Tissue plasminogen activator resistance is an early predictor of posttraumatic venous thromboembolism: A prospective study from the CLOTT research group

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BACKGROUND: Venous thromboembolism (VTE) remains a frequent postinjury complication with well established but nonmodifiable risk factors. We hypothesized that fibrinolysis shutdown (SD) as measured by thromboelastography (TEG) would be an independent risk factor for VTE in trauma patients.

METHODS: A subgroup of patients enrolled in the CLOTT-2 (Consortium of Leaders in the Study of Traumatic Thromboembolism 2), multicenter prospective cohort study had kaolin TEG and tissue plasminogen activator (tPA)–TEG data at 12 and 24 hours postadmission. Patients underwent a screening duplex venous ultrasound examination during the first week unless clot was already detected on computed tomography. Injury factors associated with early fibrinolysis SD (defined as kaolin TEG Ly30 ≤0.3%) and/or tPA resistance (tPA-R) (defined as kaolin TEG with tPA 75 ng Ly30 <2.1%) were investigated as was the association of the TEG measurements with the development of VTE.

RESULTS: A total of 141 patients had both TEG measurements at 24 hours, and 135 had both TEG measurements at 12 hours. Shutdown was evident at 12 hours in 71 of 135 (52.6%) patients and in 62 of 141 (44%) at 24 hours. Tissue plasminogen activator resistance was found in 61 of 135 (45.2%) at 12 hours and in 49 of 141 (34.3%) at 24 hours. Factors significantly associated with SD included receiving >4 U of FFP in the first 24 hours, the presence of a major brain injury or pelvic fracture, and the need for major surgery. In contrast, factors significantly associated with early tPA-R included >4 U of red blood cells transfused in the first 24 hours and the presence of a major chest injury or long bone fracture. Deep vein thrombosis was detected in 15 patients and pulmonary clots in 5 (overall VTE rate, 14.2%). Tissue plasminogen activator resistance at 12 hours was found to be an independent risk factor for VTE (hazard ratio, 5.57; 95% confidence interval, 1.39–22.39).

CONCLUSION: Early development of a hypercoagulable state as defined by tPA-R at 12 hours after admission represents a potentially modifiable risk factor for postinjury VTE. (*J Trauma Acute Care Surg.* 2022;93: 597–603. Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.)

LEVEL OF EVIDENCE: Therapeutic/Care Management; Level II.

KEY WORDS: VTE; TEG; fibrinolysis shutdown; tPA resistance.

Trauma-induced coagulopathy has been recognized for decades and remains a common cause of hemorrhagic shock and death after major injury.[1–3] More recently, with increased use of thromboelastography (TEG) in trauma patients, an exaggerated hypercoagulable condition termed fibrinolysis shutdown (SD) has been observed frequently on admission with an associated mortality of 22%.[4–6] Fibrinolysis SD can be further classified by TEG analysis into those patients lacking hypersensitivity to tissue plasminogen activator (tPA; tPA resistant).[7] Patients who exhibit tPA resistance (tPA-R) have a fivefold higher incidence of death compared with patients without fibrinolysis SD.[7] While the association between a hypercoagulable state and the development of venous thromboembolism (VTE) has been suggested, no large-scale prospective study focusing specifically on fibrinolysis SD, tPA-R, and VTE after injury has been conducted.[8,9]

The CLOTT (Consortium of Leaders in the Study of Traumatic Thromboembolism) research group was funded by the Department of Defense to conduct prospective, multicenter studies on posttraumatic pulmonary clots. Our first study (CLOTT-1) included data on 7,880 injured patients and demonstrated that most early pulmonary clots are primary pulmonary thrombi (PT) and not embolic.[10] The CLOTT-2 contains a subset of those patients who were the most critically injured and required intensive care unit (ICU) care. The objective of CLOTT-2 was to describe the relationship between fibrinolysis SD, tPA-R, and VTE in these ICU patients. We hypothesized that early development of SD or tPA-R would identify patients at high risk for VTE.

PATIENTS AND METHODS

Study Design and Data Collection

The CLOTT-1 was a prospective, multicenter observational cohort study conducted over a period of 2 years (2018–2020) at 17 major trauma centers in the United States.[10] A comprehensive electronic case report form was designed exclusively for the study, and research coordinators received training on data collection before the start of the study. Periodic quality checks were performed by the principal investigator (M.M.K.), and the data were entered into the secure central Research Electronic Data Capture system at the University of California San Francisco. Patients were screened for inclusion in CLOTT-1 if they were admitted to any of the 17 participating trauma centers and had at least 1 of our previously identified risk factors for VTE.[11] These risk factors included shock on admission (systolic blood pressure, <90 mmHg), major head injury (Abbreviated Injury Scale [AIS], ≥3), major chest injury (AIS, ≥3), major abdominal injury (AIS, ≥3), pelvic fracture, long bone fracture (lower extremity above the ankle), a major named venous injury requiring ligation or repair, the need for major surgery on admission (surgery lasting at least 1 hour and requiring general anesthesia), and the presence of coagulopathy on admission (international

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This study was presented at the Western Trauma Association Meeting, February 29, 2022, in Big Sky, Montana.

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normalized ratio, >1.5). Additional data collected included the use of blood products during the first 24 hours, prophylactic measures used, missed doses of anticoagulants, imaging procedures related to deep vein thrombosis (DVT) and pulmonary clots, VTE events, treatment of VTE including complications related to treatment, injuries identified, and outcomes. Because these studies were funded by the Department of Defense, only patients in the deployable age range (18–40 years) were eligible for inclusion.

Patients enrolled in CLOTT-1 were also eligible for inclusion in CLOTT-2 if they were severely injured and required at least 48 hours of ICU care. The CLOTT-2 investigators have active coagulation laboratories and included the following five institutions: the University of California, San Francisco and the Zuckerberg San Francisco General Hospital, the Ernest E. Moore Shock Trauma Center at Denver Health and the University of Colorado, Oregon Health Sciences University, the University of Maryland, and the University of Texas Health Science Center, Houston. Enrollment into CLOTT-2 required signed consent by the patient or their surrogate, and the study was approved by each of the 5 institutional review boards as well by the US Department of Defense Human Research Protection Office. The CLOTT-2 patients had serial blood samples collected for TEG analysis within 6 to 12 hours of admission and then at 24, 36, and 48 hours and again on days 5 and 7. Each patient underwent a duplex venous ultrasound examination for DVT at day 3, unless prior imaging had identified DVT or pulmonary clots that required treatment. The ultrasound examination included surveillance for clot in both lower and upper extremities, but to exclude clots associated with central venous lines, we defined DVT in this study as any clot visualized in the vena cava or below. All patients were followed until discharge from the hospital or for up to 30 days. The primary outcome of interest for CLOTT-2 was the development of a hypercoagulable state by TEG assays and its association with any VTE event (DVT, pulmonary emboli, or de novo PT). Secondary outcomes included the trauma-related factors associated with fibrinolysis SD and/or tPA-R as defined hereinafter.

TEG Assays

Citrated Kaolin TEG

Blood was collected in 3-mL citrated blood tubes at as many of the time intervals described previously as possible. Samples were kept at room temperature and assayed within 2 hours after the blood draw in accordance with manufacturer's recommendations. Kaolin TEG assays were run according to the manufacturer's instructions on the TEG 5000 Thrombelastograph Hemostatis Analyzer (Haemonetics, Braintree, MA). The following variables were recorded from the tracing of the TEG: R time (minutes), angle (degrees), maximum amplitude (MA; mm), and the percent lysis 30 minutes after MA (Ly30). For the purposes of this study, we defined fibrinolysis SD as kaolin TEG Ly30 ≤0.3%.[6,7,12]

tPA Challenge TEG

The research coordinators from the other four centers were trained in person in Denver to correctly perform tPA challenge TEGs developed by the University of Colorado trauma research group (H.B.M., E.E.M., A.S.).[7,13] Paired samples collected at the same time as the citrated kaolin TEG were analyzed. Human single chain tPA (Molecular Innovation, Novi, MI) was diluted in 5% bovine serum albumin in phosphate-buffered saline to a final concentration of 10 μg/μL. Individual aliquots of tPA (prepared for a final concentration of 75 ng/mL) were stored at −80°C and thawed immediately before use. From the sample collected from the patient, 500 μL of whole blood was pipetted into a customized vial containing the tPA and mixed by gentle inversion. A 340-μL aliquot of this mixture was then transferred to a 37°C TEG cap preloaded with 20 μL of 0.2 mol/L CaCl2 and analyzed on the same TEG 5000 machine. For the purposes of this study and based on our continued research in our laboratories, we defined tPA-R as kaolin TEG with tPA 75 ng Ly30 <2.1%.[7,14] Residual plasma from these blood samples was stored at −80°C for later analysis as described in the discussion hereinafter.

There was no attempt to calculate a sample size as the relationship between SD, tPA-R, and VTE was unknown. The patients included in the study represent a subset of the critically injured patients admitted to the five trauma centers during times when research staff were available to process blood samples. Thus, there is the potential for bias by not including all critically injured patients during the study period.

TABLE 1. Number and Percent of Patients Demonstrating SD and tPA-R Among 135 Patients at 12 Hours Postadmission

12-h SD	12 h tPA-R No	12 h tPA-R Yes	Total
No	41 (64.1%)	23 (35.9%)	64 (47.4%)
Yes	33 (46.5%)	38 (53.5%)	71 (52.6%)
Total	74 (54.8%)	61 (45.2%)	135 (100%)

$p = 0.04$ denotes significant differences between the columns.

SD (+), meets criteria for fibrinolysis SD (kaolin TEG Ly30 ≤0.3%); SD (−), does not meet criteria for fibrinolysis SD; tPA-R (+), meets criteria for tPA-R (kaolin TEG with tPA 75 ng Ly30 <2.1%); tPA-R, does not meet criteria for tPA-R.

SD, fibrinolysis shutdown.

Statistical Methods

All analyses were conducted in SAS versus 9.4 (SAS Institute, Cary, NC). Unadjusted comparisons used χ^2, Fisher's exact, or Wilcoxon rank sum test for independent samples as appropriate. Competing risk analysis was used for assessing risk factors for VTE censoring for death and discharge or missing samples. Generalized linear models or linear mixed models were used to evaluate temporal trends of categorial or continuous variables, respectively. Statistical significance was set to $p < 0.05$. There were no adjustments for multiple outcomes, as all study outcomes were prespecified hypotheses, to avoid increased type II errors.[15,16] There were no missing values for the clinical risk factors or the outcomes. There were some missing values of tPA-A challenge TEG over time. These values were missing not at random; thus, imputation techniques were not appropriate. Patients with at least two values available during the observational period were included in the statistical models for temporal trends. The linear mixed models used to analyze temporal trends allow for missing observations (i.e., it will not exclude patients with missing values). This manuscript was prepared in accordance with the Strengthening and Reporting of Observational

TABLE 2. Trauma-Related Factors Associated With Early SD and tPA-R

Factor	No SD or tPA-R in 1st 24 h n = 41/179 (22.9%)	SD at 12 h n = 84/169 (49.7%)	tPA-R at 12 h n = 61/135 (45.2%)	SD at 24 h n = 76/180 (42.2%)	tPA-R at 24 h n = 49/141 (34.8%)
Shock, n = 35/240	6/30 (20.0%)	9/23 (39.1%)	12/20 (60.0%)	11/25 (44.0%)	12/24 (50.0%)
TBI, n = 123/240	19/92 (20.7%)	48/89 (53.9%)	26/70 (37.1%)	47/94 (50.0%)	27/73 (37.0%)
Spinal cord injury, n = 14/240	3/11 (27.3%)	5/10 (50.0%)	5/10 (50.0%)	4/10 (40.0%)	2/8 (25.0%)
Chest injury, n = 119/240	15/83 (18.1%)	48/82 (58.5%)	35/62 (56.5%)	45/86 (52.3%)	27/63 (42.9%)
Abdominal injury, n = 85/240	13/69 (18.8%)	30/55 (54.6%)	27/48 (56.3%)	31/67 (46.3%)	24/56 (42.9%)
Pelvic fracture, n = 65/240	10/53 (18.9%)	25/40 (62.5%)	21/34 (61.8%)	29/54 (53.7%)	20/47 (42.6%)
Long bone fracture, n = 69/240	6/56 (10.7%)	29/49 (59.2%)	28/43 (65.1%)	29/56 (51.8%)	25/49 (51.0%)
Venous injury, n = 29/240	4/21 (19.1%)	9/16 (56.3%)	7/12 (58.3%)	11/23 (47.8%)	6/17 (35.3%)
RBCs >4 U in 1st 24 h, n = 48/240	3/37 (8.1%)	15/26 (57.7%)	17/22 (77.3%)	19/33 (57.6%)	19/28 (67.9%)
Plasma >4 U in 1st 24 h, n = 47/240	3/36 (8.3%)	17/26 (65.4%)	17/22 (77.3%)	19/33 (57.6%)	20/27 (74.1%)
Platelets >2 U in 1st 24 h, n = 17/240	1/12 (8.3%)	6/11 (54.6%)	6/8 (75%)	7/10 (70.0%)	5/7 (71.4%)
Cryo >2 U in 1st 24 h, n = 7/240	1/6 (16.7%)	1/1 (100%)	1/1 (100%)	2/3 (66.7%)	3/3 (100%)
Major surgery first day, n = 185/240	29/141 (20.6%)	65/121 (53.7%)	50/103 (48.5%)	66/133 (49.6%)	42/107 (39.3%)

The definitions for each of these trauma-related factors are contained in the methods section of the manuscript with n = number with each factor. In the other columns, the numerator represents the number negative or positive for SD or tPA-R over the total number of samples obtained at that time period for patients with that particular factor.

Cryo, cyroprecipiate; SD, fibrinolysis shutdown.

studies in Epidemiology checklist (Supplemental Digital Content, Supplementary Data 1, http://links.lww.com/TA/C568).

RESULTS

Two hundred forty patients were enrolled in CLOTT-2: 80% were male and 80% were resulting from blunt trauma, with a median age of 29 years. Although most patients had several blood samples for analysis during the first week, a preliminary look at the data suggested that the signal for an association between VTE, SD, and/or tPA-R was strongest during the first 24 hours postinjury (data not shown). Therefore, we first chose to concentrate on the patients with paired blood samples collected during the first 24 hours postinjury. This included 141 patents with paired samples at 24 hours and 135 with paired samples at 12 hours postinjury. The VTE rate in these 141 patients was 14.2% (15 DVT), 4 patients with pulmonary clot only (PT), and 1 patient with both DVT and pulmonary clot (pulmonary emboli). More than 70% of the pulmonary clots were detected by day 4 of hospitalization as were 48% of the DVTs. Table 1 contains the data for the 12-hour samples, which were available for 135 patients. At 12 hours, 71 of 135 (52.8%) met the definition of SD, and 38 of those 71 (53.5%) also demonstrated tPA-R versus only 23 of 64 (35.9%) of those without SD ($p = 0.04$). At 12 hours, a total of 61 of 135 (45.2%) met the definition of tPA-R, and 38 of the 61 (62.3%) also showed SD versus 33 of 74 (44.8%) of those without tPA-R ($p = 0.04$). At 24 hours, 62 of 141 patients (44.0%) had SD, and of these patients, 30 of 62 (48.8%) also had SD at 12 hours. Similarly, of the 49 of 141 (34.8%) who had tPA-R at 24 hours, 61.2% had tPA-R at 12 hours.

We next investigated the trauma-related factors associated with the development of SD or tPA-R at 12 and/or 24 hours. The data on all 240 patients who had at least 1 sample for analysis within the first 24-hour period and the association between the risk factors and the development of SD or tPA-R are shown in Table 2. In the final adjusted analysis, we used only the data from patients who had paired samples at both 12 and 24 hours. As can be seen in Table 3, the adjusted relative risk (RR) for development of SD at 12 hours was significant in patients who received >4 U of FFP on the day of admission (RR, 1.04; $p < 0.0001$). At 24 hours, SD was independently associated with traumatic brain injury (RR, 1.73; $p = 0.0007$), pelvic fracture (RR, 1.39; $p < 0.0001$), and the need for major surgery as defined previously (RR, 2.37; $p < 0.001$). In contrast, tPA-R at 12 hours was associated with the need for >4 U of red blood cell (RBC) transfusions in the first 24 hours (RR, 1.03; $p = 0.03$), the presence of a major chest injury (RR, 1.47; $p = 0.02$), and a long bone fracture (RR, 1.79; $p = <0.001$). At 24 hours, both long bone fracture and the need for RBC transfusion remained significant for their association with tPA-R (Table 4).

TABLE 3. Independent Injury-Related Risk Factors for the Development of Fibrinolysis SD at 12 and 24 Hours

Time Period	Variable	RR	Lower Confidence Limits (95%)	Upper Confidence Limits (95%)	*p*
SD at 12 h	Plasma >4 U in 1st 24 h	1.04	1.03	1.06	<0.0001
	Shock (<90 mm Hg)*	0.61	0.38	0.98	0.94
SD at 24 h	TBI	1.71	1.25	2.34	0.0007
	Pelvic fracture	1.39	1.23	1.56	<0.0001
	Major surgery 1st day	2.37	2.20	2.55	<0.0001

*Shock is associated with hyperfibrinolysis not SD.[8]

TBI, traumatic brain injury with AIS ≥3.

TABLE 4. Independent Injury-Related Risk Factors for Development of tPA-R at 12 and 24 Hours

Time Period	Variable	RR	Lower Confidence Limits (95%)	Upper Confidence Limits (95%)	*p*
12 h	RBCs >4/first 24 h	1.036	1.003	1.07	0.03
	Chest injury	1.47	1.08	2.02	0.02
	Long bone fx	1.79	1.50	2.13	<0.0001
24 h	RBCs >4 first 24 h	1.03	1.02	1.05	<0.0001
	Long bone fx	1.80	1.20	2.68	0.0045

Fx, fracture.

Finally, competitive risk models were developed to examine the independent association between early tPA-R, SD, and the development of VTE (Table 5) Of note, 73.5% of these patients had either SD or tPA-R before development of VTE. As can be seen in the final unadjusted analysis (Table 5), tPA-R at 12 hours emerged as an independent significant risk factor for the development of VTE (hazard ratio, 5.57) when adjusted for other injury-related risk factors listed in Table 2.

DISCUSSION

This CLOTT-2 study documents that more than half of critically injured patients demonstrate failure of clot lysis as early as 12 hours postadmission. Furthermore, those with the most severe hypercoagulable state (tPA-R) are more than five times more likely to develop VTE after injury. Our results suggest that patients most at risk for early tPA-R have chest injuries, long bone fractures, or have required >4 U of RBC transfusions within the first 24 hours of admission.

Most previous investigations on clotting disorders after injury have focused on the development of trauma-induced coagulopathy. Seminal work by Brohi et al.[1] documented a 46% mortality among patients who were coagulopathic after injury versus only 10.9% among those without clotting abnormalities. Coagulopathy after trauma is related to injury severity and the presence of hypoperfusion resulting in death from hemorrhage.[1,3] In fact, recognition of failure to maintain hemostatic clot (acute fibrinolysis) in trauma patients led to the interest in the administration of tranexamic acid (TXA) in the prehospital setting in both military and civilian patients.[17,18]

On the other end of the spectrum, failure to lyse clot after injury has received less attention. The first work in trauma demonstrating fibrinolysis activation with subsequent SD was from Innes and Sevitt[19] in 1964, with subsequent work from the Netherlands terming this "fibrinolytic shutdown" following myocardial infarction and after trauma.[20] These investigators and others described a rapidly developing pattern of fast-acting inhibitor of tissue-type plasminogen activator after trauma leading to fibrinolysis SD.[20,21] Higher levels of plasminogen-activator inhibitor 1(PAI-1) have also been detected in the plasma of patients who developed VTE after total hip surgery compared with those without VTE.[22] More recently, fibrinolysis SD has been correlated with thromboembolic events in patients with severe COVID-19 and the recognition that the inflammation associated with this disease leads to both macroclots and microclots in the arterial as well as the venous system.[23–26] Collectively, this work prompted the large-scale, multinational research projects by the National Institutes of Health focusing on the use of anticoagulants in patients with COVID-19 infections.[24,25]

The current ubiquitous use of TEG in trauma resuscitation has greatly facilitated the study of fibrinolysis SD. Most of the pioneering work in this area has been led by the investigators in Denver (including authors H.B.M., E.E.M., and A.S.) focusing on both the underlying mechanistic pathology and the clinical implications of SD after injury. In a seminal paper, these authors described three distinct fibrinolysis phenotypes identified in a series of trauma patients based on TEG analysis: physiologic fibrinolysis, hyperfibrinolysis, and fibrinolysis SD.[6] In that study, the mortality rate among those with physiologic fibrinolysis was 3% compared with 44% in the hyperfibrinolysis group and 17% in patients with SD.[6] In a multicenter evaluation of 2,540 severely injured patients (Injury Severity Score, >15), SD was the most commonly observed phenotype.[5] The odds ratio of death in those patients with hyperfibrinolysis was highest (odds ratio, 3.3; 95% confidence interval [CI], 2.4–4.6; $p < 0.0001$) but was also elevated in patients with SD (odds ratio, 1.6; 95% CI, 1.3–2.1; $p = 0.0003$) compared with those with physiologic fibrinolysis. Investigations by these authors documented a fivefold increase in mortality in trauma patients who lacked hypersensitivity to tissue plasminogen activator resistance (tPA-R).[7] Using enzyme-linked immunosorbent assay assays, they demonstrated that SD was associated with the activity of tissue PAI-1, which blocks the conversion of plasminogen to plasmin and thus prevents the breakdown of fibrinogen to fibrin split products during clot lysis.[27]

Other investigators have contributed to our understanding of SD. Meizoso et al.[5] reported that persistent SD (lasting for 1 week) was an independent predictor of mortality with an odds ratio of death as high as 8.48 (95% CI, 1.35–53.18; $p = 0.022$). In their study, the authors found

TABLE 5. Independent Hazard Ratios of SD and tPA-R at 12 and 24 Hours for the Development of VTE (Adjusted for VTE Risk Factors)[11]

Parameter	Hazard Ratio	Lower 95% Confidence Limits	Upper 95% Confidence Limits
SD at 12 h	1.29	0.4990	3.403
tPA-R at 12 h*	5.57	1.391	22.39
SD at 24 h	0.57	0.22	1.46
tPA-R at 24 h	1.821	0.600	5.543

*Statistically significant risk factor ($p < 0.05$).

the highest rates of SD in patients with penetrating injuries and those receiving blood product transfusions. Animal models suggest that the severity of SD is associated with the degree of tissue injury.[28]

Platelets have been shown to not only be contributors to clot formation but also central to regulation of clot lysis.[29–31] Specifically, platelets release PAI-1 in parallel with the endothelium. A secondary analysis of data from the Pragmatic Randomized Optimal Platelet and Plasma Ratios trial study group suggested that platelet dysfunction and suppressed clot lysis contributed to the development of VTE after injury.[32] Given the role of platelets in both clot formation and lysis, the use of antiplatelet drugs for VTE prevention deserves further investigation especially in patients with SD. A retrospective study by Brill et al.[33] found that preinjury aspirin (ASA) use was associated with lower VTE rates after injury when combined with in-hospital heparinoid prophylaxis.

Our study has documented that approximately half of critically injured patients have SD or tPA-R (or both) as early as 12 hours postinjury. In a smaller bi-institutional study evaluating clot risk in trauma patients with blunt solid organ injury, low fibrinolytic activity was also associated with clot complications at 12 hours, while clot strength was associated with thrombotic complications 48 hours after admission.[34] In contrast, another study evaluating TEG and stroke from blunt cerebral vascular injury did not find an association with prolonged clot lysis (Ly30), but there was an association with clot strength measured by MA on TEG analysis.[35] It is possible that vascular beds in various anatomical positions differ in their responses to risk factors for thrombotic events.

In our study, patients most at risk for the development of a very early (12 hours postinjury) hypercoagulable state are those who require transfusions and those with chest trauma or long bone fractures. At 24 hours, brain injuries, pelvic fractures, and those undergoing major surgery during the first 24 hours are most at risk for displaying SD or tPA-R. A review of the data in Tables 2, 3, and 4 would suggest that transfusions of RBCs, plasma, platelets, and cryoprecipitate could be implicated as causative factors contributing to the inability to lyse clot. Perioperative blood transfusions have been associated with postoperative VTE in other fields of surgery with an estimated odds ratio of 2.95.[36,37] It has been proposed that transfused blood acts as a modulator of the inflammatory cascade and thus contributes of hypercoagulation. A previous study in trauma patients by Schultz et al.[38] found that patients who had been transfused with RBCs had more than three times the risk of developing VTE compared with those without transfusions, although the number of units of blood is not mentioned in that study. Gearhart et al.[39] also found that transfusion of >4 U of RBCs conferred a significant risk of developing VTE. However, it is unclear whether it is the RBCs themselves (or FFP as we have shown here) that contribute to the hypercoagulable state or that these transfusions merely reflect the severity of shock and tissue injury that drives the hypercoagulability.

None of the patients in CLOTT-2 had a venous ligation, but there were overall 29 patients with a major venous injury that required repair. Given the known association between a venous injury and DVT, we reanalyzed our data excluding those 29 patients, and found that the association of VTE with tPA-R at 12 hours remained statistically significant (14 of 20 VTE cases [70%] had tPA-R at 12 hours vs. 38 of 103 patients (36.9%) with no VTE [$p = 0.006$]).

As mentioned previously, there is great enthusiasm for the use of TXA early in the resuscitation of patients at risk for hemorrhagic shock. However, given the fact that nearly 50% of multiply injured patients admitted to the ICU met our definition of SD and/or tPA-R, we strongly advise that TXA in patients at risk for SD be withheld unless there is evidence of pathologic fibrinolysis, as TXA in patients with SD can worsen the hypercoagulable state. The use of TXA and its association with VTE and SD are the subject of ongoing research by the CLOTT group.

Our study has several limitations. While we intended to obtain blood samples at multiple intervals throughout the first week of admission, many samples were missed. Discharge from the ICU or hospital before the 7-day blood draw accounted for many missed samples. Not all centers had 24/7 research staff to collect samples and perform the TEG assays. Research was also impacted by the COVID-19 pandemic during which many research projects were curtailed or completely suspended. Another limitation is the age range of our enrolled patients (18–40 years), which questions the generalization to older trauma patients. Additional research from CLOTT will focus on patients with delayed development of a hypercoagulable state (after the first 24 hours), on patients who resolve this state but redevelop it again (observed anecdotally), and on associations with platelet biology. The plasma samples stored from these patients are currently being analyzed for tPA, PAI-1, tPA/PAI-1, plasminogen, plasmin-antiplasmin, thrombin-activated fibrinolysis inhibitor, clotting factor XIII, and biomarkers that may provide further insight into the biological mechanisms of posttraumatic clotting disorders. Given the potential contribution of platelets to the inability to lyse clot, the addition of ASA as a prophylactic agent deserves further attention.

Despite these limitations, this study contributes to our understanding of clotting disorders following injury. Importantly, establishing that severe fibrinolysis SD defined by tPA-R is significantly associated with posttraumatic VTE identifies for the first time a potentially modifiable VTE risk factor after injury. Based on our study, we recommend the use of ASA together with chemical thromboprophylaxis at 12 hours postinjury in patients at risk for SD to target the platelet contribution to tPA-R. Additional basic research will likely yield other targeted interventions that could further customize our approach to VTE prevention guided by injury type and coagulation profile.

AUTHORSHIP

M.M.K., E.E.M., L.Z.K., C.E.W., B.R.B., L.N.K., M.K.M., H.B.M., and A.S. contributed in the study design. M.M.K., E.E.M., L.N.K., C.E.W., M.K.M., and B.R.B. contributed in the data acquisition. M.M.K., E.M.M., H.B.M., L.Z.K., and A.S. contributed in the interpretation of data.

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