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Thrombin generation profiles as predictors of symptomatic venous thromboembolism after trauma: A prospective cohort study

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Abstract

Background—Reliable biomarkers predictive of venous thromboembolism (VTE) after acute trauma are uncertain. The objective of the study was to identify risk factors for symptomatic VTE after trauma, including individual plasma coagulum characteristics as reflected by thrombin generation.

Methods—In a prospective, case-cohort study, trauma patients were enrolled over the 4.5 year period, 2011–2015. Blood was collected by venipuncture into 3.2% trisodium citrate at 0, 6, 12, 24 and 72 hours after injury, and at hospital discharge. Platelet poor plasma was stored at –80°C until analysis. Thrombin generation, as determined by the calibrated automated thrombogram (CAT) using 5 pM tissue factor (TF)/4 uM phospholipid (PS), was reported as peak height (nM thrombin) and time to peak height (ttPeak [minutes]). Data are presented as median [IQR] or hazard ratio (HR) with (95% CI).

Results—Among 453 trauma patients (ISS=13.0 [6.0, 22.0], hospital LOS=4.0 [2.0, 10.0] days, age=49 [28, 64] years, 71% male, 96% with blunt mechanism, mortality 3.2%), 83 developed symptomatic VTE within 92 days after injury (35 [42%] after hospital discharge). In a weighted,

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Author Contributions:

M.S.P., W.S.H., A.A.A., and J.A.H. designed this study. M.S.P. and J.A.H. conducted the literature search. M.S.P., T.M.J.F., A.M.H., D.H.J., and S.P.Z. participated in patient enrollment. A.X. performed the assays. M.S.P., A.X., T.M.J.F., A.M.H., and S.K.D. contributed to data collection. G.S., T.M.J.F., A.M.H., and S.K.D. designed the database. M.S.P., G.S., W.S.H., K.R.B., A.A.A., and J.A.H. performed data analysis. M.S.P., G.S., A.X., W.S.H., A.A.A., and J.A.H. contributed to data interpretation. M.S.P., G.S., A.X., A.M.H., D.H.J., S.P.Z., W.S.H., K.R.B., A.A.A., and J.A.H. prepared the manuscript. M.S.P., D.H.J., S.P.Z., K.R.B., A.A.A., and J.A.H. critically revised the manuscript.

multivariate Cox model that included clinical and CAT characteristics available within 24 hours of admission, increased patient age (1.35 [1.19,1.52] per 10 years, $P<0.0001$), body mass index (BMI) ≥ 30 kg/m² (4.45 [2.13,9.31], $p<0.0001$), any surgery requiring general anesthesia (2.53 [1.53,4.19], $P=0.0003$) and first available ttPeak (1.67[1.29, 2.15], $p<0.00001$) were independent predictors of incident symptomatic VTE within 92 days after trauma (C-statistic=0.799).

Conclusion—The individual's plasma coagulome (as reflected by thrombin generation) is an independent predictor of VTE after trauma. Clinical characteristics and ttPeak can be used to stratify acute trauma pts into high and low risk for VTE.

Keywords

Trauma; venous thromboembolism; thrombin; calibrated automated thrombogram

Background

The overall incidence of VTE remained relatively unchanged at about 1 per 1000 between 1980 and 2000, and actually increased during 2001–2005.^{1–4} Likewise, after trauma, the incidence of pulmonary embolism has more than doubled during the period of 2007–2009 as compared to an earlier period (1994–2001).⁵ While the Joint Commission, CMS and the Surgeon General recommend assessment of the risk of VTE for every hospitalized patient, our rudimentary understanding of mechanisms contributing to VTE has precluded development of reliable biomarkers and standardized screening procedures to identify the individual at high risk for VTE. Previously identified clinical risk factors for VTE after trauma have a low predictive value for the individual patient; fatal VTE may occur despite a presumed low risk profile. Hence, there is critical need for identifying the high VTE-risk individual trauma patient to decrease the risk of bleeding complications⁶ or infection^{7,8} from the use of anticoagulant-based prophylaxis in the low VTE-risk individual.

To assess an individual patient's coagulation phenotype, we used the Calibrated Automated Thrombogram (CAT) to quantify the kinetics of plasma thrombin generation.^{9,10} The plasma procoagulant system is a “cascade” of enzymatic reactions triggered by exposure of blood to tissue factor (TF) released after trauma, ultimately resulting in the generation of thrombin. Thrombin cleaves plasma fibrinogen to produce insoluble fibrin and activates platelets which cohere with fibrin to produce a thrombus (clot). CAT is a rapid, sensitive method to quantify in real time plasma thrombin generation in response to TF and procoagulant phospholipid (PS). Compared to traditional clotting assays, such as the prothrombin time (PT) and activated partial thromboplastin time (aPTT) which yield only 1 time point and evaluate clot formation early in the process of thrombin generation, CAT provides the total amount of thrombin generated and the peak rate of thrombin generation in an individual's plasma. Thus, CAT is a global measure of an individual's plasma coagulome. Patients with clinical risk factors for VTE (obesity, increasing age, oral contraceptives) have increased total thrombin generation,^{11–13} and importantly, total thrombin generation has been shown to be an independent predictor of recurrent VTE.¹⁴ CAT parameters include lagtime (initiation phase of clotting; minutes), peak height (maximal amount of thrombin generated; nM), and time to peak thrombin (reflects rate of thrombin generation; ttPeak, minutes). Theoretically, “hypercoagulable” TIC would be reflected by a shorter lagtime, higher peak height, and

shorter ttPeak, and indeed, comparing all trauma patients to healthy volunteers, we have recently shown that the CAT parameters followed this pattern.¹⁵ The overall *hypothesis* is that the VTE risk prediction score, which utilizes a novel plasma biomarker (ttPeak), will improve the accuracy of identifying patients at high risk for symptomatic VTE than using clinical parameters alone.

Methods

Study Design, Setting and Population

In a prospective case-cohort study, all trauma patients transported to the Mayo Clinic Emergency Department (ED) by ambulance or air transport, as a level 1 or 2 trauma activations, from February 2011 to June 2014 were considered for study inclusion (Table 1). Exclusion criteria were prior history of VTE, age < 18 years, anticoagulation (e.g., heparin, warfarin) or antithrombotic therapy (excluding aspirin or non-steroidal anti-inflammatory drugs), preexisting coagulopathy, more than 12 hours from time of injury, no blood drawn within first 12 hours after injury, active cancer, sepsis, renal failure, burn injuries or declined consent by the patient or legal guardian (Figure 1). The time of injury (TOI) was determined by the pre-hospital medical providers based on information at the injury scene. If the TOI was unclear, the pre-hospital medical providers estimated the time and relayed this information to the emergency communication center. A trauma alert page was then sent to the hospital and laboratory staff as to the TOI. We collected demographic, baseline and time-dependent clinical characteristics, including injury severity score (ISS), patient age and sex, body mass index (BMI), hospital length of stay (LOS), all-cause mortality, and start and stop of anticoagulant-based thromboprophylaxis and other medications affecting coagulation, trauma injury severity score (TRISS), all injury codes from Mayo Clinic Trauma Registry, transfusion data in the prehospital and for entire index hospitalization, IVC filter placement, and standard admission laboratory data (PT, aPTT, INR, hemoglobin, platelet count). Transfusion therapy was mainly based on Mayo Clinic Trauma Center transfusion guidelines but also at the discretion of the medical provider. This study was approved by the Mayo Clinic Institutional Review Board. All patients were followed forward in time from time of injury (TOI) to the earliest of symptomatic, objectively-diagnosed VTE, death or other loss to follow-up, or 92 days (about 3 months) post hospital discharge.

Power Analysis for the Prospective Case-Cohort Study

When the study was designed, it was anticipated that the total number of patients with a recognized VTE within 3 months of the date of trauma will be a minimum of 80 patients. Assuming the minimum of 80 patients are identified, with a two-sided test at alpha-level=0.05 there will be 80% (90%) power to detect a hazard ratio (HR) 1.87 (2.07) for an association with a binary risk factor present in 50% of study population. For a binary risk factor present in 20% of study population, this study could detect a HR 2.19 (2.48).

Diagnostic Criteria for Venous Thromboembolism

A diagnosis of incident DVT or PE, either in hospital or within 92 days (365/4, or about 3 months) after hospital discharge, required objectively-diagnosed, symptomatic DVT or PE.

The diagnosis of VTE required symptoms or signs of acute DVT or PE and a positive diagnostic imaging study (i.e., whole leg compression venous duplex ultrasonography; pulmonary angiography, computed tomographic pulmonary angiography). The Mayo Clinic Trauma Center utilizes a VTE prophylaxis clinical practice guideline (CPG), which was constructed with input from the Eastern Association for the Surgery of Trauma and the American College of Chest Physicians (ACCP) guidelines.¹⁶ Routine screening leg venous duplex ultrasonography in search of asymptomatic DVT was not performed. Incident symptomatic, objectively-diagnosed arm DVT, proximal or isolated distal leg DVT (occlusive or non-occlusive), and PE were included as outcome events.

The end of day 1 was chosen as a time point, prior to which development of VTE would be considered non-preventable. This was based on our Institutional and Trauma Division's guidelines to assess for risk of venous thromboembolism within 24 hours of admission. This is consistent with Centers for Medicare and Medicaid Services (CMS) recommendation that patients be assessed for VTE risk within 24 hours after admission. Hence, we reasoned that if the VTE occurred before hospital day 1, it would be considered non-preventable. Lastly, the objective of this study is to assess the utility of CAT assay in predicting eventual development of VTE. As a rural trauma center, about 50% of our trauma patients are from outside Olmsted County and hence, do not arrive immediately from the scene. Hence, this window of opportunity to collect samples was needed as long as they presented within 12 hours from the time of injury.

Selection of Cohort Patients

During the first 12 months of study enrollment, all the trauma patients enrolled into the study without development of VTE were considered as cohort members during the first 12 months of study. Close to 97% of cohort patient samples that were acquired during this time period were analyzed. During the subsequent years of enrollment, on a quarterly basis, an average of 3 non-VTE patients had their samples analyzed. Cohort members who developed VTE > 92 days after hospital discharge were excluded as cases but were available for selection as a cohort member. CAT assays were performed on stored plasma samples for VTE cases and members of the cohort.

Sample Collection and Processing

Blood samples were collected at baseline (0–2 hours), 6 hours, 12 hours, and days 1 and 3 after injury, and at hospital discharge. When patients were unable to provide consent at the time of the trauma, consent was obtained from the patient or legal guardian within 30 days of hospital discharge; samples were discarded when consent could not be obtained. A total of 18mL of whole blood was collected by antecubital venipuncture or via existing indwelling catheters into 4.5 mL citrated Vacutainer tubes (0.105M buffered sodium citrate, 3.2% Becton Dickinson, Plymouth, UK) and processed to platelet free plasma by double centrifugation (3000g, 15 minutes) as recommended by the ISTH vascular biology SSC Collaborative Workshop¹⁷, and stored in multiple aliquots at –80 degrees Celsius until analysis. All samples were processed within 1 hour of collection.

Calibrated Automated Thrombogram (CAT) Analyses

Thrombin generation was measured with the Calibrated Automated Thrombogram (CAT, Thrombinoscope BV, Maastricht, The Netherlands), utilizing a Fluoroskan Ascent plate reader (390 nm excitation, 460 nm emission, Thermo Electron Corp, Vantaa, Finland), as previously described by Hemker et al^{18–19} and Owen et al²⁰. Assays of trauma patient samples were performed in triplicate. Corn trypsin inhibitor (25mg/mL) 25uL (50ug/mL final concentration) was added to each plasma sample prior to CAT analyses. Thrombin generation was initiated using two different reagents: addition of 20uL of either PPP (5 pM tissue factor and 4 uM phospholipid, Stago, US) or PRP (1 pM tissue factor, Stago, US) reagent. Then, 80uL of citrated platelet poor plasma was added to each well of U bottom 96-well microtiter plates (Nunc, Thermo Fischer Scientific, Rochester, NY) using a single channel pipette. After an incubation period (10 minutes at 37 °C), 20 uL of warmed FLUCA reagent (Fluca kit, TS50, Thrombinoscope, BV), which contains the fluorogenic substrate and CaCl₂ was added to each well via an automated dispenser. Thrombin generation curves were recorded continuously for 90 minutes at a rate of 3 readings per minute. Separate wells containing the thrombin calibrator, which corrects for inner filter effects and quenching variation among individual plasmas were also measured in parallel. A dedicated program, Thrombinoscope, was used to calculate thrombin activity over time. The parameters derived were: lagtime (LT), time to peak (ttPeak), and peak height (PH). We did not analyze samples collected after anticoagulant-based (mainly heparin) prophylaxis as prophylactic dose of heparin completely attenuated thrombin generation in CAT analyses. When unfractionated heparin was added to platelet poor plasma (Cryocheck) at final concentrations varying from 0.1 to 0.4 units per mL, we noted a significant decrease in PH values (85% decrease) and prolongation of LT (200% increase), even at the lowest dose.

Statistical Analyses

Data analysis was performed using SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina) and R version 3.2.0. Descriptive statistics are presented as median values (interquartile range). Comparisons of variables between VTE cases and cohort members were done using the Kruskal-Wallis and Fisher's exact tests. For each lab test, the first plasma sample for each patient analyzed for CAT was identified. This value was used in univariable and multivariable assessments of association with freedom from VTE using Cox proportional hazards regression, with results presented as hazard ratio(HR) with (95% CI). Weighted Cox model, using Barlow weights, was used to account for the fact that all cases but only a fraction of cohort members (370 out of total 1151) were included in the analyses.²¹ Cox modeling was done with time to VTE as the endpoint, and death as a censoring event. We also performed univariable and multivariable Cox proportional hazards regressions using the most "hypercoagulable" (shortest ttPeak) value within the first 24 hours for each patient who had multiple samples. Clinical variables adjusted for in multivariable analysis were those easily obtainable within 24 hours of admission, and strongly related to symptomatic VTE ($P < 0.05$) on a univariate basis. For each model, the concordance (c-) statistic adapted to survival analysis was used as a descriptive measure of predictive power. Risk scores were derived based on the linear combination of variables using their coefficients in the Cox proportional hazards regression. In order to describe the

impact of CAT parameter (in particular, ttPeak) on reclassification, we used a ROC analyses of the binary version of the outcome (VTE: yes or no) to select a score cutoff with highest sensitivity and specificity. The alpha-level was set at 0.05 for statistical significance.

Results

Over the 4.5 year period, Feb 2011–September 2015, 2954 patients with acute trauma were screened for study enrollment; 1960 met study inclusion criteria of which 1234 (63%) consented to participate (Figure 1). Of the 726 patients (37%) of eligible patients, 423 (or their surrogates) could not be reached for consent. A total of 303 patients declined to participate. The 1720 patient who were not enrolled patients were of similar median age (48[28,68]), sex (69% men) and mechanism of injury (93% blunt) as compared to the 1234 enrolled. Of those 1234 enrolled, 453 trauma patients had their plasma analyzed and their median ISS was 13.0 [6.0, 22.0], hospital LOS was 4.0 [2.0, 10.0] days, age was 49 [28, 64] years. Overall, 71% were male, 95% had blunt mechanism, and mortality rate of 3.1%.

In an effort to ensure that the analyzed group were representative of all patients enrolled into our study, the 370 (after excluding the 83 VTE patients) patients whose plasma samples were analyzed were compared to the 781 (all of whom being non-VTE patients) who were enrolled but did not have their plasma analyzed. there were no statistical differences between the 2 groups: Age (47[26, 62], 47[30, 62]; $p = 0.300$), male sex % (70, 70; $p = 0.870$), % blunt mechanism of injury (96, 96, $p = 0.838$), ISS (12[5, 19], 11[6, 17]; $p = 0.807$).

Of these, 83 developed VTE within 92 days after trauma; 11 patients developed VTE within day 1 and 35 patients (42%) developed VTE after hospital discharge. At end of day 1 of admission, 34% and 57% of patients had been placed on chemo- and mechanical prophylaxis, respectively. Overall, 67.5% VTE patients and 52.7% random cohort patients, whose plasma samples were analyzed, received chemoprophylaxis during their hospital admission ($p = 0.015$). For mechanical prophylaxis, there were no significant differences between these two groups (56.3% and 65.4%, respectively; $p = 0.164$). Demographic and baseline characteristics of venous thromboembolism (VTE) cases and cohort members are outlined in Table 2. We analyzed 1600 samples collected from the 83 patients who developed VTE and 370 cohort members without VTE. Overall, 236 patients had their first blood sample drawn at baseline, 191 had 6 hours, and 24 at 12 hours. The median time of first blood draw was 2.3 hours (1.25, 5.85). There were a total of 35 IVC filters placed in VTE patients: 22 as “prophylaxis”, 2 after acute PE, 8 after acute DVT, and 3 after both PE and DVT. Among the 22 patients who had a “prophylactic” IVC filter placed, 2 developed PE and 20 developed DVT after filter placement. Of the 83 VTE cases, 11 patients developed VTE by the end of day 1 and were considered to have non-preventable VTE (2 patients with diagnosis of PE, 3 for DVT, 6 for both PE and DVT). Hence, the following analyses were based on the remaining 72 VTE cases. In a univariable analysis of the prospective cohort data, several clinical variables which were available within 24 hours of admission, as well as ttPeak and Lagtime data obtained from first blood sample in each patient after injury were predictors of incident VTE (Table 3). For our multivariable model, we chose clinical variables that were significantly and strongly associated with VTE and easy to obtain within 24 hours after admission. This model showed that increasing patient

age (1.35 [1.19,1.52] per 10 years, $P<0.0001$), body mass index (BMI) 30 kg/m^2 (4.45 [2.13,9.31], $p<0.0001$), any surgery that required general anesthesia (2.53 [1.53,4.19], $P=0.0003$) and first available ttPeak value (2.26 [1.61,3.18] per 1 (minute) decrease, $p<0.0001$) were independent predictors of incident (first-lifetime time) VTE within 92 days after trauma (Table 4). The statistical strength of each variable's partial association in this model suggests that this is a very robust model. The model with ttPeak included had a C-statistic of 0.799 (95% CI: [0.731, 0.867]); without retention of ttPeak in the model, the C-statistic was 0.754 (95% CI: [0.686, 0.822]).

In a receiver operator characteristic curve analysis, based on simplifying the end-point from time-to-event to a binary variable, the optimal VTE risk score cutoff gave the best sensitivity and specificity of 74% and 73%, respectively. Hence, a patient at end of day 1 of admission who has a VTE risk score greater than the optimal cutoff would be considered to be at "High-Risk" for VTE in the ensuing 92 days. This definition led to 52 true positives, 18 false negatives, 87 false positives and 239 true negatives. The addition of ttPeak more accurately predicted VTE than using clinical variables alone (in our case, BMI, Age, Any Surgery). Specifically, we were able to identify (net) 5 more true VTE cases and 5 more true VTE negative patients, reducing the rate of misclassification by 8.7% (10/115) due to ttPeak datum in the model (Table 5). We also tested the most hypercoagulable CAT variable (ttPeak). Similar to using the first ttPeak value, we again observed an improvement (9.6%) in misclassification when each patient's most hypercoagulable ttPeak value (C-statistic of 0.794) was used, when compared to the clinical-only model. Almost 56% of patients who had multiple blood samples had the most hypercoagulable sample during their first blood draw.

Discussion

This is the first study to show that an individual's plasma coagulome as reflected by thrombin generation (CAT) can be used to predict VTE after trauma. Current guidelines recommend VTE prophylaxis for all trauma patients, a patient population at high risk for bleeding. The literature is replete with well-designed work elucidating risk factors for VTE in trauma patients.^{22–25} However, follow-up often is limited to the duration of hospitalization. In our prospective case-cohort study, we observed that 42% of cases developed VTE after hospital discharge. We recently published a retrospective population-based, case-cohort study of Olmsted County trauma patients, in whom post-discharge symptomatic VTE rate was 62%.²⁶ These observations are very similar to findings by Godat et al., who showed that the period of VTE risk after acute injury persists for 3 months after injury.²⁷ Additionally, the peak endogenous thrombin generation formed more quickly as compared to non-VTE patients very early after injury. In particular, ttPeak value appears to be an independent predictor of VTE and helped us to identify (net) 5 more true VTE cases and 5 more true VTE negative patients; reducing the rate of misclassification by 8.7%. Hence, acceleration of thrombin generation is important in generating the hypercoagulable milieu needed for thrombotic complications. In our multivariable model, Z-scores for the beta-coefficients were on the order of 3 to 4, indicating high power to detect them again in a new sample of similar size. Currently, no appropriately validated tools, that takes into account individual patient's coagulation phenotype, are available to identify the individual

trauma patient at high risk for VTE. Our multivariable model shows that ttPeak improves the concordance statistic by 4.5% from 0.754 to 0.799. Interestingly, our initial focus on generating the model was using the most hypercoagulable ttPeak (shortest) value as most patients had multiple samples drawn during the study. What we have found is that using each patient's first available ttPeak value, as long as it is within first 12 hours of injury, is as superior as picking the most hypercoagulable ttPeak value. Hence, CAT can be laboratory test, with a translatable potential.

Our study has several limitations. First, our overall consent rate was approximately 63%. As with all observational studies, sampling bias was a concern in our study and potentially affect the estimate of the relative risk in our observational study. However, the enrolled patients were reflective of the excluded population of 1720 patients (18 years old) seen at our Trauma Center during the study period in regards to median age (48[28,68]), sex (69% men) and mechanism of injury (93% blunt). When patients or their legal surrogates were approached in person, our overall consent rate has been 80%. When patients are approached via mail, after their initial discharge, the overall consent rate has been consistently 20%. The best way to prevent sampling bias is achieving a high response rate, which requires intensive efforts. In this present study, we devised a standard protocol, in which surveys were mailed close to 3 months after injury, followed by phone calls utilizing a standard template of questions (to reduce the risk of measurement bias). Second, 97% cohort patients were chosen from those enrolled during the first 12 months of the study. Due to budget limits and lack of additional power added by analyzing all the cohort samples, plasma samples from few cohort patients were analyzed after initial 12 months of the study enrollment. Hence, our cohort patients were not truly randomly selected throughout the study period. To ensure that the 370 cohort patients (whose plasma samples were analyzed) were representative of all cohort patients enrolled into our study, we compared the ISS, age, male sex % and mechanism of injury and found no significant differences between the two groups. Lastly, we could have added more variables such as ISS and TRISS into the final model, potentially improving the concordance rate. We intentionally wanted to generate a model that utilizes clinical variables that are easily obtainable and CAT assay, which theoretically would be ordered as a batched laboratory test. We hope that our model is generalizable as a result of its use of easy to obtain clinical variables.

In a future study, we plan to validate our VTE risk assessment tool ("24 hour tool") which incorporate readily obtainable clinical risk factors, and the individual patient's plasma coagulum (as reflected in ttPeak via CAT). In our completed prospective cohort study, 42% VTE cases occurred after hospital discharge. Universally extending prophylaxis beyond discharge will increase the bleeding risk period. A better strategy is to intensively prophylax the high VTE-risk individual who would benefit most, and potentially avoid the complications of anticoagulant-based VTE prophylaxis in the low VTE-risk individual with trauma. If we are able to validate this tool, we hope to perform a clinical trial to test the efficacy (non-inferiority for efficacy [endpoints will be DVT, PE or sudden death]) and safety (superiority for safety [endpoints will be bleeding and wound infection]) of the VTE risk prediction tool in individual with acute trauma.

Conclusion

Our VTE risk tool, which incorporates easily obtainable clinical risk factors, as well as the individual patient's procoagulant phenotype (as reflected by thrombin generation via CAT), will more accurately predict the occurrence of symptomatic VTE after injury. Alarming, we have observed that 42% of our cases developed VTE after hospital discharge. However, universally extending prophylaxis beyond discharge will increase the bleeding risk period. A better strategy would be to target more effective prophylaxis to the trauma patients at high risk individual. Our VTE risk assessment tool needs to be validated.

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Figure 1.
Enrollment Flow Diagram

Table 1**Adult Trauma Activation Criteria**Level 1 Trauma Resuscitation Criteria:

- 1 Threatened or Compromised Airway or Intubated Patient
- 2 Respiratory Distress
- 3 Respiratory rate < 10 or >30
- 4 Flail chest (known or suspected)
- 5 Heart rate > 120 and/or heart rate > systolic blood pressure
- 6 Confirmed Systolic BP < 90 at any time
- 7 Transfer patients receiving blood to maintain vital signs
- 8 StO₂ 70%
- 9 GCS 12 associated with injury
- 10 Paralysis, loss of sensation, and/or suspected spinal cord injury
- 11 Unstable pelvic fracture (known or suspected)
- 12 Bilateral femur fractures (known or suspected)
- 13 Traumatic amputation/crushed, degloved, mangled or pulseless injury extremity (excluded isolated hand/foot)
- 14 Patients with tourniquets to control hemorrhage
- 15 Burns > 10% body surface area (2nd/3rd degree) or with any known or potential airway compromise
- 16 High voltage electrocution including lightening
- 17 Gunshot wound proximal to elbow or knee OR any penetrating injury to head, neck, torso, axilla, or groin
- 18 Patients with known, intracranial bleed on anticoagulation (excluding ASA)

Level 2 Trauma Resuscitation Criteria:

- 1 Two or more proximal long bone fractures (if bilateral femur = RED) (known or suspected)
- 2 Death of restrained passenger in same vehicle
- 3 Major auto deformity (intrusion into passenger compartment)
- 4 Auto vs Pedestrian/bicyclist/motorcyclist thrown, run over, or with significant (> 20 mph) impact
- 5 Ejection (separated from) from motorized vehicle (car, motorcycle/motocross, snowmobile, ATV)
- 6 Fall > 20 feet
- 7 Suspected brain injury in patient on anticoagulation therapy (excluding ASA)

Table 2

Demographic and Baseline Characteristics of Venous Thromboembolism (VTE) Cases and Cohort Members

Characteristic	VTE Cases (N=83)	Cohort Members (N=370)	P-value
Age at Trauma, median (IQR); years	56.0 (46.0, 67.0)	46.5 (26.0, 62.0)	0.0001 ¹
Male sex; n (%)	62 (74.7%)	259 (70.0%)	0.43 ²
BMI, median (IQR); kg/m ²	29.5 (25.9, 35.3)	27.0 (23.7, 30.7)	0.0003 ¹
Injury Severity Score (ISS), median (IQR); %	21.0 (11.0, 29.0)	12.0 (5.0, 19.0)	<0.0001 ¹
Mechanical prophylaxis [#] ; n (%)	38 (45.8%)	220 (59.5%)	0.027 ²
Chemoprophylaxis [#] ; n (%)	27 (32.5%)	128 (34.6%)	0.79 ²
IVC filter after trauma; n (%)	35 (42.2%)	35 (9.5%)	<0.0001 ²

[#] Prophylaxis provided by 24 hours from time of injury¹ Kruskal-Wallis test² Fisher's exact test

Table 3

Univariate Analyses of Characteristics as Potential Predictors of Symptomatic, Incident Venous Thromboembolism After Acute Trauma

Characteristic	N ^{\$}	HR (95% CI)	p-value
Age, per 10 years	429	1.23 (1.10, 1.37)	<0.001
Injury Severity Score	429	1.06 (1.04, 1.08)	<0.001
Any Surgery	429	2.92 (1.80, 4.75)	<0.001
Any blood transfusion	429	3.71 (2.34, 5.90)	<0.001
Any Fracture	429	1.99 (1.12, 3.43)	0.013
Total bed rest	381	4.21 (2.28, 7.78)	<0.001
25 BMI < 30 (kg/m ²)	399	2.45 (1.15, 5.22)	0.021
BMI ≥ 30 (kg/m ²)	399	4.78 (2.30, 9.91)	<0.001
TRISS per 10% Decrease	419	1.26 (1.17, 1.36)	<0.001
No Mechanical prophylaxis	429	1.63 (1.03, 2.59)	0.038
No Chemoprophylaxis	429	1.02 (0.62, 1.66)	0.94
First ttPeak (per minute decrease)	426	2.02 (1.45, 2.80)	<0.001
First Lagtime (per minute decrease)	427	1.66 (1.06, 2.60)	0.028
Worst (shortest) ttPeak [*] (per minute decrease)	426	1.80 (1.42, 2.30)	<0.001
Worst (shortest) Lagtime [*] (per minute decrease)	427	1.90 (1.15, 3.13)	0.012
Platelet Count (per 10 ⁹ /L) [#]	419	0.98 (0.95, 1.02)	0.28
Hemoglobin (per 1 g per dL decrease) [#]	419	1.11 (1.01, 1.23)	0.027
Abnormal INR (outside of normal range of 0.8 – 1.2) [#]	420	1.37 (0.50, 3.77)	0.54
IVC Filter Placement During Day 1 of Hospitalization ^{&}	429	2.49 (1.20, 5.20)	0.015

^{\$} N equals to number of data points available from VTE cases and from those whose followup was **greater** than 1 day (Total of 429 Patients)

^{*} Using reagents 5 pM TF/4 uM PS; Any Surgery – any procedure performed in the operating room and requires general anesthesia; Any Fracture – includes upper, lower pelvic and spine fractures; TRISS – trauma injury severity score (probability of survival)

[#] Admission labs

[&] To note, only 23 patients (15 no-VTE and 8 Yes-VTE) had IVC filters placed by end of day 1 of hospitalization

Table 4

Final Multiple Variable Cox Proportional Hazard Model - 24-Hour Independent Predictors of VTE after Trauma[#]

Characteristic	HR	95% CI	Beta Coefficient	Z-Statistic	p-value
25 BMI < 30 (kg/m ²)	1.94	(0.90, 4.18)	0.664	1.70	0.089
BMI ≥ 30 (kg/m ²)	4.45	(2.13, 9.31)	1.493	3.97	<0.0001
Age, per 10 years	1.35	(1.19, 1.52)	0.296	4.69	<0.0001
Any Surgery	2.53	(1.53, 4.19)	0.928	3.61	0.0003
First ttPeak* (min)	2.26	(1.61, 3.18)	-0.815	-4.69	<0.0001

[#] Concordance Statistic of clinical variables is 0.754 (Increases to 0.799 with addition of ttPeak)

* Using reagents 5 pM tissue factor (TF)/4 uM phospholipid (PS)

* HR is per 1-minute decrease in ttPeak

Table 5

Reclassification of Cases and Cohort Patients with addition of first available ttPeak^{*} to the Multivariable Clinical Model:

	True VTE (Yes)	True VTE (No)
Low to High-Risk	8	19
High to Low-Risk	3	24

* Using reagents 5 pM tissue factor (TF)/4 uM phospholipid (PS)