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Clot activators do not expedite the time to predict massive transfusion in trauma patients analyzed with tissue plasminogen activator thrombelastography

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ABSTRACT

Background: Trauma patients with hypersensitivity to tissue plasminogen activator mediated fibrinolysis quantified by tissue plasminogen activator thromboelastography are at increased risk of massive transfusion. The tissue plasminogen activator thromboelastography assay has been tested in trauma patients using native thromboelastography with no exogenous activator. We hypothesize that adding an activator will expedite the time to results.

Methods: Healthy whole blood was assayed with and without exogenous plasmin, which acts to deplete inhibitors of fibrinolysis, mimicking trauma blood. Samples were assessed using native, kaolin, and rapid thromboelastography with and without tissue plasminogen activator. The tissue plasminogen activator thromboelastography indices of time to maximum amplitude and lysis at 30 minutes were contrasted between healthy blood with and without plasmin using the three different activators. The activators were then used with a tissue plasminogen activator thromboelastography in 100 trauma patients to assess performance in predicting massive transfusion.

Results: In healthy blood, regardless of activator, lysis at 30 minutes did not increase with plasmin alone, but did increase with tissue plasminogen activator ($P = .012$). Adding tissue plasminogen activator and plasmin increased lysis at 30 minutes ($P = .036$). Time to maximum amplitude was reduced with tissue plasminogen activator and plasmin compared with tissue plasminogen activator alone ($P = .012$). Activated thromboelastographies had increased lysis at 30 minutes ($P = .002$), but no difference in time to maximum amplitude compared with native thromboelastographies. In trauma patients, native tissue plasminogen activator thromboelastography had greater performance in predicting massive transfusion than activated tissue plasminogen activator thromboelastographies with no difference in time to maximum amplitude.

Conclusion: Adding an activator to tissue plasminogen activator thromboelastography does not expedite time to maximum amplitude in healthy blood depleted of fibrinolysis inhibitors. Activated tissue plasminogen activator thromboelastographies are inferior to native tissue plasminogen activator thromboelastography for predicting massive transfusion and do not reduce the time to results.

Introduction

Thrombelastography (TEG) is a viscoelastic point-of-care hemostatic assay that can be used to risk stratify trauma patients for a massive transfusion and guide point of care resuscitation.[1–4] TEG

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measures the viscoelastic properties of coagulation by placing whole blood samples in a cup, in which a pin is inserted that can measure resistance to rotation and oscillation over time. This enables assessment of clot formation and degradation, both of which can drive pathologic bleeding after trauma.[3] Tissue factor and kaolin can be added to the TEG cup to activate the intrinsic and extrinsic clotting pathways to expedite the time to obtain results. This dual activated rapid TEG (r-TEG) generates results 10 to 20 minutes earlier than a kaolin (k-TEG, only activating the intrinsic pathway) or citrated native TEG (cn-TEG, no activator added). Although rapid activation of the coagulation system to expedite results to treat a bleeding trauma patient is an appealing strategy, overactivation of the coagulation system from exogenous activators can suppress the sensitivity of TEG to quantify fibrinolysis.[5] Accurate quantification of fibrinolysis is clinically relevant because excessive fibrinolysis has repeatedly been demonstrated to predict major bleeding and mortality in trauma, but can take up to an hour to obtain results.[6–8]

Recent work has demonstrated that the addition of exogenous tissue plasminogen activator (tPA) to a cn-TEG can improve TEG's sensitivity for predicting massive transfusion in regards to fibrinolytic response to this plasminogen activator.[9] Trauma patients with a reduced time to obtain maximum clot strength (TMA) in a tPA-cn-TEG inversely correlates with fibrinolysis which enables predicting massive transfusion within 15 minutes. Proteomic analysis supports that the patients with shortened TMA with the tPA-cn-TEG are depleted of the most abundant fibrinolysis inhibitor, alpha2-antiplasmin (α2AP).[10] α2AP is a potent inhibitor of plasmin (the active protease in fibrinolysis), which renders free circulating plasmin's half-life to less than a second, a rate exponentially faster than enzymatic fibrin(ogen) degradation.[11] Therefore, exogenous plasmin can be added to citrated whole blood from a healthy individual to sequester α2AP to replicate this proteomic aberrance appreciated in trauma patients with shortened TMA on tPA-cn-TEG at risk of massive transfusion. We hypothesize that exogenous plasmin will reduce the TMA of tPA-cn-TEG of healthy individuals serving as a model of trauma patients at risk of massive transfusion and that adding a clot activator (tissue factor or kaolin) will further reduce TMA, expediting the time to identify trauma patients at risk for massive transfusion.

Methods

Healthy volunteer population

Under Colorado Institution Review Board approval, healthy adult volunteers with no known history of coagulopathy were recruited for the study ($n = 8$). Seven of the volunteers (87.5%) were male. Ages ranged from 18 to 38 years with a median age of 25 years (23–34). No subjects were taking aspirin, ibuprofen, or birth control at the time of the experiment.

Trauma patient population

Adult trauma patients meeting criteria for the highest level of activation at our level 1 trauma center (Denver Health Medical Center) from July 2017 until January 2018 ($n = 100$) were prospectively enrolled with multiple t-PA challenge TEG assays (see Methods below). All patients had samples collected under protocols approved by the Colorado Multiple Institutional Review Board for prospective evaluation of coagulation in response to trauma. The patients enrolled in this study were consecutive trauma activations in which research blood was available, excluding patients known to be on anticoagulation (Coumadin, therapeutic heparin, or oral thrombin or Xa inhibitors), pregnant, or >18 years of age. Patient demographics, injury mechanism, laboratory results, and transfusion requirements were recorded by professional research assistants who provide onsite, continuous coverage of the emergency department. Injury severity was measured by the maximum Abbreviated Injury Scale scores for the head or neck, chest, abdomen, and extremities; the Injury Severity Score; and the Glasgow Coma Scale score.

Blood collection

Healthy control blood samples were collected via venous blood draw and stored in 3.5-mL tubes containing 3.2% citrate in the laboratory as detailed below. Trauma patient blood samples were collected by trained health care professionals and stored in citrate tubes that were identical to those used for healthy volunteers, and viscoelastic assays were then run by trained research assistants as previously described.[9]

Viscoelastic assays

All healthy volunteer viscoelastic assays were conducted by the first author (C.W.) with extensive experience in multiple types of TEG assays, and all trauma patient samples were assayed by trained professional research assistants who also have extensive experience running viscoelastic assays. Citrated blood samples were analyzed using the TEG 5000 thrombelastograph hemostasis analyzer (Haemonetics, Braintree, MA). Citrated native (cn), kaolin (k), and rapid (r) TEGs were performed according to instructions provided by the manufacturer (Haemonetics). The following indices were obtained from the tracings of the TEG: reaction time (R-time, minutes), angle (degree), maximum amplitude (MA, millimeters), time to maximum amplitude (TMA, minutes), and percent clot lysis 30 minutes after maximum amplitude (LY30, %).

Healthy volunteers tPA viscoelastic assays

Modified tPA-containing assays were also run in parallel with the 3 TEG activators (cn, k, r) without tPA. The methods for exogenous tPA TEG challenge have been previously described by Moore et al.[9] In brief, in the modified assay 500 μL of whole blood was pipetted into a customized vial containing lyophilized tPA (Molecular Innovations, Novi, MI) to a final concentration of 150 ng/mL tPA and mixed by gentle inversion. A 340 μL aliquot of this mixture was then transferred to a 37°C TEG cup preloaded with 20 μL of 0.2 mol/L $CaCl_2$. The rationale for using 150 ng/mL concentration in healthy volunteers was due to previous work in trauma identifying this concentration to provide results within 15 minutes to determine if a patient was at risk of massive transfusion.[9]

Ex vivo trauma induced coagulopathy (plasmin TEG)

Recent work has demonstrated that patients with hyperfibrinolysis have a significant depletion of plasmin inhibitors relative to other severely injured patients.[10] In particular, the primary inhibitor of plasmin, α2AP, was found to be depleted by ≤42% in patients with hyperfibrinolysis. α2AP forms one of the most rapid biochemical reactions in the body to inhibit unbound plasmin, rendering plasmin's half-life to 0.1 seconds.[12] Therefore, the exogenous addition of plasmin to whole blood acts as a depletor of α2AP, rather than a profibrinolytic agent. Based on predicted concentrations of plasminogen in a healthy individuals (176 micrograms/mL[13]), 8.3 micrograms of human plasmin (Haematologic Technologies Inc, Essex Junction, VT) were added to 500 μL of whole blood to cause a predicted 20% depletion of α2AP (which is roughly half

the concentration of circulating plasminogen, with mass-based calculations accounting for additional loss of N-terminal peptide fragments during the activation of plasminogen to plasmin). This concentration was selected based on preliminary experiments in which serial concentrations of plasmin added to a healthy volunteer TEG demonstrated that amount of plasmin, up to a 10% of maximal predicted plasmin generation, did not result in significant changes in the TEG angle, MA, TMA or LY30 compared with non-plasmin samples (Supplementary Fig 1). This plasmin-spiked sample was then assayed with cn-TEG, r-TEG, and k-TEG, with and without tPA. We standardized the abbreviations of this assays owing to the complexity of mixtures with plasmin (P), followed by t-PA, and activator. For example, a dual activated TEG with plasmin and tPA would be abbreviated P-tPA-r-TEG versus a citrated native TEG with no plasmin or tPA would be abbreviated cn-TEG. When contrasting groups, we listed the whole assay and highlighted the different variables between groups.

Trauma patient tPA viscoelastic assays

Trauma patients underwent a panel of 8 TEG assays. These included (1) cn-TEG, (2) k-TEG, (3) r-TEG, (4) cn-TEGs supplemented to 75 ng/mL tPA, (5) cn-TEGs supplemented to 150 ng/mL tPA, (6) k-TEG supplemented to 75 ng/mL tPA, (7) r-TEG supplemented to 150 ng/mL tPA, and (8) functional fibrinogen TEG assay. Eight TEG channels (4 TEG devices) maximized the number of channels available to assess trauma blood within 2 hours of injury, but we were unable to run k-TEG and r-TEG at both tPA concentrations. The rationale for using the lower dose concentration tPA TEG for k-TEG was that kaolin is a weaker activator of coagulation relying on the intrinsic pathway and more likely to show a fibrinolytic response with the lower concentration of tPA. The higher concentration of tPA was used in the rapid TEG because it had dual amplification of both the extrinsic pathway with tissue factor and intrinsic pathway with kaolin, and the most likely assay to produce the quickest results to predict which patient was at risk of massive transfusion. These assays were contrasted to assess their performance for predicting massive transfusion (see Statistical Analysis) and to assess whether the addition of activators could reduce the time to obtain results.

Massive transfusion definitions

We used the 3 common definitions of massive transfusion (MT) including ≥10 units of red blood cells (RBC) at 24 hours from injury (MT24), ≥10 units of RBC 6 hours from injury (MT6), and >4 units of RBC per hour (MT hourly).[14] Multiple definitions of MT were used to identify if TEG assays were consistent in predicting major bleeding because the optimal solitary definition of MT remains debated.[15] A patient was also considered to have a MT in any definition if the patient died of hemorrhagic shock within the initial 24 hours postinjury. All patients requiring blood transfusions underwent TEG-based resuscitation using previously defined cutoff for blood products[2] and received an empiric 1:2 ratio of plasma to red blood cells in the setting of uncontrolled bleeding until initial TEG results were available.[16]

Statistical analysis

SPSS (IBM, Armonk, NY, version 22) was used for statistical analysis. TEG measurements are presented as median and interquartile range. Comparisons of MT patients versus non-MT patients were conducted with Mann-Whitney *U* tests and χ^2 tests. All tests compared between assays were performed with a Wilcoxon rank-sum test for healthy volunteers and trauma patients. If multiple comparisons were used, a Friedman test was used with a Bonferroni adjustment using the area under the receiver operating characteristic (AUROC) curves with 95% confidence intervals (95% CI), we compared the predictive performance for all 3 MT definitions with 2 TEG results, LY30 and MA. Regarding LY30, the following 7 assays were utilized: cn-TEG, t-PA-cn-TEG 75 ng/mL tPA and 150 ng/mL tPA, k-TEG, tPA-k-TEG, r-TEG, and tPA-r-TEG. Two assays, r-TEG and functional fibrinogen used for MA, were evaluated to predict MT. All tests were 2-tailed with α set to 0.05.

Results

Trauma population

The study included 100 trauma patients who were predominantly men (83%), with a median age of 39 years (30–51) and new injury severity score of 25 (12–41). Penetrating injuries occurred in 39% of patients. Massive transfusion occurred in 16% to 19% of patients based on the definition (19% MT24, 16% MT6, and 18% MT hourly). The overall mortality rate was 14%. Differences in patient demographics that underwent a MT24 versus non-MT are listed in Table I.

Healthy volunteer plasmin versus nonplasmin TEG

Examples of TEGs in the presence or absence of tPA or plasmin are shown in Fig 1. The addition of exogenous plasmin to healthy volunteer blood reduced the R-time for all activators (P-cn-TEG 13 min vs 12 min cn-TEG; $P = .036$; P-k-TEG 7 min vs 4 min k-TEG; $P = .012$; P-r-TEG 3 min vs 2 min r-TEG; $P = .036$), but did not increase fibrinolysis (LY30 P-cn-TEG 0.5% vs 0.8% cn-TEG; $P = .600$; P-k-TEG 0.7% vs 0.4% k-TEG; $P = .999$; P-r-TEG 0.5% vs 0.3%, r-TEG; $P = .248$).

Exogenous tPA at a concentration of 150 ng/mL expectedly increased LY30 for all activators (tPA-cn-TEG 64% vs 0.5% cn-TEG; tPA-k-TEG 48% vs 0.7% k-TEG; tPA-r-TEG 47% vs 0.5% r-TEG; $P = .012$ for all). Compared with tPA-TEG, the addition of plasmin to tPA-containing assays increased LY30 for all activators (P-tPA-cn-TEG 78% vs 64% tPA-cn-TEG; $P = .036$; P-tPA-k-TEG 87% vs 48% tPA-

Table I
Patient demographics: Overall versus non-MT24 versus MT24

	Overall	Non-MT	MT24
Age (y)	39 (30–51)	39 (30–53)	31 (27–34)
Male	83%	84%	78%
ED SBP (mmHg)	122 (100–142)	130 (103–143)	95 (56–127)
ED HR	92 (78–112)	96 (80–114)	123 (100–151)
ED GCS	14 (9–15)	15 (11–15)	5 (3–14)
NISS	25 (12–41)	17 (12–27)	46 (30–55)
AIS head	0 (0–4)	0 (0–3)	5 (0–5)
AIS chest	0 (0–3)	0 (0–3)	1 (0–3)
AIS Abd	0 (0–3)	0 (0–0)	0 (0–3)
Penetrating	39%	36%	53%
INR	1.1 (1.0–1.3)	1.1 (1.0–1.1)	1.3 (1.2–2.7)
PTT	26.7 (24.9–31.6)	24.9 (22.6–28.4)	42.3 (29.5–78.3)
Fibrinogen	232 (193–281)	247 (222–304)	180 (80–221)
D-Dimer	1.29 (0.45–12.34)	1.16 (0.48–7.38)	16.95 (8–20)
ED coagulopathy	1 (1–2)	1 (1–2)	3 (2–4)
Required OR	67%	65%	69%
Mortality	14%	4%	58%

AIS, Abbreviated Injury Score; *ED GCS*, presenting Glasgow Coma Score; *ED HR*, presenting heart rate; *ED SBP*, presenting systolic blood pressure; *INR*, international normalized ratio; *MT24*, trauma patients who received ≥10 units of packed red blood cells in 24 hours; *NISS*, new Injury Severity Score; *Non-MT*, trauma patients who did not receive massive transfusion; *OR*, operating room; *PTT*, partial thromboplastin time.
Unless presented as a percentage, the data is presented as the median followed by the 25th to 75th percentile range in parenthesis.

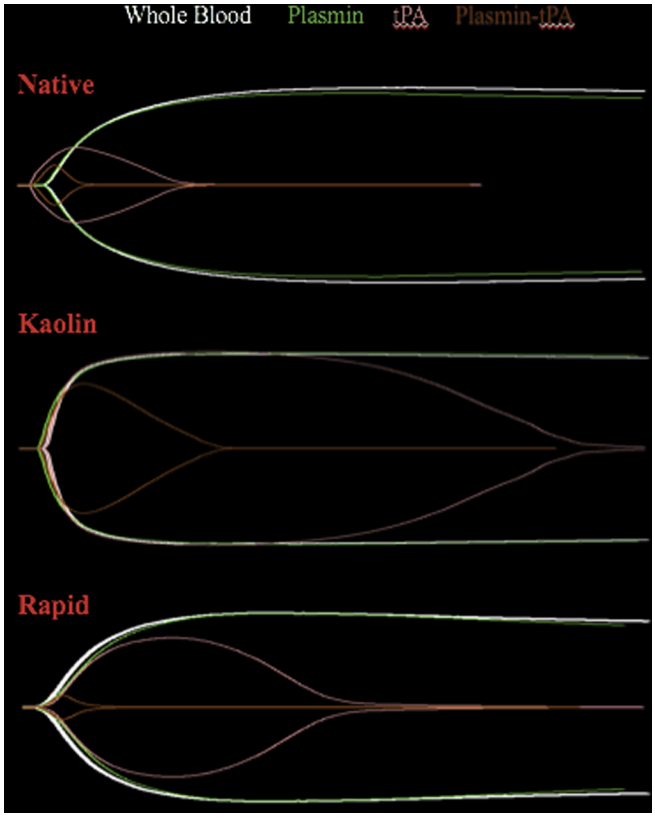

Fig 1. Superimposed representative tracings of healthy volunteer native, kaolin, rapid TEGs in the presence or absence of plasmin or tPA. Compared with healthy whole blood (white tracing), the addition of plasmin (green tracing) reduced R-time but did not affect LY30 or TMA for all activators. The addition of tPA alone (pink tracing) reduced LY30 relative to whole blood for all activators. The addition of tPA and plasmin (orange tracing) increased LY30 and reduced MA and TMA relative to tPA alone for all activators. The tracings under each activator were compiled from a single representative volunteer's assays.

k-TEG; $P = .012$; P-tPA-r-TEG 87% vs 47% tPA-r-TEG; $P = .012$). In addition to increasing LY30, the addition of plasmin to tPA-containing assays also reduced maximum amplitude (Table II).

TMA was not reduced in kaolin or rapid TEGs compared with cn-TEGs after tPA and plasmin addition (P-tPA-cn-TEG 8 min vs P-tPA-k-TEG 8 min vs P-tPA-r-TEG 7 min, $P = .072$; Fig 2). Similarly, the final clot strengths generated by the different activators, measured by maximum amplitude (MA), were similar (P-tPA-cn-TEG 19 mm vs P-tPA-k-TEG 20 mm vs P-tPA-r-TEG 27 mm; $P = .798$; Table II).

Trauma population MT versus non-MT TEG

All 8 TEG assays were completed in 93 of 100 patients. Seven patients were missing ≥1 TEG assays and were not included in the analysis between patients. When assessing the TEG variables' ability to predict massive transfusion, the tPA-TEG variables had higher AUROC for all 3 definitions of MT (Fig 3, *A* to *C*). Regardless of the MT definition, the tPA-cn-TEGs (at both tPA concentrations) had the highest AUROC in comparison wtih tPA-k-TEG and tPA-r-TEG (Table III).

Time to maximum amplitude

The time to maximum amplitude was different between the various TEG assays (Fig 4; $P < .001$). The assay with the shortest TMA was the tPA-r-TEG at 150 ng/mL (15 min [12–19]) followed by the tPA-k-TEG at 75 ng/mL tPA (15 min [12–21]) and tPA-cn-TEG at 150 ng/mL (31 min [28–33]). Only tPA-cn-TEGs (75 ng and 150 ng) demonstrated a difference in TMA between patients who received a MT24 (Fig 4). Analyzing TMA with a tPA-r-TEG vs tPA-cn-TEG (150 ng/mL) to predict MT24 demonstrated that the tPA-cn-TEG retained a higher AUROC (Fig 4), although the median TMA was not significantly different between the 2 assays in patients who received a MT24 (tPA-r-TEG 13 min [8–14] vs tPA-cn-TEG 11 min [9–12]; $P = .813$).

Discussion

The addition of plasmin to healthy whole blood does not increase fibrinolysis, but does result in a reduction in clotting time. The addition of tPA to these plasmin spiked samples does, however, cause a marked increase in LY30, reduction in clot strength, and reduction in TMA that far exceeds the changes observed in tPA assays lacking exogenous plasmin. These in vitro observations translate to trauma patients who underwent a massive transfusion because the tPA-cn-TEG TMA and LY30 both accurately predicted who was at risk of massive transfusion. The tPA-cn-TEG provided results in a similar amount of time as other activated tPA-TEGs

Table II
Median results for native, kaolin, and rapid TEG assays using whole blood from healthy volunteers (95% confidence interval)

TEG indices	cn-TEG	P-cn-TEG	tPA-cn-TEG	P-tPA-cn-TEG
R-time (min)	13 (12–16)	12 (9–14)	9 (6–12)	5 (4–7)
Angle (degrees)	38 (30–47)	39 (35–53)	45 (35–61)	56 (49–61)
MA (mm)	58 (51–67)	52 (48–56)	30 (25–43)	19 (16–32)
TMA (min)	39 (37–43)	37 (29–39)	19 (14–25)	8 (6–14)
LY30 (%)	0 (0.45–1.1)	0.75 (0.03–3.3)	64 (52–67)	78 (56–92)
TEG indices	k-TEG	P-k-TEG	tPA-k-TEG	P-tPA-k-TEG
R-time (min)	7 (6–11)	4 (4–5)	6 (5–6)	4 (4–4)
Angle (degrees)	62 (60–63)	59 (43–63)	65 (63– 68)	58 (56–64)
MA (mm)	58 (55–64)	58 (53–64)	49 (35–57)	20 (13–35)
TMA (min)	27 (23–33)	27 (22–30)	18 (13–23)	8 (6–12)
LY30 (%)	0.70 (0.03–2.0)	0.35 (0–3.4)	48 (26–60)	87 (74–95)
TEG indices	r-TEG	P-r-TEG	tPA-r-TEG	P-tPA-r-TEG
R-time (min)	3 (1–3)	2 (2–3)	2 (1–2)	2 (1–2)
Angle (degrees)	54 (49–65)	55 (46–61)	59 (49–65)	56 (43–60)
MA (mm)	55 (49–61)	54 (50–60)	51 (38–56)	27 (15–35)
TMA (min)	21 (18–24)	22 (20–23)	15 (14–17)	7 (5–9)
LY30 (%)	0.45 (0–0.95)	0.35 (0.03–3.6)	47 (39–56)	87 (80–93)

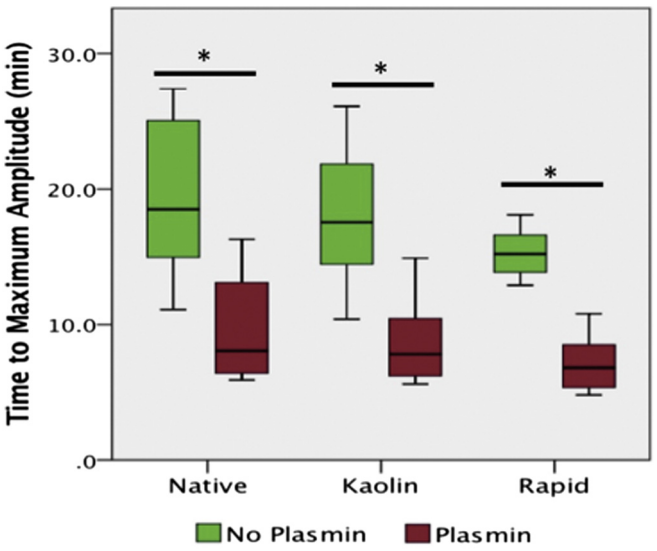

Fig 2. The addition of plasmin to tPA TEGs reduced TMA for all activators. Compared with tPA alone (green boxes), adding plasmin and tPA (red boxes) reduced TMA for native, kaolin, and rapid TEGs. No difference in TMA was observed between activators after the addition of plasmin to tPA TEGs. *$P < .05$.

(kaolin and rapid), but retained the highest performance for identifying patients at risk of MT.

Previous work has demonstrated the clinical utility of a tPA-cn-TEG in identifying patients at risk of massive transfusion.[9] This is a clinically important finding because early knowledge of patients who are likely to need a massive transfusion when it is otherwise clinically unclear allows emergency departments to quickly triage trauma patients and initiate goal-directed resuscitation, which has been shown to improve mortality by ≤50% compared to conventional coagulation studies.[4,12] Although it was anticipated that a clot activator would further expedite the time to obtain results, our in vitro data did not support this hypothesis. A potential mechanism to explain these results could be related to increased thrombin concentrations altering fibrin polymerization architecture resulting in differences in sensitivity to fibrinolysis.[17] It has previously been appreciated that a cn-TEG is more sensitive for detecting tPA mediated fibrinolysis than an activated TEG.[18] Therefore, although a cn-TEG has a slower onset to clot formation, the rate of clot degradation may occur more rapidly in the presence of tPA, resulting in maximum clot strength being obtained in a shorter amount of time. This is supported by the data in Table II in which the time from the end of R to TMA in a tPA-cn-TEG is 10 minutes vs 13 minutes in the tPA-r-TEG. The converse is true when no exogenous tPA is added, in which the time from R to TMA is 26 min in a cn-TEG vs 18 min in a r-TEG.

An additional finding to support that TEG activators in the presence of tPA have less impact on TMA was observed in the plasmin only TEG group. Despite plasmin not causing increased fibrinolysis, the R-time was significantly reduced. This can potentially be explained by the work of Lee and Mann demonstrating the effects of plasmin on factor Va.[19] Plasmin was shown to induce factor V activation to approximately 20% to 30% of the maximum activation. Plasmin also degrades tissue factor pathway inhibitor, increasing the potential for extrinsic activation of coagulation.[20] This paradoxical concept of plasmin promoting a higher rate of thrombin generation might also provide an explanation for previous studies which identified a high prevalence of hypercoagulability after trauma.[21,22] This is an important concept to take into consideration in future studies because local plasmin has the potential to prime the coagulation system for increased thrombin generation systemically but increased clot breakdown locally.

Recently there has been a description of patients with high D-Dimer levels and low lysis activity measured by viscoelastic assays that are thought to have occult hyperfibrinolysis.[23] This unique population of patients with low tPA levels but high D-Dimer had increased mortality compared with patients with low levels of D-Dimer and low systemic fibrinolytic activity. A similar patient population has also previously been described in which patients had a low LY30 in a rapid TEG but increased sensitivity to tPA mediated fibrinolysis measured by tPA-cn-TEG.[10] This patient population had a moderate depletion of plasminogen and α2AP, with low levels of tPA compared with the overall patient population. This group of patients is of particular interest for future research in trauma. Although a patient may have systemically low levels of fibrinolysis activity measured by viscoelastic assay, plasmin generation at a local site of tissue injury could be representative of fibrin burden and a biomarker of injury versus a pathologic increase in fibrinolysis at the site of injury. In addition, whether such patients would benefit from antifibrinolytic therapy is unclear and is in need of additional study. For example, recent work has demonstrated that tranexamic acid in vitro does not

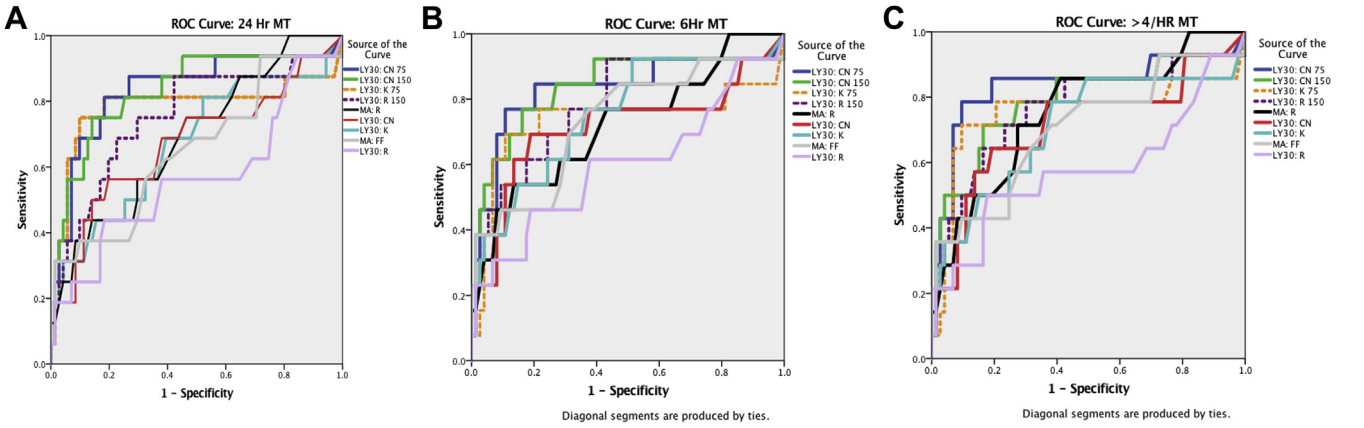

Fig 3. tPA challenge TEGs are superior for predicting massive transfusion in trauma patients. Three common definitions of massive transfusion: (*A*) ≥10 units of red blood cells (RBC) at 24 hours from injury (MT24), (*B*) ≥10 units of RBC 6 hours from injury (MT6), and (*C*) >4 units of RBC per hour (MT Hrly). The predictive ability of 9 separate TEG assays were evaluated in the context of each definition. Regardless of the definition, assays with exogenous tPA (citrated native TEGs supplemented to 75 ng/mL (LY30, tPA-cn-TEG 75) and 150 ng/mL t-PA (LY30, tPA-cn-TEG 150), kaolin-TEG supplemented to 75 ng/mL t-PA (LY30, tPA-k-TEG 75), and r-TEG supplemented to 150 ng/mL (LY30, tPA-r-TEG150) had greater AUROC curves compared with assays without additional tPA. Citrated native tPA challenge TEGs showed the highest AUROC in *A*, *B*, and *C*.

Table III
AUROC of 9 TEG variables for 3 common massive transfusion definitions

Test result variable		Area	STD error	Asymptotic sig.	Asymptotic 95% confidence interval	
					Lower bound	Upper bound
LY30 tPA-cn-TEG 75 ng/mL	MT24	0.835	0.066	<0.001	0.705	0.965
	MT6	0.83	0.079	<0.001	0.675	0.985
	MT Hrly	0.834	0.078	<0.001	0.681	0.986
LY30 tPA-cn-TEG 150 ng/mL	MT24	0.832	0.066	<0.001	0.702	0.965
	MT6	0.837	0.076	<0.001	0.688	0.987
	MT Hrly	0.79	0.081	0.001	0.631	0.948
LY30 tPA-k-TEG 75 ng/mL	MT24	0.783	0.088	<0.001	0.61	0.955
	MT6	0.734	0.104	0.007	0.53	0.937
	MT Hrly	0.753	0.096	0.003	0.565	0.942
LY30 tPA-k-TEG 150 ng/mL	MT24	0.754	0.076	0.002	0.605	0.902
	MT6	0.782	0.079	0.001	0.627	0.937
	MT Hrly	0.767	0.082	0.002	0.606	0.927
MA r-TEG	MT24	0.689	0.074	0.019	0.544	0.836
	MT6	0.72	0.084	0.012	0.556	0.885
	MT Hrly	0.755	0.074	0.003	0.611	0.899
LY30 cn-TEG	MT24	0.671	0.085	0.034	0.505	0.836
	MT6	0.713	0.097	0.015	0.524	0.902
	MT Hrly	0.713	0.089	0.012	0.54	0.887
LY30 k-TEG	MT24	0.671	0.082	0.033	0.511	0.832
	MT6	0.751	0.08	0.004	0.594	0.908
	MT Hrly	0.707	0.089	0.014	0.533	0.882
MA ff-TEG	MT24	0.653	0.083	0.057	0.491	0.816
	MT6	0.733	0.086	0.008	0.565	0.902
	MT Hrly	0.705	0.086	0.016	0.536	0.874
LY30 r-TEG	MT24	0.567	0.09	0.405	0.39	0.744
	MT6	0.6	0.099	0.253	0.405	0.794
	MT Hrly	0.58	0.101	0.346	0.382	0.777

ff, functional fibrinogen *TEG*; *LY30*, lysis at 30 minutes after maximum amplitude (%); *MA*, maximum amplitude (mm); *MT6*, trauma patients who received ≥10 units of RBC 6 hours from injury; *MT24*, trauma patients who received ≥10 units of red blood cells (RBC) at 24 hours from injury; *MT Hrly*, trauma patients who received >4 units of RBC per hour; *tPA-cn-TEG 75 ng/mL*, citrated native TEG with 75 ng/mL of exogenous tPA; *tPA-cn-TEG 150 ng/mL*, citrated native TEG with 150 ng/mL of exogenous tPA; *tPA-k-TEG 75 ng/mL*, kaolin TEG with 75 ng/mL of exogenous tPA; *tPA-k-TEG 150 ng/mL*, kaolin TEG with 150 ng/mL of exogenous tPA; *r*, rapid TEG.

improve clot strength in patients with low LY30, high D-Dimer levels, and depletion of α2AP,[24] a finding that may have major implications in therapeutic decision making if confirmed in clinical study of tranexamic acid in these patients.

There is a theoretical danger of continued local plasmin generation with a concomitant depletion of α2AP. In the setting of overt hyperfibrinolysis in trauma, α2AP levels can be depleted by >40%.[10] Sequestration of α2AP has been associated with significantly increased lysis of plasma clots and whole blood thrombi in both in vitro human and in vivo animal studies.[13,14,25,26] Mutch et al showed that, of the inhibitory SERPINs, α2AP plays the most significant role in preventing lysis of plasma and whole blood clots. Although both α2AP and PAI-1 neutralize tPA mediated fibrinolysis in a synergistic fashion,[25] α2AP complexation with plasmin represents the fastest protease reaction in the body[13] and serves as the master brake on the system (Supplemental Fig 2). In contrast to other studies, which have completely neutralized α2AP activity using neutralizing antibodies,[25,26] in our experimental model we used the addition of exaugenous plasmin to reduce α2AP in a more physiologically relevant way. In so doing, we were able to consistently demonstrate that plasmin alone did not increase fibrinolysis, but increased sensitivity to tPA mediated fibrinolysis. This translates to our clinical observations that the tPA-cn-TEG unmasks patients at risk of massive bleeding, presumably owing to these patients having decreased α2AP. In vitro, the combination of plasmin plus tPA TEGs (P-tPA-cn-TEG) reduced TMA and increased LY30 compared with tPA TEG alone (tPA-cn-TEG). Trauma patients who were bleeding had the same pattern of increased LY30 and a reduction in TMA with tPA-cn-TEGs. Similar to our previous publication,[9] the tPA-cn-TEG has the highest sensitivity for predicting massive transfusion, which was confirmed using three different commonly used definitions of massive transfusion (Table III).

In conclusion, exogenous plasmin resulted in shorter clotting times in healthy volunteers without measurable changes in the other clot properties measured by TEG, including fibrinolysis. A tPA-cn-TEG successfully discriminates a plasmin versus non-plasmin spiked blood sample presumably by unmasking increased susceptibility to fibrinolysis activation in the setting of depletion of fibrinolysis inhibitors. This increased susceptibility to tPA mediated fibrinolysis is a risk factor for massive transfusion but adding clot activators (kaolin or tissue factor) does not expedite the time to obtain these results in simulated trauma and decreases sensitivity for predicting massive transfusion in injured patients. This study underscores a clinically relevant question of how to identify patients with pathologic fibrinolytic activity in trauma. Plasmin in the absence of tPA does not cause appreciable reduction in clot strength, questioning the utility of defining pathologic hyperfibrinolysis in trauma with plasmin antiplasmin complexes. Future studies on fibrinolysis at the local microvascular level are needed to further understand the significance of plasmin generation in the setting of tissue injury because plasmin generation could be pathologic or protective.

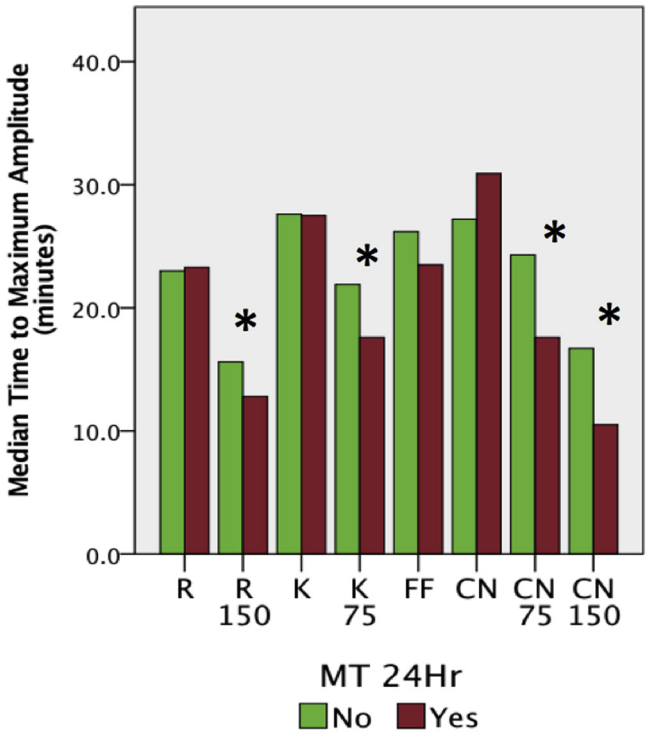

Fig 4. Trauma patients transfused with 10 or more units of RBCs in 24 hours (MT 24Hr) have a reduced TMA compared with non-MT 24Hr patients with tPA TEGs; 75 ng/mL or 150 ng/mL of exogenous tPA were added to rapid (tPA-r-TEG 150), kaolin (tPA-k-TEG 75), and citrated native (tPA-cn-TEG 75, tPA-cn-TEG 150) TEGs. A significant difference in TMA between MT 24Hr patients and non-MT 24Hr was observed only with tPA TEGs. No significant difference was observed between different activators. *$P < .05$.

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Disclosure

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.surg.2019.05.011.

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