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ORIGINAL ARTICLE

Viscoelastic hemostatic fibrinogen assays detect fibrinolysis early

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Abstract
Purpose Viscoelastic hemostatic assays are emerging as the standard-of-care in the early detection of post-injury coagulopathy. TEG and ROTEM are most commonly used. Although similar in technique, each uses different reagents, which may affect their sensitivity to detect fibrinolysis. Therefore, the purpose of this study is to determine the ability of each device to detect fibrinolysis.
Methods TEG (Rapid, Kaolin, Functional Fibrinogen) and ROTEM (EXTEM, INTEM, FIBTEM) were run simultaneously on normal blood as well as blood containing tPA from healthy volunteers ($n = 10$). A two-tailed, paired *t*-test and ANOVA were used to determine the significance between parameters obtained from normal blood and blood with tPA, and individual TEG and ROTEM assays, respectively.
Results TEG detected significant changes in clot strength and 30-min lysis after the addition of tPA ($p < 0.0001$). All ROTEM assays detected changes in the 30-min lysis ($p < 0.0001$), but only INTEM detected changes in clot strength ($p < 0.05$). Kaolin and Rapid TEG assays detected greater changes in clot strength and lysis, but INTEM and EXTEM had decreased lysis onset times compared to TEG ($p < 0.001$). Functional Fibrinogen and FIBTEM assays detected lysis sooner than other TEG/ROTEM assays, and were comparable.
Conclusions TEG assays detect greater changes in clot strength compared to ROTEM. Despite this, Functional Fibrinogen and FIBTEM assays detect fibrinolysis sooner than their corresponding intrinsic and extrinsic assays. Therefore, fibrinogen assays should be employed in actively bleeding trauma patients in order to provide timely antifibrinolytic therapy.

Keywords Thrombelastography · Thromboelastometry · Fibrinogen · Trauma-induced coagulopathy · Fibrinolysis

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Introduction

Trauma-induced coagulopathy (TIC) is an early hypocoagulable state associated with increased transfusion requirements, septic complications, prolonged hospitalizations, and mortality [1–4]. This coagulopathy is common following severe injury, and is observed in approximately one-third of trauma patients. However, the exact mechanism of TIC is unresolved, but is likely multifactorial involving the endothelial release of tPA, the activation of protein C, and the inhibition of platelets (Fig. 1) [5, 6].

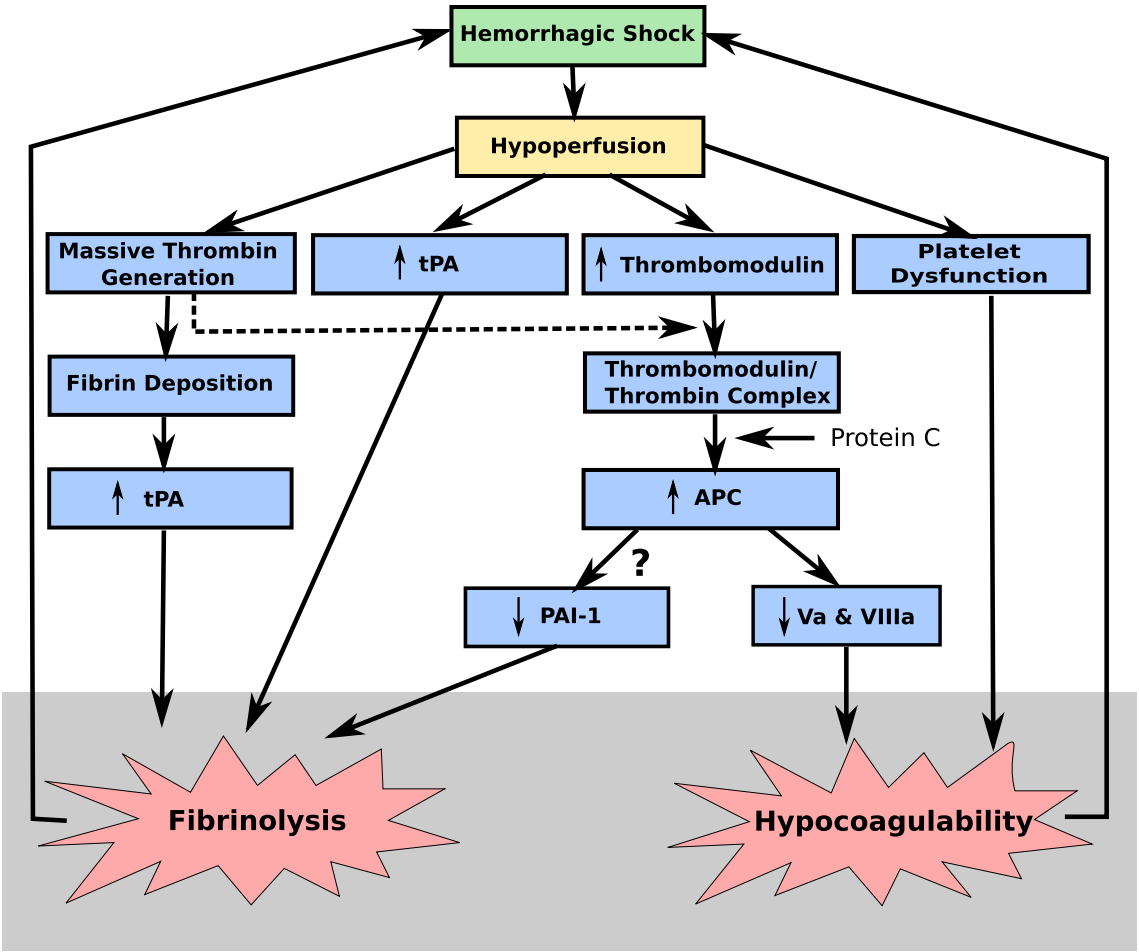

Fig. 1 Mechanisms involved in trauma-induced coagulopathy (TIC)

Fibrinolysis is a rare component of TIC, occurring in approximately 2–10 % of trauma patients, and is associated with >75 % mortality, despite treatment [7–9]. Historically, a percent fibrinolysis of 7.5–15 % was considered clinically relevant; however, recent data suggest that percent fibrinolysis as low as 3 % is associated with increased mortality [10]. With the publication of the CRASH-2 and MATTERs trials, some investigators have advocated for the empiric treatment of the bleeding trauma patient with tranexamic acid [11, 12]. However, since fibrinolysis is uncommon, and the potential complications of widespread tranexamic acid use are unknown, caution may be warranted.

Accordingly, the early detection of fibrinolysis may allow for a more selective use of antifibrinolytic therapy in trauma patients. However, commonly used plasma-based assays [activated partial thromboplastin time (aPTT) and prothrombin time/international normalized ratio (PT/INR)] do not assess fibrinolysis, and euglobulin lysis times require a minimum of 4 h before a result is obtained. An alternative is the employment of viscoelastic hemostatic assays, which use whole blood to provide a global assessment of coagulation, including the dynamics of clot formation and breakdown. Recent data continue to support the superiority of these assays in detecting coagulopathies and predicting massive transfusions in trauma patients compared to both aPTT and PT/INR [2, 13, 14]. Subsequently, viscoelastic hemostatic assays are emerging as the standard-of-care in the early detection of post-injury coagulopathy, including fibrinolysis. There are currently two viscoelastic hemostatic assay devices available, which are the TEG® 5000 and the ROTEM® delta. Although with slightly differing mechanics, these devices measure similar parameters, but employ different activators and reagents.

Each device assesses the intrinsic and extrinsic activation pathways of coagulation, as well as the platelet-independent fraction of clot integrity, which assesses primarily the fibrinogen contribution to clot formation. Thrombelastography or TEG® uses kaolin, tissue factor, and a platelet GPIIb/IIIa receptor antagonist for their kaolin (intrinsic) TEG, Rapid (extrinsic) TEG, and

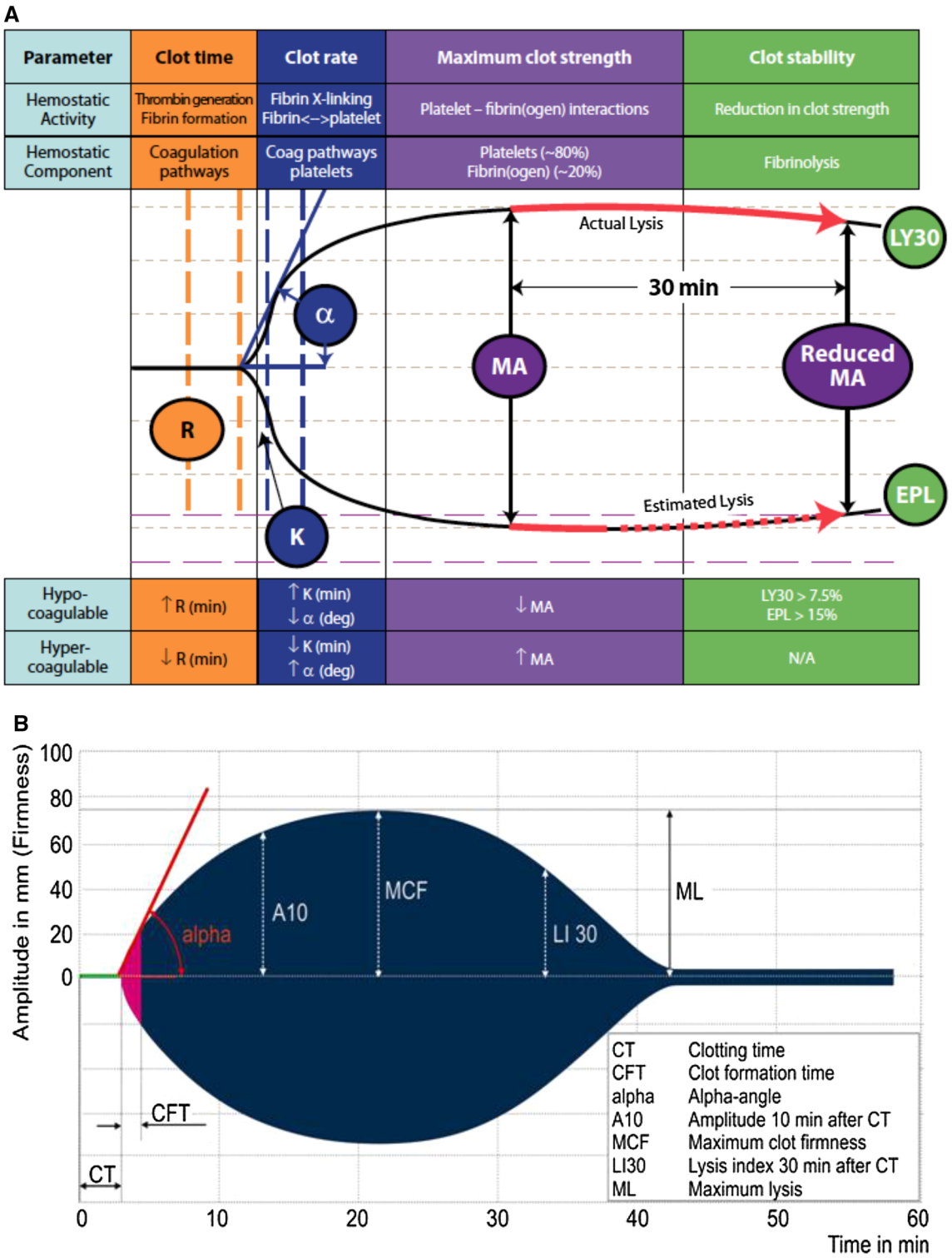

Fig. 2 Parameters for both TEG (**a**) and ROTEM (**b**)

Functional Fibrinogen assays, respectively. Thromboelastometry, or ROTEM®, uses ellagic acid, tissue factor, and cytochalasin D for their INTEM (intrinsic), EXTEM (extrinsic), and FIBTEM (fibrinogen) assays, respectively. Therefore, based on these differences, the purpose of this study is to determine the ability of each device, and the difference in their reagents, to detect fibrinolysis.

Materials and methods

These in vitro studies were performed on citrated whole-blood samples obtained from healthy volunteers ($n = 10$), and was approved under a protocol by the Colorado Multiple Institutional Review Board, and have, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All volunteers were pre-screened, had no known coagulopathies per history and baseline viscoelastic hemostatic assays, and were not taking any medications that could affect the coagulation assays. Venipuncture was performed with a 21-gauge needle in an antecubital vein, and blood was collected into two separate 3.5-mL plastic Vacutainers® containing 3.2 % citrate. In one citrated whole-blood sample, 35 μg of a 100 ng/mL solution of human tissue plasminogen activator (tPA) (Molecular Innovations, Inc., Novi, MI, USA) was added to 1 mL of blood and gently inverted three times to mix. All assays were then run per the manufacturers' guidelines [15, 16]. Prior studies were conducted to determine the 100 ng/mL concentration of tPA needed to allow for significant clot formation, as well as significant clot breakdown, in order to achieve complete lysis. Both TEG (Kaolin, Rapid, and Functional Fibrinogen) and ROTEM (INTEM, EXTEM, and FIBTEM) assays were run on normal blood and blood containing tPA. A single operator ran all assays simultaneously within 20 min of collection and all parameters were recorded. TEG and ROTEM parameters are illustrated in Fig. 2.

Since the purpose of this study was to evaluate each device and their respective reagents' ability to assess fibrinolysis, we compared values pertaining specifically to fibrinolysis. Both TEG and ROTEM have similar parameters for clot strength, which are maximal amplitude (MA) and maximal clot firmness (MCF), respectively, but differ in other lysis parameters. For TEG, we specifically evaluated the clot lysis index at 30 min (CL30), which is the index 30 min following MA, and clot lysis time (CLT), the time from MA to 2 mm of amplitude following MA. For ROTEM, we specifically evaluated MCF, lysis index at 30 min (LI30) following clotting time (CT), and lysis onset time (LOT), which is the time from CT to a 15 % reduction in MCF. Therefore, direct comparisons between TEG and ROTEM parameters are limited, and we chose to compare the changes from baseline normal blood samples compared to those with the addition of tPA.

Statistical analysis

All values are reported as the mean values ± standard error of the mean. Comparison of TEG/ROTEM parameters at baseline and with the addition of tPA were made using the Student's *t*-test to determine significance. Changes in parameters between TEG and ROTEM were compared by analysis of variance (ANOVA) using the Bonferroni/Dunn test for multiple post hoc comparisons, which was dependent upon the equality of variance. *p*-values <0.05 were considered statistically significant.

Table 1 Change in initial TEG and ROTEM parameters after the addition of tPA. There is no significant change in the parameters prior to the determination of clot strength. Statistical significance determined by the Student's *t*-test

Assay	Parameter	*p*-Value
TEG		
Kaolin		
ΔR	0.00 ± 0.38	0.94
ΔK	0.20 ± 0.31	0.51
ΔAngle	1.88 ± 2.76	0.51
Rapid		
ΔR	0.20 ± 0.12	0.19
ΔK	0.20 ± 0.23	0.49
ΔAngle	2.96 ± 2.43	0.26
Fibrinogen		
ΔR	0.30 ± 0.36	0.93
ΔK	1.55 ± 1.55	0.43
ΔAngle	2.60 ± 2.73	0.36
ROTEM		
INTEM		
ΔCT	8.70 ± 4.60	0.09
ΔCFT	2.50 ± 3.63	0.51
ΔAngle	0.20 ± 0.61	0.75
EXTEM		
ΔCT	1.10 ± 1.67	0.53
ΔCFT	4.00 ± 2.67	0.17
ΔAngle	0.80 ± 0.61	0.22
FIBTEM		
ΔCT	2.80 ± 3.37	0.43
ΔCFT	0.00 ± 0.00	0.12
ΔAngle	2.20 ± 12.87	0.92

Results

To examine the effect of tPA on TEG and ROTEM lysis parameters, in vitro studies were conducted on blood from healthy volunteers ($n = 10$) with a mean age of 33 ± 7 years, 50 % of them being male. Regarding all TEG and ROTEM parameters, there were no significant differences in values with the addition of this concentration of tPA from the time of clot initiation to the time prior to the clot strength parameter (Table 1). However, tPA did evoke significant changes in both TEG and ROTEM clot

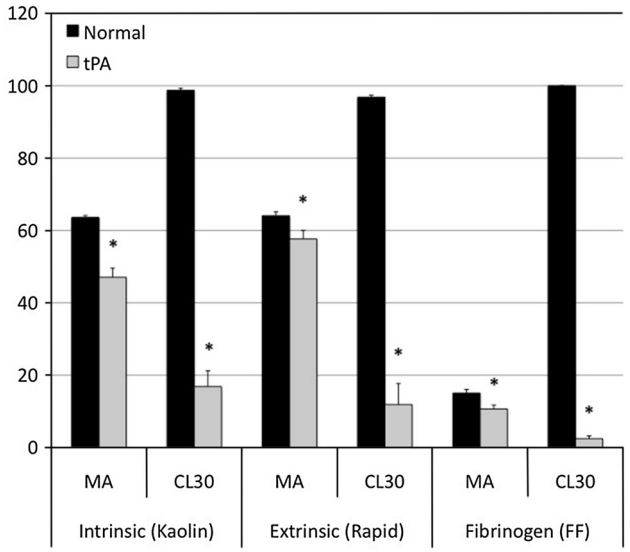

Fig. 3 Clot strengths (*MA*) and lysis (*CL30*) parameters for Kaolin, Rapid, and Functional Fibrinogen TEG assays without and with the addition of tPA. All clot strengths ($p < 0.01$) and lysis parameters ($p < 0.0001$) had a significant decrease, demonstrating significant lysis

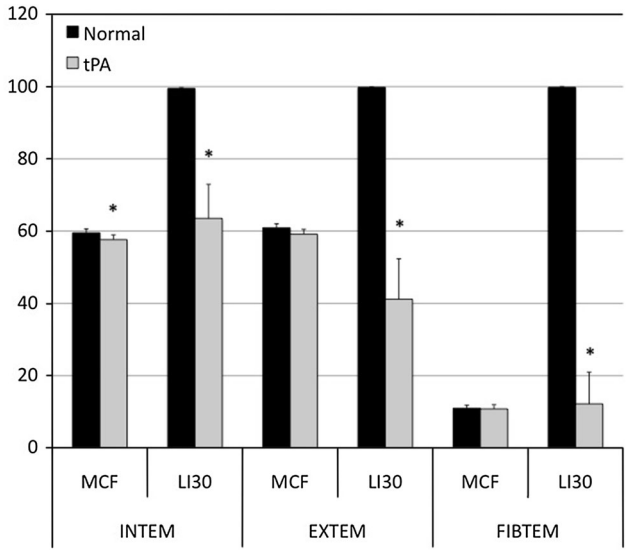

Fig. 4 Clot strengths (*MCF*) and lysis (*LI30*) parameters for INTEM, EXTEM, and FIBTEM ROTEM assays without and with the addition of tPA. Only the INTEM clot strength had a significant decrease following the administration of tPA ($p < 0.05$). All lysis parameters had a significant decrease, demonstrating significant lysis ($p < 0.001$)

strength parameters. For the MA parameter in Kaolin, Rapid, and Functional Fibrinogen TEG assays, the values decreased from 63.60 ± 1.69, 64.08 ± 3.46, and 15.07 ± 3.13 to 47.06 ± 8.00, 57.65 ± 7.53, and 10.68 ± 3.30, respectively ($p < 0.001$) (Fig. 3). For ROTEM, the INTEM MCF was the only assay for clot strength, which revealed a significant change ($p < 0.05$).

Table 2 Comparison of changes in clot strength and lysis parameters after the addition of tPA between TEG and ROTEM devices

TEG	Kaolin	Rapid	FF
ΔMA (mm)	16.5 ± 2.5[a, b]	6.4 ± 2.0[b]	4.4 ± 0.9
ΔCL30 (mm)	81.8 ± 4.2	84.9 ± 6.1	97.5 ± 0.8[c]
CLT (s)	2,095 ± 404	1,849 ± 485	865 ± 342[c]
ROTEM	INTEM	EXTEM	FIBTEM
ΔMCF (mm)	1.9 ± 0.8	1.8 ± 0.9	0.2 ± 0.9
ΔLI30 (mm)	36.0 ± 9.5	58.6 ± 11.1	87.6 ± 8.8[c]
LOT (s)	1,711 ± 102	1,588 ± 105	1,321 ± 89[c]

[a] $p < 0.05$ compared to Rapid TEG and FF assays

[b] $p < 0.05$ compared to ROTEM equivalent assay

[c] $p < 0.05$ compared to intrinsic and extrinsic assays of the same device

MCF parameter changes in the INTEM, EXTEM, and FIBTEM assays with the addition of tPA decreased from 59.5 ± 3.41, 60.9 ± 3.48, and 11.00 ± 2.71 to 57.6 ± 4.22, 59.1 ± 4.28, and 10.8 ± 3.71, respectively (Fig. 4).

Regarding fibrinolysis parameters, both TEG and ROTEM detected significant changes in fibrinolysis. The CL30 parameter for Kaolin, Rapid, and Functional Fibrinogen assays decreased from 98.70 ± 1.79, 96.83 ± 1.81, and 100 ± 0.00 to 16.86 ± 13.83, 11.9 ± 18.34, and 2.48 ± 2.45, respectively, after the addition of tPA, reflecting significant fibrinolysis ($p < 0.0001$) (Fig. 3). For ROTEM, the LI30 parameter significantly decreased after the addition of tPA from 99.50 ± 0.71, 99.80 ± 0.42, and 99.8 ± 0.63 to 63.50 ± 29.85, 41.20 ± 35.07, and 12.2 ± 27.82 for INTEM, EXTEM, and FIBTEM, respectively ($p < 0.001$) (Fig. 4). TEG CLT and ROTEM LOT were also significantly shortened following the addition of tPA for all assays. However, no significant fibrinolysis was observed in the normal blood samples. Therefore, for these samples, CLT and LOT could not be measured in the allotted time. The TEG CLT parameters for the blood samples containing tPA were 2,094.60 ± 127.83, 1,848.60 ± 153.31, and 865.20 ± 107.00 for the Kaolin, Rapid, and Functional Fibrinogen assays, respectively. The ROTEM LOT for blood samples containing tPA were 1,711.20 ± 102.10, 1,588.30 ± 105.58, and 1,321 ± 89.49 for the INTEM, EXTEM, and FIBTEM assays, respectively.

Next, we compared changes in individual parameters between TEG and ROTEM assays (Table 2). Kaolin TEG demonstrated a greater decrease in clot strength compared to the other TEG assays ($p < 0.001$) and to all other ROTEM assays ($p < 0.0001$). Since each device measures different fibrinolysis parameters at 30 min, this comparison could not be made; however, both TEG and ROTEM

fibrinogen assays had the greatest lysis at 30 min compared to their respective intrinsic and extrinsic assays ($p < 0.0001$). Again, TEG and ROTEM parameters to detect time to significant lysis are significantly different, and a direct comparison cannot be made. Nonetheless, the fibrinogen assays for TEG and ROTEM had the shortest CLT and LOT, respectively, compared to their respective intrinsic and extrinsic assays ($p < 0.0001$).

Discussion

These data demonstrate the advantage of fibrinogen assays in detecting hyperfibrinolysis early. Whether using a TEG or ROTEM device, the fibrinogen assay detects fibrinolysis 1.30–2.42 times faster compared to their respective intrinsic and extrinsic assays. This difference is likely due to the platelet-independent effect of the fibrinogen assays. TEG employs a GPIIb/IIIa receptor antagonist, while ROTEM uses cytochalasin D, which binds actin filaments and blocks both the assembly and disassembly of individual actin monomers. While these reagents have different mechanisms of action, both ultimately prevent platelet activation. Therefore, without the contribution of activated platelets to clot strength, the weak fibrin network is rapidly broken down in response to tPA, allowing for the earlier detection of fibrinolysis.

However, it is interesting that small doses of tPA has little to no effect on the overall clot strength with most TEG and ROTEM assays. The assay which demonstrated the most significant decrease in clot strength following tPA administration was the TEG Kaolin assay. This assay appeared to be more sensitive in detecting a tPA-induced decrease in clot strength compared to the other TEG assays and all ROTEM assays. The reason for this is unclear, but may be related to the difference in reagents. TEG uses kaolin, while ROTEM uses ellagic acid. Both induce the intrinsic pathway through contact activation, but may also have other untoward effects. Pre-clinical data suggest that kaolin induces a platelet-related response through the release of platelet factor 3, now known as platelet-derived microparticles, and ellagic acid has platelet inhibitory effects through COX inhibition [17–19]. Therefore, the kaolin TEG assay may have an artificially higher clot strength compared to other TEG assays, as well as the INTEM assay, allowing for a greater change in clot strength following the addition of tPA.

Clot strength preservation in the Functional Fibrinogen and FIBTEM assays following tPA administration also demonstrates that an adequate clot can form, despite platelet inhibition. Therefore, such clot preservation suggests that the platelet is still able to contribute its phospholipid surface for thrombin generation and clot formation, but is not able to form the strong platelet–fibrin network for adequate prolonged hemostasis in the setting of tPA [20]. This observation also supports other clinical and preclinical studies demonstrating that acutely injured patients may have adequate clot strength despite significant platelet inhibition, which likely contributes to post-injury coagulopathies [6, 21].

One limitation of this study is that it is an in vitro study using blood from healthy volunteers, and may not necessarily reflect the complex mechanism of hypocoagulabilty following severe trauma. TIC is multifactorial (Fig. 1), and we only evaluated the tPA component. The significant decrease in time to detect fibrinolysis in the fibrinogen viscoelastic hemostatic assays may be further decreased with the activation of protein C and the consumption of PAI-I, as well as the inhibition of factors Va and VIIIa. In addition, it is difficult to compare the concentration of tPA used in our study to what is seen clinically in severely injured patients [5]. We employed an active form of tPA, while tPA concentrations detected clinically are evaluated through enzyme-linked immunosorbent assays, which do not necessarily reflect activity. The concentration of tPA used in this study was pre-determined to allow for adequate clot formation, as well as the minimal amount needed in order to achieve complete lysis within a reasonable time period. Furthermore, an euglobulin lysis time and other plasma-based assays, including von Clauss fibrinogen levels, were not performed in this study. Prior studies have confirmed accurate detection and strong correlations with fibrinolysis and fibrinogen levels with viscoelastic hemostatic assays [22, 23]. Therefore, these studies were not performed.

Clinically, the ability of fibrinogen viscoelastic hemostatic assays to detect fibrinolysis early may allow for the selective administration of antifibrinolytics in a timely manner. Historically, empiric administration of antifibrinolytics has had untoward consequences, as seen with aprotinin in cardiothoracic surgery, which was associated with increased mortality, as well as increased cardiovascular and cerebrovascular events [24]. Similarly, tranexamic acid has also been associated with seizures, as well as seizures with intractable ventricular fibrillation [25–27]. Despite this, the CRASH-2 trial demonstrated a reduction in overall mortality with the empiric administration of tranexamic acid in bleeding trauma patients without evidence of increased fatal and non-fatal vascular occlusive events [11]. However, seizures were not reported as a primary or secondary outcome in this trial. Furthermore, the number needed to treat to prevent one death in the CRASH-2 trial was calculated to be 68 patients. Therefore, many patients will likely be subjected to unnecessary treatment with potential adverse outcomes. A subanalysis of the CRASH-2 trial did find one

correlation with poor outcomes [28]. Tranexamic acid was only shown to reduce mortality if given within 3 h of injury. If given after 3 h, there was an associated increase in mortality, which raises additional questions [28]. Furthermore, the MATTERs trial did reveal a significant increase in vascular occlusive events associated with tranexamic acid use [12]. Therefore, with ongoing debate and a questionable safety profile of empiric tranexamic acid use, the selective use of antifibrinolytics may be warranted.

Viscoelastic hemostatic assays have the ability to detect clinically significant coagulopathies in trauma early, and the individual fibrinogen assays have the ability to detect fibrinolysis even earlier. Further studies are needed so as to determine if this conclusion correlates clinically to the severely injured trauma patient. If so, the selected use of antifibrinolytics could be achieved in a timelier manner, preventing untoward effects of empiric antifibrinolytic use. Regardless, fibrinogen viscoelastic hemostatic assays should be used in the initial evaluation of the bleeding trauma patient.

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Conflict of interest J. N. Harr, E. E. Moore, T. L. Chin, M. P. Chapman, A. Ghasabyan, J. R. Stringham, A. Banerjee, and C. C. Silliman report no conflict of interest.

Ethical standard statement This study was approved under a protocol by the Colorado Multiple Institutional Review Board, and has, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All healthy volunteers gave their informed consent prior to their inclusion in the study.

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