Journal of Trauma and Acute Care Surgery

TISSUE PLASMINOGEN ACTIVATOR RESISTANCE IS AN EARLY PREDICTOR OF POST-TRAUMATIC VENOUS THROMBOEMBOLISM: A PROSPECTIVE STUDY FROM THE CLOTT RESEARCH GROUP

--Manuscript Draft--

Manuscript Number:	JT-D-22-00048R2
Full Title:	TISSUE PLASMINOGEN ACTIVATOR RESISTANCE IS AN EARLY PREDICTOR OF POST-TRAUMATIC VENOUS THROMBOEMBOLISM: A PROSPECTIVE STUDY FROM THE CLOTT RESEARCH GROUP
Article Type:	Original Article
Section/Category:	2022 WTA Podium
Keywords:	VTE, TEG, Fibrinolysis shutdown, TPA Resistance
Corresponding Author:	M. Margaret Knudson, MD University of California San Francisco San Francisco, CA UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of California San Francisco
Corresponding Author's Secondary Institution:	
First Author:	M. Margaret Knudson, MD
First Author Secondary Information:	
Order of Authors:	M. Margaret Knudson, MD
	Authors and Affiliations
	Hunter B. Moore, MD
	Ernest E Moore, MD
	Lucy Z Kornblith@ucsf.edu, MD
	Lazlo N Kiraly, MD
	Michelle K McNutt, MD
	Charles F Wade, PhD
	Brandon R Bruns, MD
	Angela Sauaia, MD, PhD
Order of Authors Secondary Information:	
Manuscript Region of Origin:	UNITED STATES
Abstract:	

Cover Letter

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • SANTA CRUZ

M. Margaret Knudson, M.D., FACS
Professor of Surgery
Chair, DSMB

Department of Surgery
Peggy.Knudson@ucsf.edu

Raul Coimbra MD, PhD
Editor-in-Chief
Journal of Trauma and Acute Care Surgery

Dear Dr. Coimbra:

We are pleased to submit our manuscript title: **Tissue Plasminogen Activator Resistance is an Early Predictor of Post-Traumatic Venous Thromboembolism: A Prospective Study form the CLOTT Research Group** for your review and consideration for publication in the Journal of Trauma and Acute Care Surgery. This manuscript has not been submitted elsewhere and will be presented at the Annual Meeting of the Western Trauma Association on February 25, 2022. This is a prospective observational study funded by the Department of Defense which should be considered in the category of Original Research/Therapeutic according to the description in the author instructions. I am a member of both AAST and WTA and my contact information is below. We look forward to a favorable review and we thank you and your editors for the consideration.

M. Margaret Knudson MD, FACS
Department of Surgery, ZSFGH, Ward 3a
1001 Potrero Avenue
San Francisco CA 94110
Phone: 628-206-4626
Cell: 650-823-1300
FAX 628-206-5484
Email: peggy.knudson@ucsf.edu; pknudson@facs.org

With respect,

M. Margaret Knudson MD, FACS
Professor of Surgery, University of California, San Francisco
Adjunct Professor of Surgery, Uniformed Services University
Medical Director, Military Health System Strategic Partnership, American College of Surgeons

Background: Venous thromboembolism (VTE) remains a frequent post-injury complication with well-established but non-modifiable risk factors. We hypothesized that fibrinolysis shutdown as measured by thromboelastography would be an independent risk factor for VTE in trauma patients.

Methods: A subgroup of patients enrolled in the CLOTT-2, multi-center prospective cohort study had kaolin TEG and tPA-TEG data at 12- and 24- hours post admission. Patients underwent a screening duplex venous ultrasound exam during the first week unless clot was already detected on computed tomography. Injury factors associated with early fibrinolysis shutdown (SD) (defined as kaolin TEG Ly30≤0.3%) and/or tPA resistance (tPA-R) (defined as kaolin TEG with tPA 75ng Ly30<2.1%) were investigated as was the association of the TEG measurements with the development of VTE.

Results: 141 patients had both TEG measurements at 24 hours and 135 had both at 12 hours. SD was evident at 12 hours in 71/135 (52.6%) of patients and in 62/141 (44%) at 24 hours. tPA-R was found in 61/135 (45.2%) at 12 hours and in 49/141 (34.3%) at 24 hours. Factors significantly associated with SD included receiving > 4 units 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 units of RBCs transfused in the first 24 hours, and the presence of a major chest injury or long bone fracture. DVT was detected in 15 patients and pulmonary clots in 5 (overall VTE rate 14.2%). tPA-R at 12 hours was found to be an independent risk factor for VTE (hazard ratio 5.57, 95% CI 1.39-22.39).

Conclusions: Early development of a hypercoagulable state as defined by tPA resistance at 12 hours after admission represents a potentially modifiable risk factor for post-injury VTE.

Level of Evidence: II

Study Type: Original Research/Therapeutic

Keywords: VTE, TEG, Fibrinolysis shutdown, tPA resistance

TISSUE PLASMINOGEN ACTIVATOR RESISTANCE IS AN EARLY PREDICTOR OF POST-TRAUMATIC VENOUS THROMBOEMBOLISM: A PROSPECTIVE STUDY FROM THE CLOTT RESEARCH GROUP

Short title: Tissue Plasminogen Activator Resistance and VTE

Authors and Affiliations: M. Margaret Knudson MD

Department of Surgery, University of California San Francisco

Peggy.Knudson@ucsf.edu

Hunter B. Moore MD

Department of Surgery, University of Colorado

Hunter. Moore@cuanschutz.edu

Ernest E. Moore MD

Department of Surgery, EE Moore Shock Trauma Center

Denver Health

Ernest.Moore@dhha.edu

Lucy Z. Kornblith MD

Department of Surgery, University of California San Francisco

Lucy.Kornblith@ucsf.edu

Lazlo N. Kiraly MD

Department of Surgery, Oregon Health Science University

kiralyl@ohsu.edu

Michelle K. McNutt MD

Department of Surgery, U. Of Texas Medical Center, Houston

Michelle.K.McNutt@uth.tmc.edu

Charles E. Wade PhD

Department of Surgery, U. of Texas Medical Centers, Houston

Charles.E.Wade@uth.tmc.edu

Brandon R. Bruns MD

Department of Surgery, U. Texas Southwestern

Brandon.Bruns@UTSouthwestern.edu

Angela Sauaia MD, PhD

School of Public Health, U. Colorado Denver

Angela.Sauaia@cuanschutz.edu

Corresponding Author: M. Margaret Knudson MD Department of Surgery, SFGH

1001 Potrero Avenue Ward 3A;San Francisco CA 94110

Phone: 628-206-4626; FAX: 628-206-5484

Peggy.Knudson@ucsf.edu

Conflicts of Interest: Kornblith: Received personal fees from Census SAB outside of the submitted work. Bruns: Nothing to report; Knudson: Nothing to report; Kiraly: Received grants

from the CNTR; Wade: has stock in Descicio LLC and grants from Grifols outside the submitted work; Moore H:: nothing to report; Sauaia: nothing to report; McNutt: nothing to report; MooreEE: has received research support from the following companies: Haemonetics, Instrumentation Labs; Hemosonics, Stago, and Diapharma.

Presentation: Western Trauma Association Meeting, Feb. 29, 2022; Big Sky MT

Acknowledgments: The authors would like to recognize the contributions to this research provided by Michael P. Chapman MD who died prematurely this past year. We would also like to express our gratitude to the staff of the Coalition for National Trauma Research for their assistance in securing funding for this work and in grant administration.

Funding Source: U.S. Army Medical Research Acquisition Activity is the awarding and administering acquisition office. This work was supported by the Assistant Secretary of Defense for Health Affairs through the Department of Defense Medical Research and Development Program under award W81XWH-1-17-1-0673. (Dr. Knudson-Principal Investigator)

Author Contributions:

Study Design: Knudson, E.Moore, Kornblith, Wade, Bruns, Kiraly, McNutt, H. Moore, Sauaia

Data Acquisition: Knudson, E Moore, Kiraly, Wade, McNutt, Bruns

Interpretation of Data: Knudson, E Moore, H Moore, Kornblith, Sauaia

March 8, 2022

Marc de Moya MD, FACS

WTA Publications Editor

Journal of Trauma and Acute Care Surgery

Dear Dr. de Moya and co-reviewers,

We appreciate all of your efforts to improve our manuscript and your thorough secondary review. Below please find the responses to your questions and comments. Additionally we have added a statement on the use of TXA and the potentially hazards of administering it to patients already in shutdown. This was suggested by the comments given when the paper was presented at the WTA live.(Please see page 12 of the manuscript). We hope you will find our edits acceptable.

Very respectfully,

M. Margaret Knudson MD, FACS

Additional Comments to Author(s):

Reviewer #1: Thank you for resubmitting this manuscript. I appreciate the responses provided to questions in the previous review.
I have three more points that the reader would like clarified.
1. Were any of the VTEs related catheters included?

Response: Although duplex exams included both lower and upper extremities, we considered VTE as being present only from the IVC down (with some abdominal clots (IVC, iliac detected on CT imaging) in order to avoid clots associated with central lines. Additionally, there were no femoral lines in these patients when the duplex was performed. A sentence to clarify that has been added to the Methods section on page 3.

2. Why were venous injuries, specifically those involving ligation included? For these patients, for example a ligation of femoral vein, the lack of flow in this vessel will often result in a clot in that vein. This additional fact is not necessarily related to SD. One may exclude these patients in the final analysis as it adds a confounder.

Response: There were no patients with venous ligation in this series. There were 29 patients with major venous injuries requiring repair. We analyzed the data with and without these patients and found that with or without these patients, early tPA-R remained significant in its association with VTE. Please see page 11.

3. While you discussed it in the discussion, please include how SD is modifiable in the conclusion.

Response: We have edited our conclusion with what we hope are helpful suggestions for early initiation of VTE prophylaxis in patients with SD as a modifiable risk factor. Please see page 12.

Reviewer #2: Minor comments:
Please take the % sign out of LY30 in the manuscript and just keep the % as the unit, ie "defined as kaolin TEG Ly30% 0.3% should just read TEG LY30 0.3%. The symbol should be used as opposed to

Response: The % after Ly30 has been removed.
In the edit of the tPA Challenge TEG description the word is should be 'in'

Response: Corrected.

BACKGROUND

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 has been observed frequently on admission with an associated mortality of 22%.[4-6] Fibrinolysis shutdown (SD) can be further classified by TEG analysis into those patients lacking hypersensitivity to tissue plasminogen activator (tPA resistant).[7] Patients who exhibit tPA resistance (tPA-R) have a 5-fold higher incidence of death compared to patients without fibrinolysis shutdown.[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 shutdown, tPA resistance and VTE after injury has been conducted.[8,9]

The CLOTT research group (Consortium of Leaders in the study Of Traumatic Thromboembolism) was funded by the Department of Defense to conduct prospective, multi-center studies on post-traumatic pulmonary clots. Our first study (CLOTT-1) included data on 7880 injured patients and demonstrated that most early pulmonary clots are primary pulmonary thrombi and not embolic. [10] 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 shutdown, tPA resistance and VTE in these ICU patients. We hypothesized that early development of SD or tPA-R would identify patients at high-risk for VTE.

METHODS

Study Design and Data Collection

CLOTT-1 was a prospective, multicenter observational cohort study conducted over a period of two years (2018-2020) at 17 major trauma centers in the US.[10] A comprehensive electronic case report form was designed exclusively for the study and research coordinators received training on data collection prior to the start of the study. Periodic quality checks were performed by the Principal Investigator (MMK) and the data was entered into the secure central Research Electronic Data Capture (REDCap) 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 one of our previously identified risk factors for VTE.[11] These risk factors included: shock on admission (systolic blood pressure < 90 mmHg); major head injury (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 normalized ratio > 1.5). Additional data collected included the use of blood products during the first 24 hours, prophylactic measures utilized, missed doses of anticoagulants, imaging procedures related to 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 yrs.) 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. CLOTT-2 investigators have active coagulation laboratories and included the following 5 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 (HRPO). CLOTT-2 patients had serial blood samples collected for TEG analysis within 6-12 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 exam for DVT at day 3, unless prior imaging had identified DVT or pulmonary clots that required treatment. The ultrasound exam included surveillance for clot in both lower and upper extremities, but in order 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, PE, or de novo pulmonary thrombi, PT). Secondary outcomes included the trauma-related factors associated with fibrinolysis shutdown (SD) and/or tPA resistance (tPA-R) as defined below.

TEG Assays

Citrated Kaolin TEG: Blood was collected in 3 ml citrated blood tubes at as many of the time intervals described above 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 ~~and~~ lysis 30 minutes after MA (Ly30~~%~~), For the purposes **o**f this study we defined fibrinolysis shutdown (SD) as kaolin TEG Ly30~~%~~ </= 0.3%. [6,7.13]

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 (HGM, EEM, AS).[7,12] 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 *u*g/*u*l. Individual aliquots of tPA (prepared for a final concentration of 75 ng/mL) were stored at -80 degrees C and thawed immediately before use. From the sample collected from the patient, 500 *u*l of whole blood was pipetted into a customized vial containing the tPA and mixed by gentle inversion. A 340 *u*l aliquot of this mixture was then transferred to a 37 degree C TEG cap preloaded with 20 *u*l of 0.2 mol/L CaCl2 and analyzed on the same TEG 5000 machine. For the purposes of this ~~study, and~~study and based on our continued research i~~s~~ our laboratories, we defined tPA resistance (tPA-R) as kaolin TEG with tPA 75 ng Ly30~~%~~<2.1%. [7,14] Residual plasma from these blood samples were stored at -80 degrees C for later analysis as described in the discussion below.

There was no attempt to calculate a sample size as the relationship between SD, tPA-R and VTE was unknow. The patients included in the study represent a sub-set of the critically injured patients admitted to the 5 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.

Statistical Methods

All analyses were conducted in SAS vs 9.4 (SAS Institute, Cary NC). Unadjusted comparisons used Chi-square, 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 pre-specified hypotheses, in order 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 (MNAR) 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 studies in Epidemiology "STROBE" checklist. **(SDC-1)**

RESULTS

Two hundred forty patients were enrolled in CLOTT-2: 80% male, 80% 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 post injury (data not shown). Therefore, we first chose to concentrate on the patients with paired blood samples collected during the first 24 hours post injury. This included 141 patents with paired samples at 24 hours and 135 with paired samples at 12 hours post injury. The VTE rate in these 141 patients was 14.2% (15 DVT, 4 patients with pulmonary clot only (PT) and one patient with both DVT and pulmonary clot (PE). Over 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 71/135 (52.8%) met the definition of SD and 38 of those 71 (53.5%) also demonstrated tPA-R versus only 23/64 (35.9%) of those without SD (p=0.04). At 12 hours, a total of 61/135 (45.2%) met the definition of tPA-R and 38 of the 61 (62.3%) also showed SD versus 33/74 (44.8%) of those without tPA-R (p=0.04). At 24 hours, 62/141 (44.0%) patients had SD and of these patients 30/62 (48.8%) also had SD at 12 hours. Similarly, of the 49/141 (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 one sample for analysis within the first 24-hour period and the association between the risk factors and the development of SD or tPA-R is 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

units of FFP on the day of admission (RR=1.04, p <.0001). At 24 hours, SD was independently associated with traumatic brain injury (TBI), (RR=1.73, p0.0007), pelvic fracture (RR=1.39, p<.0001) and the need for major surgery as defined above (RR=2.37, p<0.001). In contrast, tPA-R at 12 hours was associated with the need for > 4 units 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)**

Finally, competitive risk models were developed in order 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 prior to 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 (HR 5.57) when adjusted for other injury-related risk factors listed in Table 2.

DISCUSSION

This CLOTT-2 study documents that over half of critically injured patients demonstrate failure of clot lysis as early as 12 hours post-admission. Further, those with the most severe hypercoagulable state (tPA-R) are over 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 units 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 and others documented a 46% mortality among patients who were coagulopathic after injury versus only 10.9% among those without

clotting abnormalities.[1] 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 shutdown was from Innes and Sevitt in 1964, with subsequent work from the Netherlands terming this "fibrinolytic shutdown" following myocardial infarction and after trauma.[19,20] These investigators and others described a rapidly developing pattern of fast-acting inhibitor of tissue-type plasminogen activator after trauma leading to fibrinolysis shutdown.[20,21] Higher levels of plasminogen-activator inhibitor-one (PAI-1) have also been detected in the plasma of patients who developed venous thromboembolism after total hip surgery compared to those without VTE.[22] More recently fibrinolysis shutdown has been correlated with thromboembolic events in patients with severe coronavirus disease (COVID-19) and the recognition that the inflammation associated with this disease leads to both macro-and micro-clots in the arterial as well as the venous system.[23-26] Collectively this work prompted the large-scale, multi-national research projects by the National Institute of Health focusing on the use of anticoagulants in patients with COVID-19 infections.[24,25]

The current ubiquitous use of thromboelastography in trauma resuscitation has greatly facilitated the study of fibrinolysis shutdown (SD). Most of the pioneering work in this area has been led by the investigators in Denver (including authors HBM, EEM and AS) focusing on both the underlying mechanistic pathology as well as 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 shutdown.[6] In that study, the mortality rate among those with physiologic fibrinolysis was 3%~~(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% 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 to those with physiologic fibrinolysis. Investigations by these authors documented a five-fold increase in mortality in trauma patients who lacked hypersensitivity to tissue plasminogen activator (tPA-R).[7] Utilizing ELISA assays, they demonstrated that SD was associated with the activity of tissue plasminogen activator inhibitor-1 (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 and others reported that persistent SD (lasting for one week) was an independent predictor of mortality with an odds ratio of death as high as 8.48 (95% CI 1.35-53.18, $p=0.022$).[5] In their study, the authors found that 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 PROPPR 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 and others found that pre-injury aspirin use was associated with lower VTE rates after injury when combined with in-hospital heparinoid prophylaxis. [33]

Our study has documented that approximately half of critically injured patients have SD or tPA-R (or both) as early as 12 hours post injury. 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 post injury) 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 post-operative 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 found that patients who had been transfused with RBCs had over three times the risk of developing VTE compared to those without transfusions

although the number of units of blood is not mentioned in that study[38]. Gearhart and others also found that transfusion of > 4 units of RBCs conferred a significant risk of developing VTE.[39] 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 re-analyzed our data excluding those 29 patients and found that the association of VTE with tPA resistance at 12 hours remained statistically significant (14 (70%) out of 20 VTE cases had tPA-R at 12 hours versus 38 (36.9%) of 103 patients with no VTE (p=0.006).

As mentioned above, 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 shutdown and/or tPA-R, we strongly advise that TXA in patients at risk for shutdown be withheld unless there is evidence of pathologic fibrinolysis as TXA in patients with shutdown can worsen the hypercoagulable state. The use of TXA and its association with VTE and SD is 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) 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 (PAP), thrombin activated fibrinolysis inhibitor (TAFI), clotting factor XIII and biomarkers that may provide further insight into the biological mechanisms of post-traumatic clotting disorders. Given the potential contribution of platelets to the inability to lyse clot, the addition of aspirin 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 shutdown defined by tPA-R is significantly associated with post-traumatic 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 post-injury in patients at risk for shutdown in order 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. .

~~**Acknowledgments:** The authors would like to recognize the contributions to this research provided by Michael P. Chapman MD who died prematurely this past year. We would also like to express our gratitude to the staff of the Coalition for National Trauma Research for their assistance in securing funding for this work and in grant administration.~~

Funding Source: U.S. Army Medical Research Acquisition Activity is the awarding and administering acquisition office. This work was supported by the Assistant Secretary of Defense for Health Affairs through the Department of Defense Medical Research and Development Program under award W81XWH-1-17-1-0673. (Dr. Knudson-Principal Investigator)

Legend: SDC-1 is the STROBE Checklist

Author Contributions:

Study Design: Knudson, E.Moore, Kornblith, Wade, Bruns, Kiraly, McNutt, H. Moore, Sauaia

Data Acquisition: Knudson, E Moore, Kiraly, Wade, McNutt, Bruns

Interpretation of Data: Knudson, E Moore, H Moore, Kornblith, Sauaia

References

1. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. *J Trauma.* 2003;54:1127-1130.
2. Brohi K, Cohen MJ, Ganter MT, Matthay MA, Mackersie RC, Pittet JF. Acute traumatic coagulopathy; initiated by hypoperfusion modulated through the protein C pathway? *Ann Surg.* 2007;245:812-818.
3. Moore EE, Moore HB, Kornblith LZ, Neal MD, Hoffman M, Mutch NH, et al. Trauma-induced coagulopathy. *Nat Review Dis Primers.* 2021;29:7(1):30.
4. Moore HB, Moore EE, Liras IN, Gonzalez E, Harvin JA, Holcomb JB, et al. Acute fibrinolysis shutdown after injury occurs frequently and increases mortality; a multicenter evaluation of 2,540 severely injured patients. *J Am Coll Surg.* 2016;222:347-356.
5. Meizoso JP, Karcutski CA, Ray JJ, Namias N, Schulman CI, Proctor KG. Persistent fibrinolysis shutdown is associated with increased mortality in severely injured trauma patients. *J Am Coll Surg.* 2017;224:575-584.
6. Moore HB, Moore EE, Gonzalez E, Chapman MP, Chin TL, Silliman CC, et al. Hyperfibrinolysis, physiologic fibrinolysis, and fibrinolysis shutdown: the spectrum of postinjury fibrinolysis and relevance to antifibrinolytic therapy. *J Trauma Acute Care Surg.* 2014;77:812-817.
7. Moore HB, Moore EE, Huebner BR, Dzieciatkowska M, Stettler GR, Nuns GR, et al. Fibrinolysis shutdown is associated with a fivefold increase in mortality in trauma patients lacking hypersensitivity to tissue plasminogen activator. *J Trauma Acute Care Surg.* 2017;83:1014-1022.

8. Brill JB, Badiee J, Zander AI, Wallace JD, Lewis PR, Sise MJ, et.al. The rate of deep vein thrombosis doubles in trauma patients with hypercoagulable thromboelastography. *J Trauma Acute Care Surg.* 2017;83:413-419.
9. Sumislawski JJ, Kornblith LZ, Conroy AS, Callcut RA, Cohen MJ. Dynamic coagulability after injury: is delaying venous thromboembolism chemoprophylaxis worth the wait? *J Trauma Acute Care Surg.* 2018;85:907-914.
10. Knudson MM, Moore EE, Kornblith LZ, Shui AM, Brakenridge S, Bruns BB, et al. Challenging traditional paradigms in posttraumatic pulmonary thromboembolism. *JAMA Surg.* Epub Dec. 15, 2021.
11. Knudson MM, Ikossi DG, Khaw L, Morabito D, Speetzen LS. Thromboembolism after trauma: an analysis of 1602 episodes from the American College of Surgeons National Trauma Data Bank. *Ann Surg.* 2004;240:490-498.
12. Moore HB, Moore EE, Gonzalez E, Wiener G, Chapman MP, Dzieciakowska M, et al. Plasma is the physiologic buffer of tissue plasminogen activator-mediated fibrinolysis: rationale for plasma-first resuscitation after life-threatening hemorrhage. *J Am Coll Surg.* 2015;220:872-879.
13. Coleman JR, Moore EE,Samuels JM, Cohen MJ, Sauia A, Sumislawski JJ, et al. Trauma resuscitation considerations: sex matters. *J Am Coll Surg.* 2019;228:760-768.
14. Moore HB, Moore EE, Gonzalez G, Huebner BJ, Sheppard F, Banerjee A, et al. Reperfusion shutdown: delayed onset of fibrinolysis resistance after resuscitation from hemorrhagic shock is associated with increased circulating levels of plasminogen activator inhibitor-1 and postinjury complications. *Blood.* 2016;128:206.(Abstract).

15. Rothman KJ. No adjustments are needed for Multiple Comparisons. *Epidemiology.* 1990:1:43-46.

16. Perneger PV. What is wrong with Bonferroni adjustments. *BMJ.* 1998;316:1236.

17. Morrison JJ, Dubose JJ, Rasmussen TE, Midwinter MJ. Military application of tranexamic acid in trauma emergency resuscitation (MATTERs) study. *Arch Surg.* 2012;147:113-119.

18. Li SR, Guyette F, Brown J, Zenati M, Reitz KM, Eastridge B, et al. Early prehospital tranexamic acid following injury is associated with a 30-day survival benefit. *Ann Surg.* 2021;274:419-426.

19. Innes D, Sevitt S. Coagulation and fibrinolysis in injured patients. *J Clin Path.* 1964;17:1-17.

20. Kluft C, Verheijen JH, Jie AFH, Rijken D.C, Preston FE, Sue-Ling HM, et al. The postoperative fibrinolytic shutdown: a rapidly reverting acute phase pattern for the fast-acting inhibitor of tissue-type plasminogen activator after trauma. *Scan J Clin Lab Invest.* 1985;45:605-610.

21. Jassis J, Hirsh J, Podor TJ. Evidence that postoperative fibrinolysis shutdown is mediated by plasma factors that simulate endothelial cell type 1 plasminogen-activator inhibitor biosynthesis. *Blood.* 1992;89:1758-1764.

22. Yukizawa Y, Inaba Y, Watanabe S, Yajima S, Kobayashi N, Ishida T, et al. Association between venous thromboembolism and plasma levels of both soluble fibrin and plasminogen-activator inhibitor 1 in 170 patients undergoing hip arthroplasty. *Acta Orthopaedica.* 2012;83:14-21.

23. Wright FL, Vogler TO, Moore EE, Moore HB, Vohlauer MV, Urban S, et al. Fibrinolysis shutdown correlation with thromboembolic events in severe COVID-19 infection. *J Am Coll Surg.* 2020;231:193-204.
24. Goligher EC, Bradbury CA, McVerry BJ, Lawler PR, Berger JS, Gong MK, et al. for the REMAP-CAP, ACTIV-4a and ATTACC Investigators. Therapeutic anticoagulation with heparin in critically ill patients with Covid-19. *N Engl J Med.* 2021;385:777-89.
25. Goligher EC, Bradbury CA, McVerry BJ, Lawler PR, Berger JS, Gong MK, et al. for the ATTACC, ACTIV-4c and REMAPP-CAP Investigators. Therapeutic anticoagulation with heparin in noncritically ill patients with COVID-19. *New Engl J Med.* 2021;385:790-802.
26. Meizoso JP, Moore HB, Moore EE. Fibrinolysis shutdown in COVID-19: Clinical manifestations molecular mechanisms and therapeutic implications. *J Am Coll Surg.*2021;232:995-1003.
27. Chapman MP, Moore EE, Moore HB, Gonzalez E, Gamboni E, Chandler JB, et al. Overwhelming tPA release, not PAI-1 degradation, is responsible for hyperfibinolysis in severely injured trauma patients. *J Trauma Acute Care Surg.* 2016;80:16-23.
28. Macho AR, Moore HB, Cap AR, Meledeo A, Moore EE, Sheppard FR. Tissue injury suppresses fibrinolysis after hemorrhagic shock in nonhuman primates (rhesus macaque). *J Trauma Acute Care Surg.* 2017;82:750-757.
29. Kornblith LZ, Kutcher MF, Redick BJ, Calfee CS, Vilardi RF, Cohen MJ. Fibrinogen and platelet contributions to clot formation: implications for trauma resuscitation and thromboprophylaxis. *J Trauma Acute Care Surg.* 2014;76:255-163.

30. Moore HB, Moore EE, Gonzalez E, Hansen KC, Dzieciakowska M, Chapman MP, et al. Hemolysis exacerbates hyperfibrinolysis whereas platelolysis shuts down fibrinolysis: evolving concepts of the spectrum of fibrinolysis in response to severe injury. *Shock.* 2015;43:39-46.
31. Harr JN, Moore EE, Chin TL, Ghasabyan A, Gonzalez E, Wohlauer MV, et al. Platelets are dominant contributors to hypercoagulability after injury. *J Trauma Acute Care Surg.* 2013;74:756-7.
32. McCully BH, Connelly CR, Fair KA Holcomb JB, Fox EE, Wade CE, et al. and the PROPPR Study Group. Onset of coagulation function recovery is delayed in severely injured trauma patients. *J Am Coll Surg.* 2017;225:42-52.
33. Brill JB, Calvo RY, Wallace JD, Lewis PR, Bansal V, Sise MJ, et al. Aspirin as added prophylaxis for deep vein thrombosis in trauma: a retrospective case-control study. *J Trauma Acute Care Surg.* 2016;80:625-630.
34. Coleman JR, Annika BK, Moore EE, Moore HB, Gonzalez E, Majercik S, et al. It's sooner than you think: blunt solid organ injury patients are already hypercoagulable upon hospital admission-results of a bi-institutional prospective study. *Am J Surg.* 2019;218:1065-73.
35. Sumislawski JJ, Moore MB, Moore EE, Swope ML, Pieracci FM, Fox CJ, et al. Not all in your head (and neck): stroke after blunt cerebrovascular injury is associated with systemic hypercoagulability. *J Trauma Acute Care Surg.* 2019;87:1082-87.
36. Wang C, Kou H, Li Z, Lan J., Shandong Q, Shandong J. Association between preoperative blood transfusion and postoperative venous thromboembolism: review meta-analysis. *Ann Vasc Surg.* 2021;73:463-472.

37. Goel R, Patel EU, Cushing MM, Frank SM, Ness PM, Takemoto CM, et al. Association of perioperative red blood cell transfusions with venous thromboembolism in a North American registry. *JAMA Surg.* 2018;153:826-833.
38. Schultz DJ, Brasel KJ, Washington L, Goodman LR, Quickel RR, Lipchik RJ. et al. Incidence of asymptomatic pulmonary embolism in moderately to severely injured trauma patients. *J Trauma.* 2004;56:727-733.
39. Gearhart MM, Luchette FA, Proctor MC, Lutomski DM, Wizken C, James L, et al. The risk assessment profile score identifies trauma patient at risk for deep vein thrombosis. *Surgery.* 2000;128:631-640.

BACKGROUND

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 has been observed frequently on admission with an associated mortality of 22%.[4-6] Fibrinolysis shutdown (SD) can be further classified by TEG analysis into those patients lacking hypersensitivity to tissue plasminogen activator (tPA resistant).[7] Patients who exhibit tPA resistance (tPA-R) have a 5-fold higher incidence of death compared to patients without fibrinolysis shutdown.[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 shutdown, tPA resistance and VTE after injury has been conducted.[8,9]

The CLOTT research group (Consortium of Leaders in the study Of Traumatic Thromboembolism) was funded by the Department of Defense to conduct prospective, multi-center studies on post-traumatic pulmonary clots. Our first study (CLOTT-1) included data on 7880 injured patients and demonstrated that most early pulmonary clots are primary pulmonary thrombi and not embolic. [10] 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 shutdown, tPA resistance and VTE in these ICU patients. We hypothesized that early development of SD or tPA-R would identify patients at high-risk for VTE.

METHODS

Study Design and Data Collection

CLOTT-1 was a prospective, multicenter observational cohort study conducted over a period of two years (2018-2020) at 17 major trauma centers in the US.[10] A comprehensive electronic case report form was designed exclusively for the study and research coordinators received training on data collection prior to the start of the study. Periodic quality checks were performed by the Principal Investigator (MMK) and the data was entered into the secure central Research Electronic Data Capture (REDCap) 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 one of our previously identified risk factors for VTE.[11] These risk factors included: shock on admission (systolic blood pressure < 90 mmHg); major head injury (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 normalized ratio > 1.5). Additional data collected included the use of blood products during the first 24 hours, prophylactic measures utilized, missed doses of anticoagulants, imaging procedures related to 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 yrs.) 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. CLOTT-2 investigators have active coagulation laboratories and included the following 5 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 (HRPO). CLOTT-2 patients had serial blood samples collected for TEG analysis within 6-12 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 exam for DVT at day 3, unless prior imaging had identified DVT or pulmonary clots that required treatment. The ultrasound exam included surveillance for clot in both lower and upper extremities, but in order 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, PE, or de novo pulmonary thrombi, PT). Secondary outcomes included the trauma-related factors associated with fibrinolysis shutdown (SD) and/or tPA resistance (tPA-R) as defined below.

TEG Assays

Citrated Kaolin TEG: Blood was collected in 3 ml citrated blood tubes at as many of the time intervals described above 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 **o**f this study we defined fibrinolysis shutdown (SD) as kaolin TEG Ly30 </= 0.3%. [6,7.13]

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 (HGM, EEM, AS).[7,12] 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 *u*g/*u*l. Individual aliquots of tPA (prepared for a final concentration of 75 ng/mL) were stored at -80 degrees C and thawed immediately before use. From the sample collected from the patient, 500 *u*l of whole blood was pipetted into a customized vial containing the tPA and mixed by gentle inversion. A 340 *u*l aliquot of this mixture was then transferred to a 37 degree C TEG cap preloaded with 20 *u*l 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 resistance (tPA-R) as kaolin TEG with tPA 75 ng Ly30<2.1%. [7,14] Residual plasma from these blood samples were stored at -80 degrees C for later analysis as described in the discussion below.

There was no attempt to calculate a sample size as the relationship between SD, tPA-R and VTE was unknow. The patients included in the study represent a sub-set of the critically injured patients admitted to the 5 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.

Statistical Methods

All analyses were conducted in SAS vs 9.4 (SAS Institute, Cary NC). Unadjusted comparisons used Chi-square, 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 pre-specified hypotheses, in order 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 (MNAR) 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 studies in Epidemiology "STROBE" checklist. **(SDC-1)**

RESULTS

Two hundred forty patients were enrolled in CLOTT-2: 80% male, 80% 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 post injury (data not shown). Therefore, we first chose to concentrate on the patients with paired blood samples collected during the first 24 hours post injury. This included 141 patents with paired samples at 24 hours and 135 with paired samples at 12 hours post injury. The VTE rate in these 141 patients was 14.2% (15 DVT, 4 patients with pulmonary clot only (PT) and one patient with both DVT and pulmonary clot (PE). Over 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 71/135 (52.8%) met the definition of SD and 38 of those 71 (53.5%) also demonstrated tPA-R versus only 23/64 (35.9%) of those without SD (p=0.04). At 12 hours, a total of 61/135 (45.2%) met the definition of tPA-R and 38 of the 61 (62.3%) also showed SD versus 33/74 (44.8%) of those without tPA-R (p=0.04). At 24 hours, 62/141 (44.0%) patients had SD and of these patients 30/62 (48.8%) also had SD at 12 hours. Similarly, of the 49/141 (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 one sample for analysis within the first 24-hour period and the association between the risk factors and the development of SD or tPA-R is 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

units of FFP on the day of admission (RR=1.04, p <.0001). At 24 hours, SD was independently associated with traumatic brain injury (TBI), (RR=1.73, p0.0007), pelvic fracture (RR=1.39, p<.0001) and the need for major surgery as defined above (RR=2.37, p<0.001). In contrast, tPA-R at 12 hours was associated with the need for > 4 units 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)**

Finally, competitive risk models were developed in order 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 prior to 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 (HR 5.57) when adjusted for other injury-related risk factors listed in Table 2.

DISCUSSION

This CLOTT-2 study documents that over half of critically injured patients demonstrate failure of clot lysis as early as 12 hours post-admission. Further, those with the most severe hypercoagulable state (tPA-R) are over 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 units 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 and others documented a 46% mortality among patients who were coagulopathic after injury versus only 10.9% among those without

clotting abnormalities.[1] 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 shutdown was from Innes and Sevitt in 1964, with subsequent work from the Netherlands terming this "fibrinolytic shutdown" following myocardial infarction and after trauma.[19,20] These investigators and others described a rapidly developing pattern of fast-acting inhibitor of tissue-type plasminogen activator after trauma leading to fibrinolysis shutdown.[20,21] Higher levels of plasminogen-activator inhibitor-one (PAI-1) have also been detected in the plasma of patients who developed venous thromboembolism after total hip surgery compared to those without VTE.[22] More recently fibrinolysis shutdown has been correlated with thromboembolic events in patients with severe coronavirus disease (COVID-19) and the recognition that the inflammation associated with this disease leads to both macro-and micro-clots in the arterial as well as the venous system.[23-26] Collectively this work prompted the large-scale, multi-national research projects by the National Institute of Health focusing on the use of anticoagulants in patients with COVID-19 infections.[24,25]

The current ubiquitous use of thromboelastography in trauma resuscitation has greatly facilitated the study of fibrinolysis shutdown (SD). Most of the pioneering work in this area has been led by the investigators in Denver (including authors HBM, EEM and AS) focusing on both the underlying mechanistic pathology as well as 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 shutdown.[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% 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 to those with physiologic fibrinolysis. Investigations by these authors documented a five-fold increase in mortality in trauma patients who lacked hypersensitivity to tissue plasminogen activator (tPA-R).[7] Utilizing ELISA assays, they demonstrated that SD was associated with the activity of tissue plasminogen activator inhibitor-1 (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 and others reported that persistent SD (lasting for one 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).[5] In their study, the authors found that 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 PROPPR 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 and others found that pre-injury aspirin use was associated with lower VTE rates after injury when combined with in-hospital heparinoid prophylaxis. [33]

Our study has documented that approximately half of critically injured patients have SD or tPA-R (or both) as early as 12 hours post injury. 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 post injury) 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 post-operative 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 found that patients who had been transfused with RBCs had over three times the risk of developing VTE compared to those without transfusions

although the number of units of blood is not mentioned in that study[38]. Gearhart and others also found that transfusion of > 4 units of RBCs conferred a significant risk of developing VTE.[39] 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 re-analyzed our data excluding those 29 patients and found that the association of VTE with tPA resistance at 12 hours remained statistically significant (14 (70%) out of 20 VTE cases had tPA-R at 12 hours versus 38 (36.9%) of 103 patients with no VTE (p=0.006).

As mentioned above, 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 shutdown and/or tPA-R, we strongly advise that TXA in patients at risk for shutdown be withheld unless there is evidence of pathologic fibrinolysis as TXA in patients with shutdown can worsen the hypercoagulable state. The use of TXA and its association with VTE and SD is 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) 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 (PAP), thrombin activated fibrinolysis inhibitor (TAFI), clotting factor XIII and biomarkers that may provide further insight into the biological mechanisms of post-traumatic clotting disorders. Given the potential contribution of platelets to the inability to lyse clot, the addition of aspirin 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 shutdown defined by tPA-R is significantly associated with post-traumatic 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 post-injury in patients at risk for shutdown in order 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. .

Legend: SDC-1 is the STROBE Checklist

References

1. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. *J Trauma.* 2003;54:1127-1130.

2. Brohi K, Cohen MJ, Ganter MT, Matthay MA, Mackersie RC, Pittet JF. Acute traumatic coagulopathy; initiated by hypoperfusion modulated through the protein C pathway? *Ann Surg.* 2007;245:812-818.
3. Moore EE, Moore HB, Kornblith LZ, Neal MD, Hoffman M, Mutch NH, et al. Trauma-induced coagulopathy. *Nat Review Dis Primers.* 2021;29:7(1):30.
4. Moore HB, Moore EE, Liras IN, Gonzalez E, Harvin JA, Holcomb JB, et al. Acute fibrinolysis shutdown after injury occurs frequently and increases mortality; a multicenter evaluation of 2,540 severely injured patients. *J Am Coll Surg.* 2016;222:347-356.
5. Meizoso JP, Karcutski CA, Ray JJ, Namias N, Schulman CI, Proctor KG. Persistent fibrinolysis shutdown is associated with increased mortality in severely injured trauma patients. *J Am Coll Surg.* 2017;224:575-584.
6. Moore HB, Moore EE, Gonzalez E, Chapman MP, Chin TL, Silliman CC, et al. Hyperfibrinolysis, physiologic fibrinolysis, and fibrinolysis shutdown: the spectrum of postinjury fibrinolysis and relevance to antifibrinolytic therapy. *J Trauma Acute Care Surg.* 2014;77:812-817.
7. Moore HB, Moore EE, Huebner BR, Dzieciatkowska M, Stettler GR, Nuns GR, et al. Fibrinolysis shutdown is associated with a fivefold increase in mortality in trauma patients lacking hypersensitivity to tissue plasminogen activator. *J Trauma Acute Care Surg.* 2017;83:1014-1022.
8. Brill JB, Badiee J, Zander AI, Wallace JD, Lewis PR, Sise MJ, et.al. The rate of deep vein thrombosis doubles in trauma patients with hypercoagulable thromboelastography. *J Trauma Acute Care Surg.* 2017;83:413-419.

9. Sumislawski JJ, Kornblith LZ, Conroy AS, Callcut RA, Cohen MJ. Dynamic coagulability after injury: is delaying venous thromboembolism chemoprophylaxis worth the wait? *J Trauma Acute Care Surg.* 2018;85:907-914.
10. Knudson MM, Moore EE, Kornblith LZ, Shui AM, Brakenridge S, Bruns BB, et al. Challenging traditional paradigms in posttraumatic pulmonary thromboembolism. *JAMA Surg.* Epub Dec. 15, 2021.
11. Knudson MM, Ikossi DG, Khaw L, Morabito D, Speetzen LS. Thromboembolism after trauma: an analysis of 1602 episodes from the American College of Surgeons National Trauma Data Bank. *Ann Surg.* 2004;240:490-498.
12. Moore HB, Moore EE, Gonzalez E, Wiener G, Chapman MP, Dzieciakowska M, et al. Plasma is the physiologic buffer of tissue plasminogen activator-mediated fibrinolysis: rationale for plasma-first resuscitation after life-threatening hemorrhage. *J Am Coll Surg.* 2015;220:872-879.
13. Coleman JR, Moore EE,Samuels JM, Cohen MJ, Sauia A, Sumislawski JJ, et al. Trauma resuscitation considerations: sex matters. *J Am Coll Surg.* 2019;228:760-768.
14. Moore HB, Moore EE, Gonzalez G, Huebner BJ, Sheppard F, Banerjee A, et al. Reperfusion shutdown: delayed onset of fibrinolysis resistance after resuscitation from hemorrhagic shock is associated with increased circulating levels of plasminogen activator inhibitor-1 and postinjury complications. *Blood.* 2016;128:206.(Abstract).
15. Rothman KJ. No adjustments are needed for Multiple Comparisons. *Epidemiology.* 1990:1:43-46.
16. Perneger PV. What is wrong with Bonferroni adjustments. *BMJ.* 1998;316:1236.

17. Morrison JJ, Dubose JJ, Rasmussen TE, Midwinter MJ. Military application of tranexamic acid in trauma emergency resuscitation (MATTERs) study. *Arch Surg.* 2012;147:113-119.

18. Li SR, Guyette F, Brown J, Zenati M, Reitz KM, Eastridge B, et al. Early prehospital tranexamic acid following injury is associated with a 30-day survival benefit. *Ann Surg.* 2021;274:419-426.

19. Innes D, Sevitt S. Coagulation and fibrinolysis in injured patients. *J Clin Path.* 1964;17:1-17.

20. Kluft C, Verheijen JH, Jie AFH, Rijken D.C, Preston FE, Sue-Ling HM, et al. The postoperative fibrinolytic shutdown: a rapidly reverting acute phase pattern for the fast-acting inhibitor of tissue-type plasminogen activator after trauma. *Scan J Clin Lab Invest.* 1985;45:605-610.

21. Jassis J, Hirsh J, Podor TJ. Evidence that postoperative fibrinolysis shutdown is mediated by plasma factors that simulate endothelial cell type 1 plasminogen-activator inhibitor biosynthesis. *Blood.* 1992;89:1758-1764.

22. Yukizawa Y, Inaba Y, Watanabe S, Yajima S, Kobayashi N, Ishida T, et al. Association between venous thromboembolism and plasma levels of both soluble fibrin and plasminogen-activator inhibitor 1 in 170 patients undergoing hip arthroplasty. *Acta Orthopaedica.* 2012;83:14-21.

23. Wright FL, Vogler TO, Moore EE, Moore HB, Vohlauer MV, Urban S, et al. Fibrinolysis shutdown correlation with thromboembolic events in severe COVID-19 infection. *J Am Coll Surg.* 2020;231:193-204.

24. Goligher EC, Bradbury CA, McVerry BJ, Lawler PR, Berger JS, Gong MK, et al. for the REMAP-CAP, ACTIV-4a and ATTACC Investigators. Therapeutic anticoagulation with heparin in critically ill patients with Covid-19. *N Engl J Med.* 2021;385:777-89.

25. Goligher EC, Bradbury CA, McVerry BJ, Lawler PR, Berger JS, Gong MK, et al. for the ATTACC, ACTIV-4c and REMAPP-CAP Investigators. Therapeutic anticoagulation with heparin in noncritically ill patients with COVID-19. *New Engl J Med.* 2021;385:790-802.

26. Meizoso JP, Moore HB, Moore EE. Fibrinolysis shutdown in COVID-19: Clinical manifestations molecular mechanisms and therapeutic implications. *J Am Coll Surg.*2021;232:995-1003.

27. Chapman MP, Moore EE, Moore HB, Gonzalez E, Gamboni E, Chandler JB, et al. Overwhelming tPA release, not PAI-1 degradation, is responsible for hyperfibinolysis in severely injured trauma patients. *J Trauma Acute Care Surg.* 2016;80:16-23.

28. Macho AR, Moore HB, Cap AR, Meledeo A, Moore EE, Sheppard FR. Tissue injury suppresses fibrinolysis after hemorrhagic shock in nonhuman primates (rhesus macaque). *J Trauma Acute Care Surg.* 2017;82:750-757.

29. Kornblith LZ, Kutcher MF, Redick BJ, Calfee CS, Vilardi RF, Cohen MJ. Fibrinogen and platelet contributions to clot formation: implications for trauma resuscitation and thromboprophylaxis. *J Trauma Acute Care Surg.* 2014;76:255-163.

30. Moore HB, Moore EE, Gonzalez E, Hansen KC, Dzieciakowska M, Chapman MP, et al. Hemolysis exacerbates hyperfibrinolysis whereas platelolysis shuts down fibrinolysis: evolving concepts of the spectrum of fibrinolysis in response to severe injury. *Shock.* 2015;43:39-46.

31. Harr JN, Moore EE, Chin TL, Ghasabyan A, Gonzalez E, Wohlauer MV, et al. Platelets are dominant contributors to hypercoagulability after injury. *J Trauma Acute Care Surg.* 2013;74:756-7.

32. McCully BH, Connelly CR, Fair KA Holcomb JB, Fox EE, Wade CE, et al. and the PROPPR Study Group. Onset of coagulation function recovery is delayed in severely injured trauma patients. *J Am Coll Surg.* 2017;225:42-52.

33. Brill JB, Calvo RY, Wallace JD, Lewis PR, Bansal V, Sise MJ, et al. Aspirin as added prophylaxis for deep vein thrombosis in trauma: a retrospective case-control study. *J Trauma Acute Care Surg.* 2016;80:625-630.

34. Coleman JR, Annika BK, Moore EE, Moore HB, Gonzalez E, Majercik S, et al. It's sooner than you think: blunt solid organ injury patients are already hypercoagulable upon hospital admission-results of a bi-institutional prospective study. *Am J Surg.* 2019;218:1065-73.

35. Sumislawski JJ, Moore MB, Moore EE, Swope ML, Pieracci FM, Fox CJ, et al. Not all in your head (and neck): stroke after blunt cerebrovascular injury is associated with systemic hypercoagulability. *J Trauma Acute Care Surg.* 2019;87:1082-87.

36. Wang C, Kou H, Li Z, Lan J., Shandong Q, Shandong J. Association between preoperative blood transfusion and postoperative venous thromboembolism: review meta-analysis. *Ann Vasc Surg.* 2021;73:463-472.

37. Goel R, Patel EU, Cushing MM, Frank SM, Ness PM, Takemoto CM, et al. Association of perioperative red blood cell transfusions with venous thromboembolism in a North American registry. *JAMA Surg.* 2018;153:826-833.

38. Schultz DJ, Brasel KJ, Washington L, Goodman LR, Quickel RR, Lipchik RJ. et al. Incidence of asymptomatic pulmonary embolism in moderately to severely injured trauma patients. *J Trauma.* 2004;56:727-733.

39. Gearhart MM, Luchette FA, Proctor MC, Lutomski DM, Wizken C, James L, et al. The risk assessment profile score identifies trauma patient at risk for deep vein thrombosis. *Surgery.* 2000;128:631-640.

Table 1: Number and Percent of Patients Demonstrating Fibrinolysis Shutdown (FD) and tPA Resistance (tPA-R) among 135 patients at 12 hours post-admission

	12 hours tPA Resistance	12 hours tPA Resistance	
12 hours fibrinolysis shutdown	**NO**	**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 denoting significant differences between the colemns

SD (+): Meets criteria for Fibrinolysis Shutdown (kaolin TEG Ly30$\leq$0.3%)

SD (-): Does not meet criteria for Fibrinolysis Shutdown

tPA-R (+): Meets criteria for tPA Resistance (kaolin TEG with tPA 75ng Ly30 < 2.1%)

tPA-R: Does not meet criteria for tPA-R

Table 2: Trauma Related Factors Associated with Early Fibrinolytic Shutdown (FD) and tPA Resistance (tPA-R)

Factor	No SD or tPA-R in 1st 24 hrs. n=41/179 (22.9%)	SD at 12 hours n=84/169 (49.7%)	tPA-R at 12 hours n=61/135 (45.2%)	SD at 24 hours n=76/180 (42.2%)	tPA-R at 24 hours 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 units in 1st 24 hrs. 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 units in 1st 24 hrs. 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 units in 1st 24 hrs. n=17/240	1/12 (8.3%)	6/11 (54.6%)	6/8 (75%)	7/10 (70.0%)	5/7 (71.4%)
Cryo > 2 units in 1st 24 hrs 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%)

Note: 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.

Table 3: Independent injury-related risk factors for the development of fibrinolysis shutdown (SD) at 12 and 24 hours

Time Period	Variable	Relative Risk	Lower Confidence limits (95%)	Upper Confidence limits (95%	P-value
SD at 12 hours	Plasma> 4 units in 1st 24hrs.	1.04	1.03	1.06	<.0001
	Shock (< 90 mm Hg)**	0.61	0.38	0.98	0.94
SD at 24 hours	TBI	1.71	1.25	2.34	0.0007
	Pelvic fracture	1.39	1.23	1.56	<.0001
	Major surgery 1st day	2.37	2.20	2.55	<.0001

TBI=Traumatic Brain Injury with AIS≥3

*Note: Shock is associated with hyperfibrinolysis not SD[8]

Table 4: Independent injury-related risk factors for development of tPA-R at 12 and 24 hours

Time Period	Variable	Relative Risk	Lower Confidence Limits (95%)	Upper Confidence Limits (95%	p-value
12 hours	RBCs>4/first 24 hours	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	<.0001
24 hours	RBCs>4 first 24 hours	1.03	1.02	1.05	<.0001
	Long bone fx	1.80	1.20	2.68	0.0045

Fx=fracture

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 hours	1.29	0.4990	3.403
tPA-R at 12 hours*	5.57	1.391	22.39
SD at 24 hours	0.57	0.22	1.46
tPA-R at 24 hours	1.821	0.600	5.543

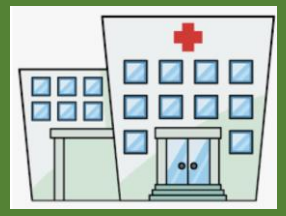
*statistically significant risk factor ($p<0.05$)

Visual Abstract

tPA Resistance: An Early Predictor of Post Traumatic VTE

Study Population

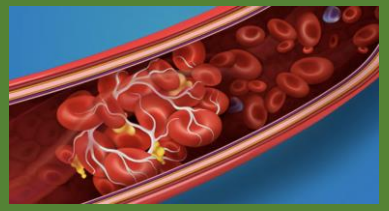
CLOTT-2: Prospective, observational

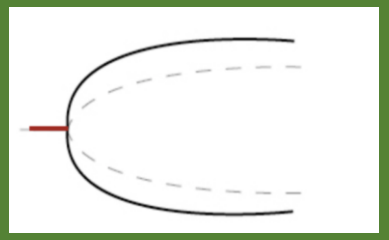


X 5

Trauma Centers

Risk Factors
Venous Thrombo
Embolic Event

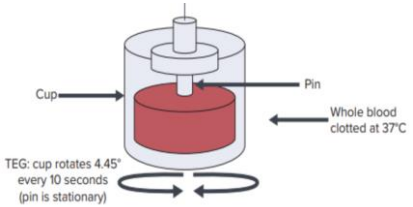




Role of Fibrinolysis?

Results

141 Patients Serial Coag Assessment



\+ tPA

From ICU Admit

<2.1% Lysis

HR VTE 5.57

95% CI 1.39-22.39

Conclusions

tPA resistance early quantifiable risk factor VTE in ICU