to early metabolic changes following trauma, and result in increased susceptibility to fibrinolysis via a loss of platelet function. The relationship between platelet function and regulation of fibrinolysis warrants future evaluation, as identifying the driving mechanism for platelet impairment will create therapeutic opportunities to prevent patients from progressing to the lethal systemic hyperfibrinolytic phenotype.

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Fig. 1. Citrated native thrombelastography (TEG) parameters contrasting trauma patients with non-trauma volunteers. The y -axis represents the measurements of the TEG parameters, and the x -axis represents the two patient populations. Each panel represents a different TEG parameter. (A) Reaction time (R -time). (B) Angle. (C) Maximum amplitude (MA). (D) Lysis at 30 min (LY30). *Extreme outlier.

Fig. 2.

Specialized thrombelastography (TEG) assays contrasting trauma patients with non-trauma volunteers. The *y*-axis represents the measurements of the TEG parameters, and the *x*-axis represents the two patient populations. Each panel represents a different specialized TEG parameter. (A) Functional fibrinogen (FF) clot strength. (B) ADP platelet mapping clot strength. (C) Sensitivity of blood to fibrinolysis quantified by the percentage of clot degradation 30 min after maximum clot strength is reached when whole blood is challenged with exogenous tissue-type plasminogen activator (t-PA). LY30, lysis at 30 min; MA, maximum amplitude. *Extreme outlier.

Table 1

Multivariate linear regression model used to predict t-PA-LY30

Viscoelastic parameter	Crude coefficient	Crude <i>P</i>	Adjusted coefficient	Adjusted <i>P</i>
ADP-MA (platelet)	− 0.765	< 0.001	− 0.502	0.001
FF-MA (fibrinogen)	− 2.623	< 0.001	0.217	0.773
<i>R</i> -time (coagulation factors)	0.721	0.559	0.978	0.505
Angle (fibrinogen/platelet)	− 0.589	0.021	0.842	0.083
MA (platelet/fibrinogen)	− 1.931	< 0.001	− 2.012	< 0.001

FF, functional fibrinogen; LY30, lysis at 30 min; MA, maximum amplitude; *R*-time, reaction time; t-PA, tissue-type plasminogen activator. Dependent variable: t-PA-LY30. The table shows the multivariate linear regression model used to predict t-PA-LY30 with native thrombelastography (TEG) parameters, i.e. *R*-time, angle, and MA, and specialized TEG assays, i.e. ADP-MA and FF-MA. The model had an overall *F*-statistic of 14.2 ($P < 0.001$) and an *R*² of 0.544.

Table 2

Correlation of thrombelastography parameters with blood product use

	RBCs	Plasma	Platelets	Cryoprecipitate
t-PA-LY30	0.627 $P < 0.001$	0.618 $P < 0.001$	0.504 $P < 0.001$	0.456 $P < 0.001$
ADP-MA	− 3.05 $P = 0.020$	− 0.295 $P = 0.026$	− 0.241 $P = 0.068$	− 0.300 $P = 0.022$
FF-MA	− 0.458 $P < 0.001$	− 0.447 $P = 0.001$	− 0.286 $P = 0.032$	− 0.436 $P = 0.001$
<i>R</i> -time	0.079 $P = 0.564$	0.067 $P = 0.623$	0.018 $P = 0.893$	− 0.019 $P = 0.889$
Angle	− 0.332 $P = 0.015$	− 0.332 $P = 0.015$	− 0.126 $P = 0.355$	− 0.222 $P = 0.101$
MA	− 0.655 $P < 0.001$	− 0.635 $P < 0.001$	− 0.416 $P = 0.001$	− 0.305 $P = 0.022$

FF, functional fibrinogen; LY30, lysis at 30 min; MA, maximum amplitude; RBC, red blood cell; *R*-time, reaction time; t-PA, tissue-type plasminogen activator. The table shows the Spearman's rho correlation and associated significance for t-PA-LY30, ADP-MA, FF-MA, *R*-time, angle, and MA, and blood product usage during the first 6 h after injury.

Table 3

Correlation of thrombelastography (TEG) parameters with clinical variables and laboratory assays

	ADP-MA	MA	FF-MA	Angle
Age ($n = 58$)	0.04, $P = 0.767$	0.03, $P = 0.981$	0.05, $P = 0.722$	0.09, $P = 0.489$
BMI ($n = 58$)	0.13, $P = 0.350$	0.19, $P = 0.185$	0.17, $P = 0.244$	0.17, $P = 0.233$
SBP ($n = 57$)	0.15, $P = 0.267$	0.12, $P = 0.390$	- 0.12, $P = 0.384$	0.09, $P = 0.493$
HR ($n = 57$)	- 0.20, $P = 0.128$	- 0.03, $P = 0.816$	- 0.09, $P = 0.497$	0.09, $P = 0.500$
GCS ($n = 57$)	0.22, $P = 0.100$	- 0.05, $P = 0.732$	0.15, $P = 0.269$	- 0.12, $P = 0.373$
Temperature ($n = 50$)	0.30, $P = 0.034$	0.32, $P = 0.829$	0.09, $P = 0.525$	0.00, $P = 0.992$
Total serum calcium ($n = 55$)	0.43, $P = 0.001^*$	0.26, $P = 0.063$	0.25, $P = 0.070$	0.01, $P = 0.493$
BD ($n = 41$)	0.51, $P = 0.001^*$	0.39, $P = 0.015$	0.37, $P = 0.020$	0.35, $P = 0.032$
HCT ($n = 58$)	- 0.11, $P = 0.386$	0.09, $P = 0.502$	- 0.03, $P = 0.830$	- 0.06, $P = 0.613$
Platelet count ($n = 58$)	0.18, $P = 0.186$	0.49, $P < 0.001^*$	0.31, $P = 0.019$	0.24, $P = 0.072$
INR ($n = 55$)	- 0.29, $P = 0.034$	- 0.36, $P = 0.008^*$	- 0.27, $P = 0.054$	- 0.12, $P = 0.384$
PTT ($n = 55$)	- 0.27, $P = 0.051$	- 0.43, $P = 0.001^*$	- 0.23, $P = 0.098$	- 0.34, $P = 0.013$
ISS ($n = 50$)	- 0.44, $P = 0.001^*$	- 0.34, $P = 0.018$	- 0.39, $P = 0.006$	- 0.18, $P = 0.230$

BD, base deficit; BMI, body mass index; FF, functional fibrinogen; GCS, Glasgow coma score; HCT, hematocrit; HR, heart rate; INR, International Normalized Ratio of prothrombin time; ISS, injury severity score; MA, maximum amplitude; n , number of patients with available data on a specific variable out of a total of 58 patients; PTT, partial thromboplastin time; SBP, systolic blood pressure. The table shows the Spearman's rho correlation with associated significance, contrasting TEG variables associated with platelet and fibrinogen function with patient demographics, clinical vital signs on presentation to the emergency department, and laboratory variables.

* $P < 0.01$.

Table 4

Correlations of metabolites with ADP-MA

	Succinate	Spermidine	Spermine	Glycocholate	Lactate
Correlation with ADP-MA	- 0.783, $P=0.013$	- 0.750, $P=0.020$	- 0.700, $P=0.036$	- 0.633, $P=0.067$	- 0.433, $P=0.244$
Trauma	856 (648–1395)	219 (179–650)	82 (57–300)	1870 (947–3522)	166 000 (72 900–4 625 000)
Non-trauma	635 (587–712)	107 (64–155)	7 (0–14)	2680 (1242–5945)	32 450 (27 400–47 825)
P -value	0.010	< 0.001	< 0.001	0.842	< 0.001

MA, maximum amplitude. Values of trauma and non-trauma volunteers are displayed as the medians of a thousand arbitrary units with the 25th–75th percentiles. P -values represent contrasts between trauma and non-trauma volunteer values with the Mann–Whitney U -test. The table shows the metabolic correlations with ADP-MA and the differences in levels between trauma and non-trauma volunteers. The top row represents metabolites previously identified in rodent models associated with metabolic changes in rodents that developed systemic hyperfibrinolysis.