Title
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TEG LYSIS SHUTDOWN REPRESENTS COAGULOPATHY IN BLEEDING TRAUMA PATIENTS: ANALYSIS OF THE PROPPR COHORT

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Abstract

Background—Thrombelastography (TEG) fibrinolysis shutdown after trauma is associated with increased mortality due to hypercoagulability-associated organ failure. However, a lack of mechanistic data has precluded the development of novel interventions to treat shutdown.

Objectives—To define the pathophysiology of TEG shutdown in severely injured, bleeding patients through secondary analysis of the PROPPR trial.

Methods—Fibrinolysis was characterized in PROPPR subjects using admission TEG lysis at 30 minutes (LY30) or plasmin-antiplasmin (PAP) levels. LY30 categories were low (< 0.9%), moderate (0.9-2.9%), or high (3%). PAP was classified as low (<1500 μg/L), moderate (1500-20,000 μg/L), or high (>20,000 μg/L). Demographics, outcomes, admission TEG values, platelet count and function, standard coagulation tests and coagulation proteins were compared.

Results—547 patients had TEG data and 549 patients had PAP data available. Low LY30 was associated with reduced platelet count and aggregation, poorer TEG clot formation, prolonged clotting times, and reduced fibrinogen and alpha2 antiplasmin. Compared to moderate PAP, low PAP subjects had similar platelet parameters, TEG values, fibrinogen, and alpha2 antiplasmin, but reduced tPA, and elevated PAI-1. D-Dimer values increased as PAP increased, however patients with low LY30 had elevated D-Dimer compared to moderate LY30 patients. Most low LY30 deaths were due to TBI (45%) and hemorrhage (42%) versus one of each cause (TBI, hemorrhage, MOF) in low PAP patients.

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Conclusions—Low TEG LY30 does not reflect shutdown of enzymatic fibrinolysis with hypercoagulability, but rather a coagulopathic state of moderate fibrinolysis with fibrinogen consumption and platelet dysfunction that is associated with poor outcomes.

Keywords

fibrinolysis; shutdown; trauma; coagulopathy; thrombelastography

Introduction

The recent characterization of the fibrinolytic system using thrombelastography (TEG) in severely injured patients has uncovered a spectrum of phenotypes including fibrinolysis shutdown, physiologic fibrinolysis, and hyperfibrinolysis (1). While hyperfibrinolysis (HF) is the least common phenotype observed, it is by far the most lethal and a substantial amount of research has been done to define its pathophysiology and guide treatment strategies (2–5). Several groups have shown that HF is driven by a rapid and robust increase in the profibrinolytic tissue plasminogen activator (tPA) in the absence of sufficient inhibitors such as plasminogen activator inhibitor (PAI-1) (3, 4, 6). Given these data, antifibrinolytic agents such as tranexamic acid (TXA), aminocaproic acid, and aprotinin, are all feasible therapies to reverse HF. In contrast, fibrinolysis shutdown (SD) is far more common than either HF or physiologic fibrinolysis (Phys) and is associated with an increased risk of death, particularly if SD persists up to or beyond a week (1, 7, 8). Despite its prevalence and association with mortality, we have a limited understanding of the mechanisms that drive SD in trauma patients and therefore no data-driven interventions that can improve outcomes in patients with this phenotype.

Current theories suggest that SD represents a hypercoagulable state, a common and appropriate response to trauma and hemorrhage, and this lack of fibrinolysis promotes aberrant fibrin deposition and increases the risk for microvascular thrombi and end-organ injury, as well as thromboembolic events. This concept is supported by the observation that the most common cause of death among SD patients is late multi-organ failure (7). However, other data within the same patient populations bring this notion into question. Moore et al reinforced in an elegant study from two institutions that the SD phenotype was very common and associated with poor outcomes, with slightly reduced early deaths from hemorrhage and increased late deaths, mostly from organ failure (7). Interestingly, while HF patients had the highest incidence of coagulopathy, as expected (32%), SD patients had a significantly higher incidence when compared to Phys (28% vs 18%). Although these patients could have experienced shutdown of fibrinolytic activity at a later time in their hospital stay, these admission data are inconsistent with the hypothesis that SD represents a hypercoagulable state.

Raza et al, during their characterization of fibrinolytic activation following trauma, described a group of patients that demonstrated elevated plasmin-antiplasmin (PAP) complex levels without evidence of fibrinolysis on viscoeslastic testing (3). They reported that only 1 in 20 patients with moderate fibrinolytic activation by PAP complex demonstrated lysis by viscoelastic testing, nonetheless, those patients had significantly

increased transfusion requirements and increased mortality. Our group similarly observed worsened outcomes in patients with moderate enzymatic fibrinolysis by PAP complex having TEG LY30 values within normal limits. This suggests TEG may be useful to identify only those patients experiencing catastrophic fibrinolytic activation in dire need of immediate clinical intervention (4, 5).

Despite our inadequate understanding of the pathophysiology of SD or the nature of what TEG is capturing concerning fibrinolysis, the concept of TEG fibrinolysis SD is being increasingly recognized as representing a deadly hypercoagulable state for which antifibrinolytic therapy is contraindicated. Given the recent analysis of TEG LY30 groups using the PROPPR trial data (9), we aimed to characterize the underlying mechanism of the TEG LY30 shutdown phenotype using both viscoelastic and molecular techniques in addition to examining the independent contribution of enzymatic fibrinolysis by PAP complex concentration. Using prospectively collected data from the multicenter PROPPR trial we further aimed to compare and contrast these mechanistic findings with outcomes and causes of death.

Materials and Methods

Study Design

This study was a retrospective analysis of data collected during the PROPPR trial (10). For this analysis, we used a data-driven approach utilizing pre-defined variables (fibrinolysis phenotypes) from the literature and applying them to this unique dataset of severely injured and bleeding trauma patients to generate new insights into fibrinolytic activation following injury. The PROPPR trial was a multi-center, pragmatic, randomized study designed to compare the effectiveness of two different ratios of blood products in trauma patients predicted to require massive transfusion. The study took place between August 2012 and December 2013 and enrolled 680 patients who were randomized to receive a 1:1:1 or 1:1:2 ratio of platelets, plasma, and red blood cells. This study was conducted with approval from the US Food and Drug Administration (NCT01545232), the Department of Defense, and in accordance with all local committees for the Protection of Human Subjects under exception from informed consent.

Study Population

The PROPPR trial design and enrollment criteria have been described in detail previously (10). Briefly, patients must have met the local criteria for highest-level trauma team activation, 15 years of age or older, received directly from the injury scene, received a minimum of 1 unit of blood products prehospital or within 1 hour of admission, and predicted to need a massive transfusion by Assessment of Blood Consumption score or physician gestalt.

Sample collection and testing

Whole blood was collected from all enrolled patients at the time of hospital admission into citrated vacutainer tubes and EDTA vacutainer tubes containing a cell preserving agent for immunophenotyping (Streck, Inc, La Vista, NE). Whole blood in the citrated tube was

maintained for 30 minutes at room temperature. After this time, kaolin-activated TEG was performed on all patients using the TEG model 5000 (Haemonetics, Braintree, MA) in accordance with the company specifications. Endpoints reported include R time (time to initiation of clot formation), K time (end of R time to 20 mm amplitude), Alpha Angle (rate of clot formation), Maximum Amplitude (MA) (clot strength), G value (clot strength, log derivative of MA) and the LY30 and LY60 values (degree of fibrinolysis at 30 and 60 minutes, respectively). In addition to reporting standard clotting variables described above, the TEG 5000 also records and reports raw clot amplitude as the total deviation in both directions (or $2 \times A$) in mm recorded every 5 seconds. Clotting velocity is also reported similarly and is defined as the integral of the total amplitude in mm/min plotted every 10 seconds. We extracted the first 60 minutes of assay time for both raw clot amplitude and velocity for all available subjects. Platelet function was also measured using the Multiplate® impedance aggregometer (Diapharma, West Chester, OH). Whole blood was diluted 50% with warm saline in a cuvette containing a stir bar and electrodes which generate an electrical signal between them. To stimulate aggregation, ADP (6.5 μM), arachidonic acid (0.5 mM), collagen (3.2 μg/mL), thrombin receptor activating peptide (TRAP) (32 μM), or ristocetin (0.8 mg/mL) were added to the diluted blood. Impedance to the electrical signal as the platelets aggregated onto the electrodes was recorded and expressed as area under the curve (AUC).

Platelet count was determined for all patients from the EDTA/fixative tube using flow cytometry analysis on a Beckman Coulter Gallios 3L 10C instrument (Beckman Coulter, Indianapolis, IN). Whole blood was incubated with APC-conjugated antibodies directed against the CD42b antigen (BD Biosciences, San Jose, CA) and the number of positive cells recorded.

Plasma was generated from citrated whole blood by centrifuging at 2,500g for 20 minutes, then aliquoted and stored at −80° C for further analysis. The ACL TOP Coagulation Analyzer (Instrumentation Laboratory, Bedford, MA) was used to measure standard coagulation tests including APTT, PT, and INR and coagulation factor levels including fibrinogen, D-Dimer, and Alpha 2-antiplasmin (Alpha 2 AP). Plasmin-antiplasmin (PAP) (Abcam, Cambridge, MA), tissue plasminogen activator (tPA) (eBiosciences, San Diego, CA), and plasminogen activator inhibitor (PAI-1) (eBiosciences) were measured by enzyme-linked immunosorbent assay.

Patient Stratification and Outcomes

Patients were stratified according to their degree of fibrinolytic activity on admission to the Emergency Department using two different techniques: TEG using the LY30 values and the PAP complex levels. The thresholds for TEG fibrinolysis are as previously described: Low LY30 = LY30 <0.9%, moderate LY30 = 0.9-2.9%, and high LY30 = 3% (1, 5, 7). The thresholds for PAP complex are also those which we and several other investigators have previously described: low PAP = PAP <1500 μg/L, moderate PAP = 1500-20,000 μg/L, and high PAP = >20,000 μg/L (3, 4).

Patient demographics, injury severity and type, resuscitation volumes, and outcomes (ventilator, intensive care unit, and hospital-free days and both 24-hour and 30-day mortality) and cause of death were collected prospectively during the PROPPR study.

Statistical Analysis

All statistical analyses were performed using STATA statistical software, version 14.1 (StataCorp, College Station, TX) and OriginPro 2017 ver. 94E (OriginLab Corp, Northampton, MA, USA). A power analysis was performed to determine if we had sufficient numbers to detect a significant difference in mortality between low LY30 and low PAP patients. Assuming a null difference in mortality of 20% and alpha=0.05, the minimum number of deaths required to achieve 80% power to avoid a type II error was 147. We had 164 deaths in this population, suggesting sufficient power to detect differences and avoid type II error. For continuous outcome variables that were normally distributed, significance was determined using a one-way ANOVA with a Bonferroni correction to determine which groups were significant. For continuous outcome variables that were not normally distributed, significance between groups was determined using Kruskal-Wallis rank sum tests using a Dunn's posthoc test with a Bonferroni correction to determine which groups were significantly different. For categorical variables, significance between groups was determined using Chi-square tests. TEG amplitude and velocity plots were grouped and the mean amplitude and velocity for each group was plotted every 5 and 1 minute, respectively, for up to 60 minutes of clotting time. Mean TEG amplitude curves were compared between all 6 groups for significant overall differences using one-way repeated measure ANOVA with Tukey adjustment for multiple comparisons to maintain an overall level of significance at $p<0.05$.

Results

Between August 2012 and December of 2013, 680 patients were enrolled in the PROPPR study. Admission TEG data were available on 547 patients and PAP complex values were available for 549 patients. Patient demographic, clinical, and outcome data were collected prospectively and therefore available for all patients.

Patient Demographics and Outcomes

Fibrinolysis stratification by TEG LY30—When stratified by TEG LY30, 61% of patients had low LY30, 17% of patients had moderate LY30, and 22% of patients had high LY30. No demographic differences were found between groups (TABLE 1). Both low and high LY30 patients were more likely to have suffered blunt mechanism injuries ($p<0.01$). Furthermore, patients in these groups had more severe injuries evidenced by lower RTS ($p<0.01$), and higher ISS values were evident in the high LY30 group ($p<0.01$). This was also true of profound head injuries by AIS score ($p<0.01$). Patients with low LY30 had worse shock compared to moderate LY30, however patients with high LY30 displayed significantly worse base excess compared to both groups and thus the most profound shock ($p<0.01$).

High LY30 patients received the most blood products over the first 24 hours. In agreement with other reports (1, 5, 7), we did observe fewer ventilator- and hospital-free days in the low LY30 patients compared to moderate LY30 patients. Overall, high LY30 patients experienced far worse outcomes with fewer ventilator-, ICU-, and hospital-free days, almost four-fold greater incidence of 24-hour mortality and more than double the incidence of 30-day mortality (all p<0.01) (TABLE 1).

Fibrinolysis stratification by PAP complex level—When stratified by PAP complex, 9.3% of patients had low PAP, 75.2% of patients had moderate PAP, and 15.5% of patients had high PAP. Patients with high PAP were more likely to be older (p=0.02) and female (0.02). (TABLE 2). Compared to low PAP, both moderate and high PAP phenotypes suffered more blunt mechanism injuries (p<0.01). The severity of injury increased as the degree of fibrinolytic activation by PAP complex increased (ISS 19 vs 26 vs 36, p<0.01). Further, RTS values, head AIS scores, and base excess values were significantly worse in the high PAP compared to low PAP patients (p<0.05, p<0.01, p<0.01, respectively) (TABLE 2).

Interestingly, the moderate PAP received the highest volume of prehospital crystalloid (p=0.01, compared to high PAP). Patients in the high and moderate PAP groups both received more blood product transfusions over the first 24 hours than the low PAP group, however high PAP patients received the most overall. Similar to the pattern observed with ISS, outcomes worsened as the degree of fibrinolytic activation by PAP complex increased. Overall, patients with low PAP were less-injured and had the best outcomes. Patients in both moderate and high PAP groups experienced fewer ventilator-, ICU-, and hospital-free days compared to low PAP; high PAP required the most prolonged clinical care. High PAP patients had the highest mortality at both 24-hours and 30 days (both p<0.01).

Neither admission LY30 nor PAP outcomes differed significantly by assigned treatment arm (p=0.40 and p=0.72, respectively). There was a significant overall effect of study site on the distribution of LY30 and PAP categories among sites (p<0.01 and p<0.01, respectively). However, on closer inspection, this effect was not likely to be clinically significant since all sites shared similar distributions of LY30 and PAP categories. Therefore, we did not include adjustments for treatment group or treatment site in our analyses.

Global Coagulation and Fibrinolysis Mechanism Assessment

Fibrinolysis stratification by TEG LY30—We observed significant differences in platelet count and function between groups when patients were grouped by LY30 (TABLE 3). Moderate LY30 patients had the highest platelet counts, followed by low LY30, and high LY30 patients (p<0.01). After adjusting for platelet count (Multiplate agonist AUC/platelet count), platelet aggregation remained different between groups. With the exception of thrombin stimulation, patients with moderate LY30 demonstrated the best platelet aggregation function to all agonists tested (TABLE 3). Both low and high LY30 patients had significantly lower platelet function compared to moderate, with high LY30 patients having the worst platelet function compared to the other two groups (all p<0.01).

All other TEG parameters demonstrated a hypocoagulable phenotype in the low LY30 group compared to the moderate group, with high LY30 being the most profoundly coagulopathic

(more prolonged R and K times, lower alpha angle and lower MA; all p<0.05). This observation is further reflected in the standard coagulation tests where the most prolonged APTT, PT, and INR times are observed in the high LY30 patients and low LY30 patients demonstrated significantly longer coagulation times compared to moderate LY30.

In order to determine differences in the mechanisms driving fibrinolytic activation and fibrinolysis in these cohorts, multiple fibrinolytic biomarkers and pathway proteins were measured. As expected, high LY30 patients had the lowest fibrinogen and Alpha 2 AP levels and the highest D Dimer, PAP complex, and tPA values. However, compared to moderate LY30, we found that low LY30 patients actually had significantly lower fibrinogen and Alpha 2AP levels, and *higher* D Dimer levels. Yet, there was no difference in PAP complex levels or any other fibrinolytic mediator (TABLE 3). Taken together, these data suggest that low LY30 patients are actually experiencing coagulopathy driven by low fibrinogen and poor platelet function in the presence of physiologic levels of enzymatic fibrinolysis.

Fibrinolysis stratification by PAP complex level—Unlike the observations made following stratification by LY30, we observed no differences in platelet count between groups when stratified by PAP complex (TABLE 4). Further, with the exception of Ristocetin-induced aggregation, which was highest in the low PAP group (p<0.01), no other differences in platelet function by aggregation were observed.

When analyzing all TEG parameters, LY30 and LY60 increased as PAP increased between groups (TABLE 4). Otherwise, there were no other differences in TEG between groups with the exception of the R time, which was shortest in the low PAP group. Similarly, no differences in standard coagulation tests were found.

Interestingly, no differences in fibrinogen levels were noted between PAP groups. However, as PAP complex increased, levels of D-Dimer increased, tPA increased, and Alpha 2 AP decreased. Further, the highest PAI-1 levels were observed in the low PAP group (TABLE 4). Therefore, PAP complex reflected canonical mediators of fibrinolytic activation and fibrinolysis and was not associated with defects in platelet count, function or fibrinogen levels.

TEG Amplitude Curve Analysis—Full raw TEG amplitude and velocity curves were analyzed by plotting the raw amplitude and velocity values against time, allowing direct visualization of clot formation and kinetics between groups. When stratified by LY30, we observed that the moderate LY30 patients demonstrated significantly increased mean (SD) overall clot amplitude during 60 minutes of clotting time at 113.7 (27.5) mm compared to both the low LY30 group at 106.4 (35.8) mm, and the high LY30 group at 61.6 (50.0) mm (ANOVA p<0.05 with Tukey adjustment). (Figure 1A) When stratified by PAP complex, the low PAP group had the highest overall amplitude averaging 111.9 (30.1) mm, which was significantly increased compared to both moderate PAP at 96.3 (43.0) mm, and high PAP at 93.5 (46.4) mm, while moderate PAP was not different than high PAP (ANOVA p<0.05 with Tukey adjustment). (Figure 1B)

TEG Velocity Curve Analysis—When stratified by LY30, mean (SD) overall mean velocity was not different between low LY30 at 1.03 (2.54) mm/min, and moderate LY30 at 1.04 (2.85) mm/min, but both were significantly increased compared to high LY30 at 0.44 (2.49) mm/min (ANOVA p<0.05 with Tukey Adjustment). (Figure 2A) When stratified by PAP complex, the mean velocity was similar for low PAP at 0.97 (2.50) mm/min and moderate PAP at 0.95 (2.74) mm/min, while high PAP was decreased at 0.77 (2.72) mm/min, which was significantly different than only moderate PAP (ANOVA p<0.05 with Tukey adjustment) (Figure 2B).

Causes of Death—The most common cause of death in the overall population was hemorrhage (52%), which is expected given that the PROPPR trial enrollment targeted severely bleeding patients, followed by TBI (38%) and MOF (11%). More than one cause of death was noted, if applicable. Using LY30, 47% of deaths occurred in high LY30 patients, followed by 45% in low LY30 patients, and lastly 8% in the moderate LY30 group. Using PAP, moderate patients were the most likely to die (64%), followed by high PAP (34%), and low PAP (2%).

The majority of low LY30 patients died of TBI (45%), followed by hemorrhage (42%) and only 13% died of MOF (Figure 3). Deaths in the moderate LY30 group were similar with 36% caused by TBI, 45% by hemorrhage, and 18% by MOF. The most common cause of death for high LY30 was hemorrhage (58%), followed by TBI (34%) and MOF (8%). For low PAP patients there were only 3 deaths contributing 1 to each main cause (33% hemorrhage, 33% TBI, 33% MOF) (Figure 3). Causes of death between moderate and high PAP were quite similar with hemorrhage being the leading cause of death in both groups (49% and 47%, respectively), followed by TBI (38% and 47%, respectively), and lastly MOF (13% and 7%, respectively) (Figure 3).

When looking only at hemorrhage-related deaths, 57% had high LY30, 8% had moderate LY30 and 35% had low LY30 (Figure 4). Among those patients who died of hemorrhage with low LY30, almost all (96%) had moderate or severe enzymatic fibrinolytic activation by PAP (Figure 4).

Low TEG LY30 Subgroup Analysis—In order to determine the effects of enzymatic fibrinolytic activation amongst low LY30 patients, we divided the low LY30 subgroup by PAP complex category by the defined criteria. Among low LY30 patients, 11% had low PAP levels, 73% had moderate PAP levels, and 16% had high PAP levels (Table 5). No differences in demographics, injury mechanism, or admission markers of physiologic derangement were observed. However, low LY30 patients who also had low PAP levels were less severely injured than those with moderate PAP levels (ISS 22 versus 26, respectively, p=0.03). There were no differences in the volume of blood products transfused over the first 24 hours, however those with high PAP levels received less prehospital crystalloids compared to those with low or moderate PAP levels. Patients with low LY30 and low PAP experienced fewer days on the ventilator, in the ICU and in the hospital (all p<0.01), however there were no observed differences in early or late mortality between subgroups. When comparing low LY30 patients who lived versus those who died, we found that both PAP and D-dimer values were significantly higher in the patients who died [median (IQR)

PAP 4,883 (2,458, 8,549) versus 11,676 (8,072, 16,632); p<0.01 and D-dimer 4,104 (746, 12,157) versus 18,717 (5,912, 38,380); p<0.01].

Discussion

The recently described TEG LY30 SD phenotype is associated with a poor prognosis in severely injured patients that is attributed to a prothrombotic state leading to death by MOF (7). However, our results suggest that TEG LY30 SD in actively-bleeding trauma patients does not represent either a prothrombotic or antifibrinolytic state. Using TEG and PAP to classify patients by degree of fibrinolysis and fibrinolytic activation, we found that 89% of low LY30 patients actually had moderate to high fibrinolytic activation by PAP. This was corroborated by significantly elevated D-Dimer in the low compared to moderate LY30 group. D-Dimer is a specific byproduct of the enzymatic cleavage of fibrin by plasmin. This result alone strongly suggests that fibrinolysis is increased rather than suppressed in TEG LY30 SD. Low LY30 patients also had reduced fibrinogen concentration, lower platelet count, reduced platelet aggregation function, and prolonged standard coagulation test values compared to moderate LY30 patients. Further examination of the full TEG amplitude and velocity curves for each LY30 group also showed an overall decreased clot formation response for the low LY30 group relative to the moderate group that was not consistent with a prothrombotic state.

The clinical outcomes reported here support this conclusion. The TEG cohorts accurately reproduced previously published results by demonstrating a U-shaped mortality distribution, with an increased relative mortality for low LY30 versus moderate LY30 (1, 7, 8). However, in this population, 42% of low LY30 patients died of bleeding. Combined, these laboratory and clinical results suggest that TEG LY30 SD more likely represents a type of coagulopathy characterized by fibrinolytic activation with concurrent fibrinogen consumption and platelet dysfunction with impaired clot formation.

Published data are also in alignment with many of the findings presented here. Moore et al showed that the incidence of coagulopathy by INR was increased in the SD patients compared to patients with physiologic lysis (7). Further, they demonstrated that platelet counts were lowest in the SD group with other markers of coagulation being the most pro-coagulable in their physiological lysis group, not the SD group. Among their SD patients, 15% died of hemorrhage.

Viscoelastic tests like TEG are reliable tools for measuring global coagulation potential at the patient bedside. However, as a global test, multiple components of the coagulation system can contribute to the behavior of TEG parameters like LY30. While it remains unclear what has caused the low LY30 in the SD patient population, changes in LY30 can potentially be generated through a combination of plasmin generation causing fibrin degradation, coagulation factor level depletion, and or changes of platelet numbers or function. Platelets, in particular, are capable of inducing TEG phenotypes that are indistinguishable from fibrinolysis without also incorporating fibrinolytic inhibitors into the assays for comparison (11). Therefore, TEG LY30 does not solely indicate the presence of enzymatic fibrinolysis and using it under the assumption that it is a specific marker of

fibrinolytic activity may have resulted in a misidentification of these coagulopathic patients. The mechanism by which patients with elevated fibrinolysis would produce a low LY30 on TEG will be the subject of future studies.

We found that examining fibrinolytic activation by PAP complex in addition to fibrinolysis by D-Dimer allowed for a clear estimate of fibrinolysis. Low PAP levels were associated with significantly reduced D-Dimer and tPA levels, increased PAI-1 and Alpha 2 AP levels, with no appreciable difference in platelet count or aggregation, fibrinogen level, or standard coagulation tests. Examination of the corresponding TEG amplitude and velocity curves suggested that similarities in the PAP-derived groups was likely due to the relative insensitivity of TEG for fibrinolysis, where the TEG is able to detect profound and clinically catastrophic fibrinolysis but cannot easily distinguish between other degrees of fibrinolysis that remain clinically-relevant. Interestingly, when comparing the amplitude curves, the high LY30 patients appeared far more hypocoagulable than the high PAP patients. This supports our above findings showing that grouping by PAP complex is largely reflective of changes in fibrinolytic activation alone whereas grouping by LY30 more likely reflected a mechanistic combination of fibrinolysis, hypofibrinogenemia, and platelet dysfunction. From this data it is clear that the PAP-derived definition of fibrinolytic activation when compared in relation to D-Dimer is more likely to identify an isolated effect of enzymatic fibrinolysis on clot formation and outcomes in trauma patients and thus, may offer more specific guidance on antifibrinolytic therapy (Schematic 1).

The findings presented here have relevant clinical ramifications concerning treatment of bleeding trauma patients. According to our data, patients having TEG LY30 SD should be treated like any other traumatic coagulopathy patient in which procoagulant interventions may be appropriate. Further, these patients do not necessarily represent a population from which TXA therapy should be withheld. Among the hemorrhage-related deaths with low LY30 (35%), all patients but one demonstrated moderate to severe fibrinolytic activation by PAP complex. These data imply that there are a significant number of patients who die of hemorrhage with fibrinolysis as a contributing mechanism that are not detected by or misinterpreted by the apparent biphasic behavior of TEG LY30. These patients may have benefited from antifibrinolytic therapy had a more sensitive point-of-care test of fibrinolysis been available and employed.

Further, the low LY30 patients also demonstrated low platelet counts and reduced fibrinogen levels resulting in poor clot formation. Platelet contributions to clot structure and function are known to regulate clot lytic susceptibility (12). In such a case, platelet transfusion and/or fibrinogen replacement may be beneficial by improving clot structure, while TXA may help support coagulation by inhibiting the action of plasmin on fibrin clot structure and improving its durability. A recent study also showed that outcomes following TXA administration were worst in trauma patients having moderate lysis by TEG LY30 (13). This makes sense in light of our data showing that the moderate LY30 patients demonstrated the best overall clot formation and therefore would likely benefit from TXA therapy the least. Importantly our data also support the conclusions of other reports that TEG may not be the most reliable tool for distinguishing which patients will benefit from TXA administration (14). Given our results, we caution against using isolated portions of a TEG curve to make

treatment decisions during bleeding situations without also holistically-evaluating the entire curve with attention paid to all reported parameters and overall clot formation.

There are several limitations to this study. First, this study is a post hoc secondary analysis of the PROPPR trial that aimed to enroll patients with substantial bleeding and severe injuries. The findings presented here are therefore not reflective of the overall trauma population, but rather a cohort of more critically injured and bleeding patients. Second, measuring PAP complex is not a current point-of-care test and therefore cannot be used clinically at this time. Developing a rapid and sensitive test for use at the bedside could be an area of future research. Finally, this analysis was done on admission samples and thus we cannot comment on dynamic fibrinolytic changes that include transition to enzymatic fibrinolytic inhibition that occur as a result of interventions later in the course of care.

In conclusion, the TEG LY30 shutdown phenotype found in severely-bleeding trauma appears at Emergency Department arrival likely represents a coagulopathic state resulting from moderate fibrinolysis with concurrent fibrinogen consumption and platelet dysfunction. Procoagulant clinical interventions such as a high ratio of plasma and platelet resuscitation should be considered to improve outcomes in these patients. The best method to identify those patients most likely to benefit from antifibrinolytic agents is still to be determined.

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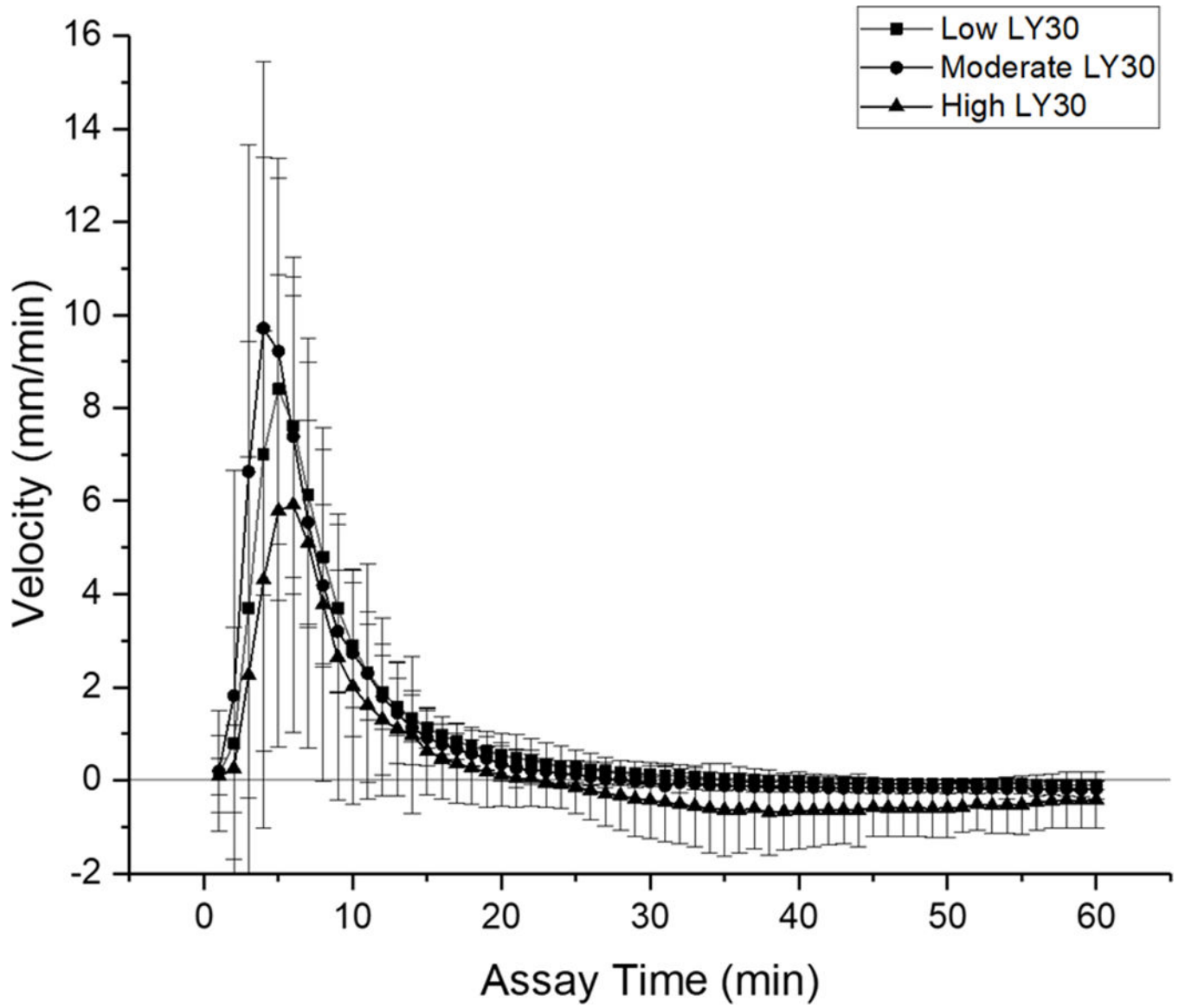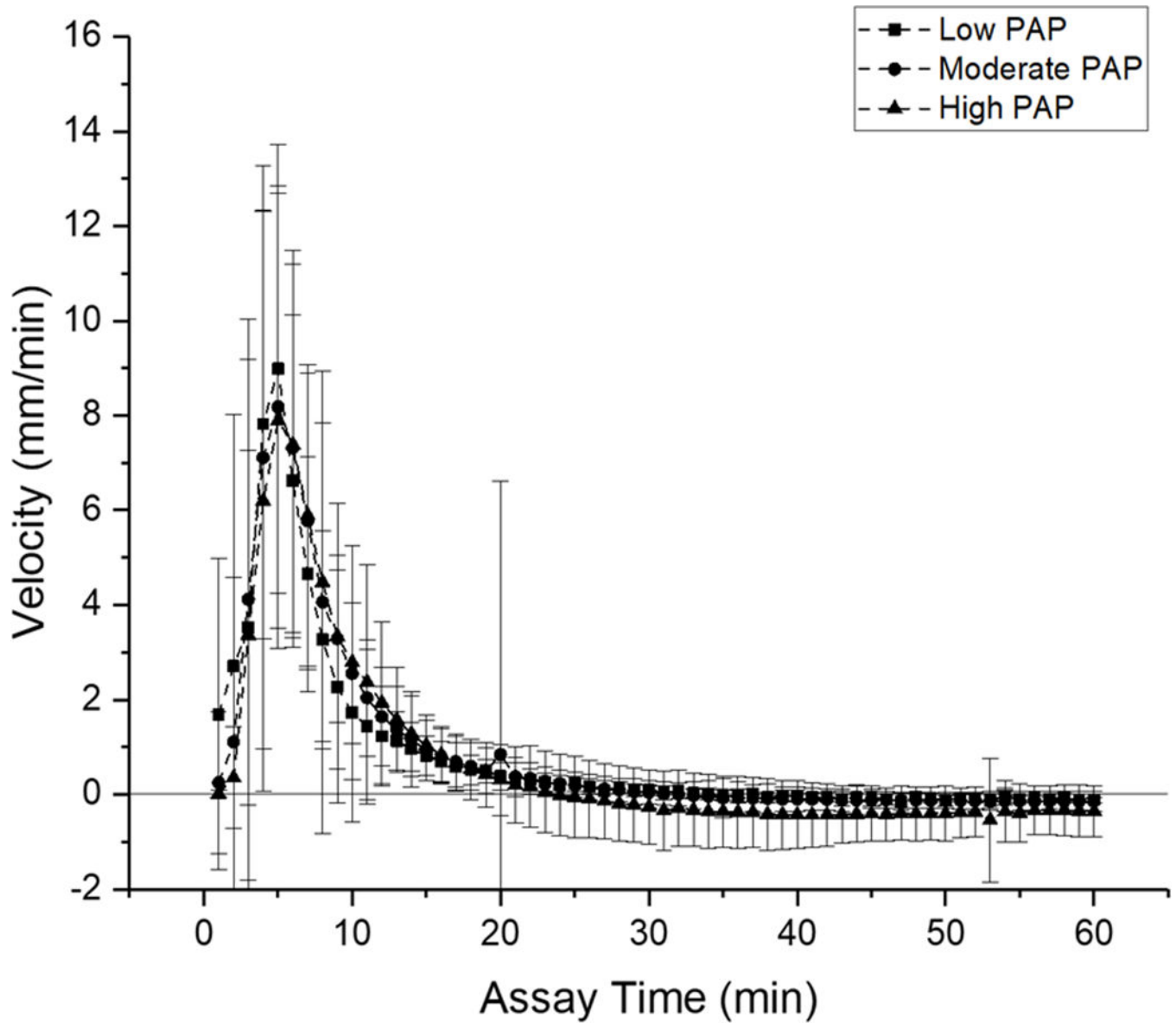
Figure 1.
Effects of fibrinolysis on clot formation. Raw TEG clot amplitude curves were plotted for A) TEG LY30 fibrinolysis phenotypes and B) PAP fibrinolysis phenotypes. Available numbers of amplitude curves were as follows: low LY30 =159, moderate LY30=47, high LY30 =72, low PAP=25, moderate PAP=198, and high PAP=81. Data are presented as means with standard deviation. Square denotes low fibrinolysis; circle denotes moderate fibrinolysis; triangle denotes high fibrinolysis.
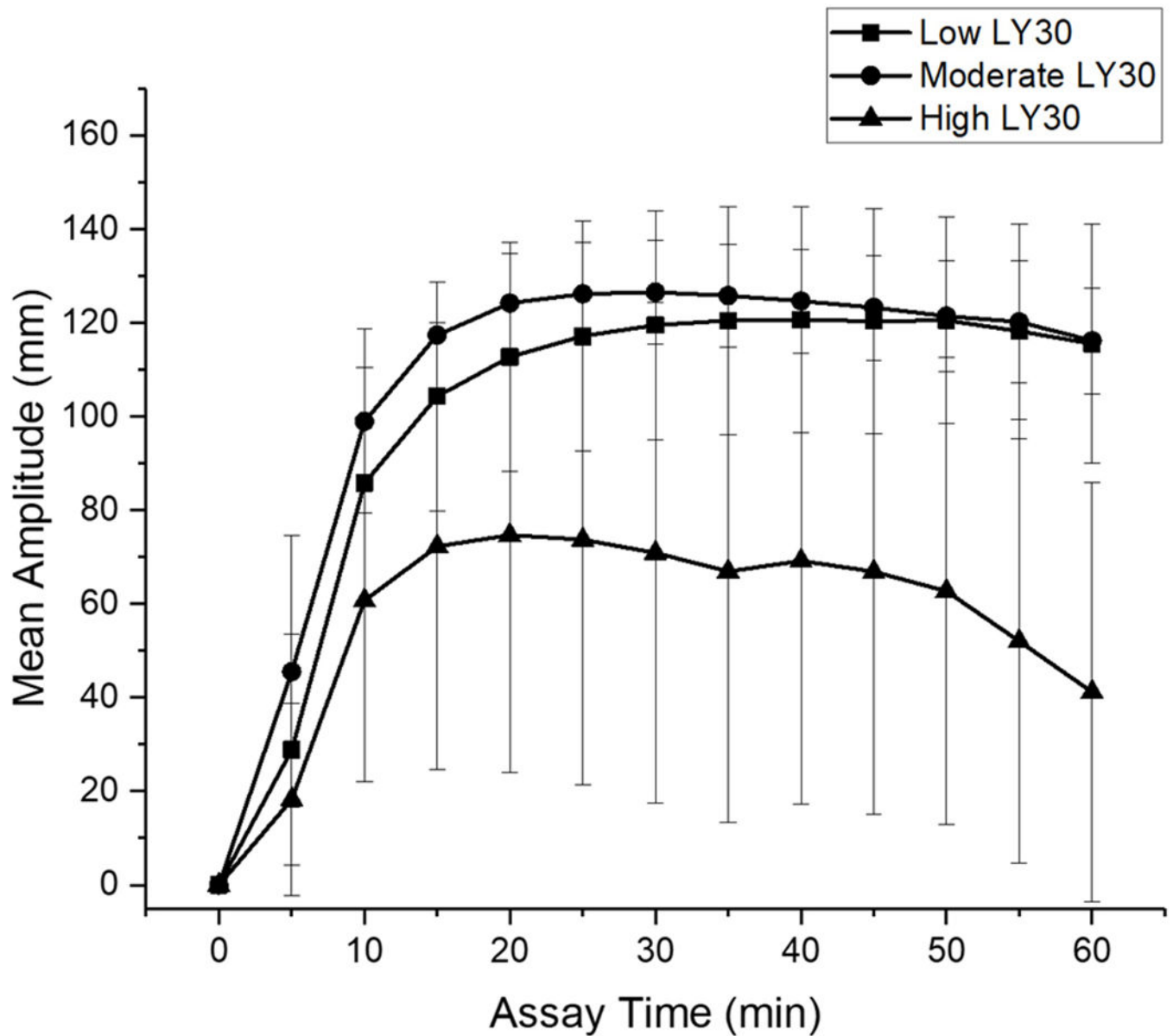
Figure 2.
Effects of fibrinolysis on clot kinetics. Raw TEG clot velocity curves were plotted for A)
TEG LY30 fibrinolysis phenotypes and B) PAP fibrinolysis phenotypes. The number of
curves available for analysis were: low LY30=136, moderate LY30=63, high LY30=61, low
PAP=17, moderate PAP=198, and high PAP=79. Data are presented as means with standard
deviation. Square denotes low fibrinolysis; circle denotes moderate fibrinolysis; triangle
denotes high fibrinolysis.
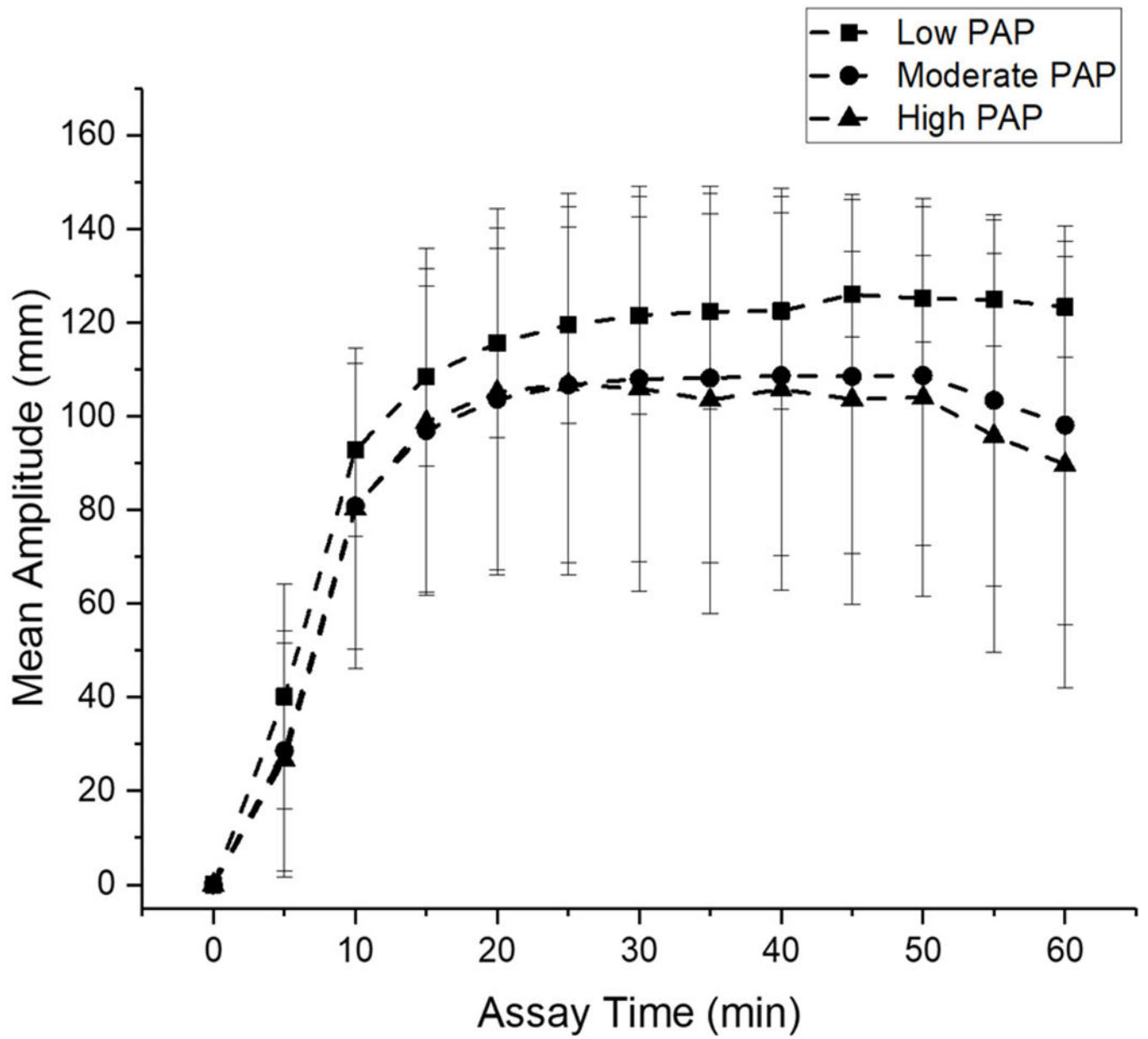
Figure 3.
Causes of mortality among fibrinolysis groups.
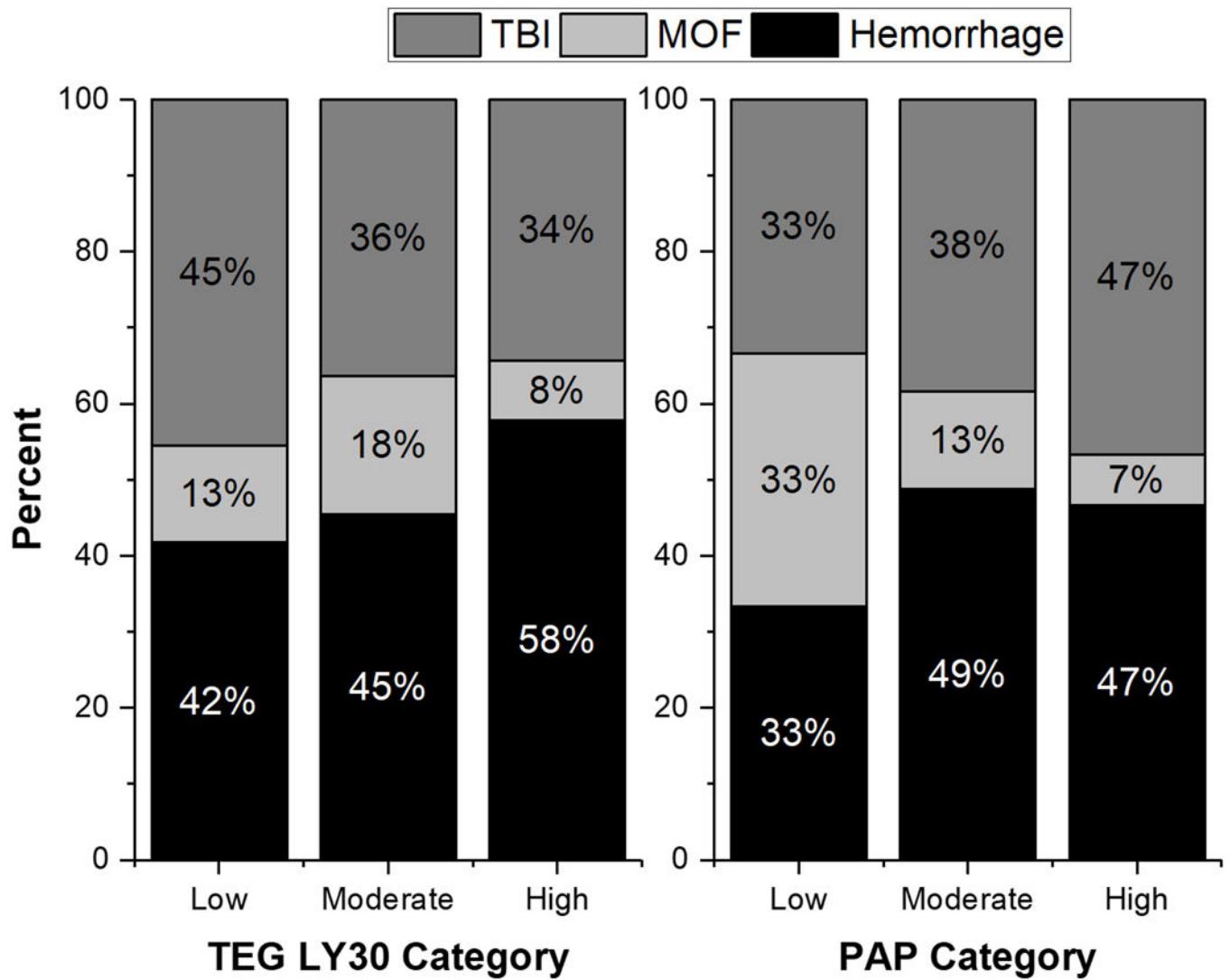
Figure 4.
TEG LY30 groups among hemorrhage-related deaths (left). Among patients who died of hemorrhage with low LY30, percentages with low and moderate to severe PAP levels are shown (right).

TEG LY30 Categories

PAP categories within Low TEG LY30
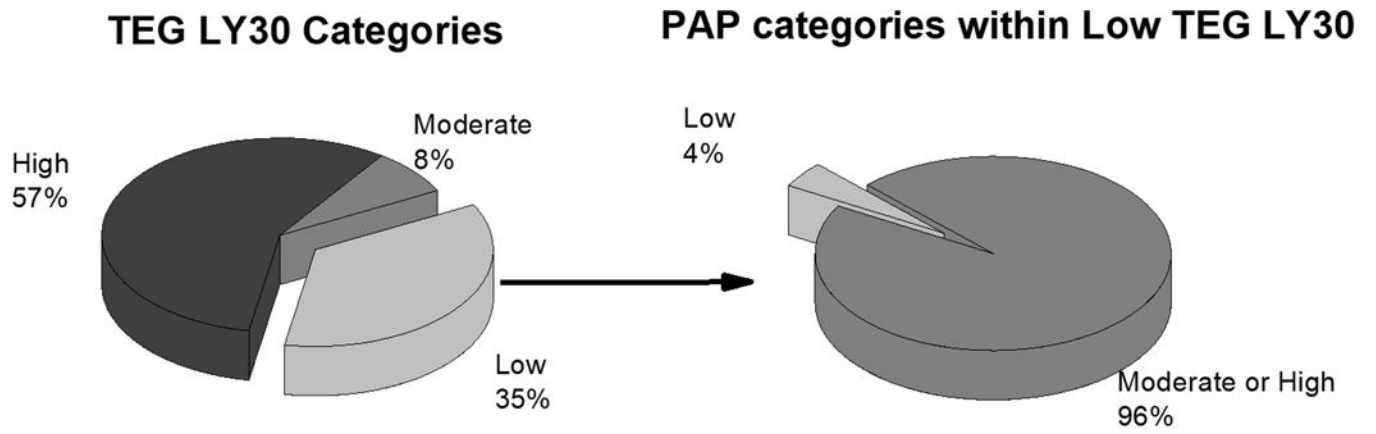
Schematic 1.
Relationships between clinical presentation and TEG variables versus enzymatic fibrinolysis

TABLE 1

Patient demographics, injury, resuscitation volumes and outcomes in patients with low LY30 (<0.9), moderate LY30 (0.9-2.9), and high LY30 (3). Median and IQR values are reported. Significance between groups was determined using Kruskal-Wallis rank sum tests using a Dunn's posthoc test with a Bonferroni correction to determine which groups were significantly different

|	Low (N=333)	Moderate (N=95)	High (N=119)	p-value
Demographics				
Age	34 (25, 52)	35 (22, 46)	34 (24, 55)	0.34
Male	271 (81%)	80 (84%)	92 (77%)	0.42
Injury				
Blunt	169 (51%)	38 (40%) [*]	75 (63%) [#]	0.01
Systolic BP	100 (80, 123)	107 (82, 125)	100 (77, 130)	0.50
RTS	6.4 (4.1, 7.6)	7.6 (6.1, 7.8) [*]	6.0 (4.1, 7.6) [*,#]	<0.01
Head AIS	0 (0, 2)	0 (0, 0)	0 (0, 4) [#]	<0.01
ISS	25 (17, 38)	25 (16, 34)	34 (22, 45) [*,#]	<0.01
Base Excess	−7.9 (−12, −4)	−6.4 (−10, −2.5) [*]	−12.0 (−18, −6.7) [*,#]	<0.01
Resuscitation Volumes				
Pre-hospital Crystalloid	0.4 (0, 1.0)	0.2 (0, 0.8)	0.3 (0, 0.8)	0.05
24 Hour RBC	9 (5, 14)	7 (4, 12)	15 (9, 24) [*,#]	<0.01
24 Hour Plasma	6 (3, 10)	4 (2, 10)	11 (4, 17) [*,#]	<0.01
24 Hour Platelets	6 (6, 12)	6 (0, 12)	12 (6, 24) [*,#]	<0.01
24 Hour Cryoprecipitate	0 (0, 0)	0 (0, 0)	0 (0, 10)	0.08
Outcomes				
Vent-free days	26 (8, 28)	27 (18, 29) [*]	0 (0, 25) [*,#]	<0.01
ICU-free days	21 (1, 26)	22 (13, 27)	0 (0, 22) [*,#]	<0.01
Hospital-free days	4 (0, 17)	11 (0, 19) [*]	0 (0, 10) [*,#]	<0.01
24 Hr Mortality, n (%)	30 (9.0%)	5 (5.7%)	42 (35.3%) [*,#]	<0.01
30-Day Mortality, n (%)	58 (17.4%)	11 (11.6%)	61 (51.3%) [*,#]	<0.01

[*] p<0.05 compared to Low

[#] p<0.05 compared to Moderate

TABLE 2

Patient demographics, injury, resuscitation volumes and outcomes in patients with low enzymatic fibrinolysis (PAP<1500 μg/L), moderate enzymatic fibrinolysis (PAP 1500-20,000), and high enzymatic fibrinolysis (PAP>20,000). Median and IQR values are reported. Significance between groups was determined using Kruskal-Wallis rank sum tests using a Dunn's posthoc test with a Bonferroni correction to determine which groups were significantly different

	Low (N=51)	Moderate (N=413)	High (N=85)	p-value
Demographics				
Age	30 (26, 42)	34 (24, 51)	44 (26, 57) [*,#]	0.02
Male	43 (86%)	338 (82%)	59 (69%) [#]	0.02
Injury				
Blunt	15 (29%)	208 (50%) [*]	67 (79%) [*,#]	<0.01
Systolic BP	102 (83, 132)	100 (79, 124)	98 (80, 124)	0.42
RTS	7.1 (4.1, 7.8)	6.4 (4.1, 7.8)	6.1 (4.1, 7.6) [*]	0.04
Head AIS	0 (0, 0)	0 (0, 2)	2 (0, 4) [*,#]	<0.01
ISS	19 (12, 26)	26 (17, 38) [*]	36 (26, 45) [*,#]	<0.01
Base Excess	−6.4 (−10.2, −2.1)	−8.0 (−12.0, −4.5)	−11 (−16.4, −4.2) [*,#]	<0.01
Resuscitation Volumes				
Pre-hospital Crystalloid	0.3 (0, 1.1)	0.4 (0, 1.0)	0.2 (0, 0.5) [#]	0.01
24 Hour RBC	7 (4, 10)	9 (5, 15) [*]	15 (9, 23) [*,#]	<0.01
24 Hour Plasma	3 (1, 8)	6 (3, 11) [*]	10 (6, 17) [*,#]	<0.01
24 Hour Platelets	6 (0, 12)	6 (6, 18) [*]	12 (6, 18) [*,#]	<0.01
24 Hour Cryoprecipitate	0 (0, 0)	0 (0, 6) [*]	0 (0, 10) [*]	0.01
Outcomes				
Vent-free days	28 (27, 29)	24 (0, 28) [*]	0 (0, 17) [*,#]	<0.01
ICU-free days	27 (23, 29)	18 (0, 26) [*]	0 (0, 12) [*,#]	<0.01
Hospital-free days	19 (2, 23)	3 (0, 16) [*]	0 (0, 1) [*,#]	<0.01
24 Hr Mortality, n (%)	1 (2.0%)	49 (11.9%)	26 (30.6%) [*,#]	<0.01
30-Day Mortality, n (%)	3 (5.9%)	84 (20.3%) [*]	45 (52.9%) [*,#]	<0.01

[*] p<0.05 compared to Low

[#] p<0.05 compared to Moderate

TABLE 3

Laboratory values by LY30 groups. Median and IQR values are reported. Significance between groups was
determined using Kruskal-Wallis rank sum tests using a Dunn's posthoc test with a Bonferroni correction to
determine which groups were significantly different

	Low (N=333)	Moderate (N=95)	High (N=119)	p-value
Platelet Count				
CD42+ (x 1000)	216 (161, 284)	251 (196, 317)[*]	203 (141, 285)[#]	<0.01
Standardized Platelet Function				
ADP (AUC)	0.13 (0.07, 0.23)	0.20 (0.13, 0.28)[*]	0.10 (0.02, 0.18)[*,#]	<0.01
AA (AUC)	0.10 (0.03, 0.19)	0.19 (0.12, 0.26)[*]	0.07 (0.0, 0.16)[*,#]	<0.01
Collagen (AUC)	0.09 (0.03, 0.15)	0.14 (0.10, 0.20)[*]	0.05 (0.0, 0.12)[*,#]	<0.01
Thrombin (AUC)	0.37 (0.27, 0.49)	0.37 (0.28, 0.48)	0.31 (0.17, 0.40)[*,#]	<0.01
Ristocetin (AUC)	0.10 (0.04, 0.20)	0.15 (0.08, 0.25)[*]	0.05 (0.01, 0.15)[*,#]	<0.01
TEG				
R Time	3.8 (2.9, 4.5)	3.2 (2.5, 3.9)[*]	4.3 (3.3, 5.5)[*,#]	<0.01
K Time	1.5 (1.2, 2)	1.2 (1.1, 1.5)[*]	1.7 (1.3, 2.3)[*,#]	<0.01
Alpha Angle	69.6 (63.8, 73.7)	74.3 (70, 76.3)[*]	67.0 (57.9, 71.7)[*,#]	<0.01
MA	61.4 (55.7, 65.9)	62.1 (58.2, 66.1)[*]	51.7 (36.7, 61.6)[*,#]	<0.01
G Value	8.0 (6.3, 9.7)	8.2 (7.0, 9.7)	5.4 (2.9, 8.1)[*,#]	<0.01
LY30	0 (0, 0.2)	1.6 (1.1, 2.0)[*]	14 (5.6, 52.8)[*,#]	<0.01
LY60	0.7 (0.1, 2.0)	4.8 (2.6, 5.9)[*]	40.6 (9.8, 71.8)[*,#]	<0.01
Standard Coag Tests				
APTT (s)	25.9 (23.7, 31.3)	24.0 (21.3, 27.4)[*]	30.8 (24.5, 44.2)[*,#]	<0.01
PT (s)	12.8 (11.2, 15)	11.4 (10.8, 12.8)[*]	13.1 (11.4, 16.2)[#]	<0.01
PT INR	1.16 (1.02, 1.35)	1.03 (0.98, 1.16)[*]	1.18 (1.04, 1.45)[#]	<0.01
Factor Levels				
Fibrinogen (mg/dL)	164 (123, 221)	208 (151, 259)[*]	134 (85, 172)[*,#]	<0.01
D-Dimer (ng/mL)	5758 (906, 16333)	1469 (743, 9226)[*]	8095 (3555, 24252)[*,#]	<0.01
PAP (µg/L)	5578 (2728, 10706)	6918 (3575, 11805)	17794 (10380, 24460)[*,#]	<0.01
tPA (ng/mL)	241 (119, 431)	253 (105, 578)	994 (507, 1529)[*,#]	<0.01
PAI-1 (ng/mL)	17.4 (9.7, 37.4)	15.2 (8.8, 32.8)	14.9 (9.2, 27.3)	0.22
Alpha 2 AP (ng/mL)	58.9 (45.5, 73.2)	69.3 (55.4, 81.7)[*]	44.5 (19.0, 60.7)[*,#]	<0.01

[*] p<0.05 compared to Low

[#] p<0.05 compared to Moderate

TABLE 4

Laboratory values by PAP groups. Median and IQR values are reported. Significance between groups was determined using Kruskal-Wallis rank sum tests using a Dunn's posthoc test with a Bonferroni correction to determine which groups were significantly different

	Low (N=51)	Moderate (N=413)	High (N=85)	p-value
Platelet Count				
CD42+ ($\times$ 1000)	198 (172, 290)	219 (156, 288)	212 (158, 284)	0.89
Standardized Platelet Function				
ADP (AUC)	0.14 (0.09, 0.23)	0.14 (0.07, 0.22)	0.15 (0.07, 0.29)	0.53
AA (AUC)	0.11 (0.03, 0.20)	0.12 (0.03, 0.20)	0.11 (0.02, 0.19)	0.97
Collagen (AUC)	0.11 (0.03, 0.19)	0.09 (0.03, 0.15)	0.11 (0.03, 0.20)	0.46
Thrombin (AUC)	0.37 (0.27, 0.49)	0.35 (0.26, 0.46)	0.35 (0.23, 0.50)	0.71
Ristocetin (AUC)	0.15 (0.08, 0.25)	0.09 (0.04, 0.18)[*]	0.08 (0.01, 0.14)[*,#]	<0.01
TEG				
R Time	3.4 (2.1, 4.0)	3.8 (2.8, 4.7)[*]	4.0 (3.2, 4.9)[*,#]	<0.01
K Time	1.3 (1.2, 1.8)	1.5 (1.2, 2.0)	1.7 (1.2, 2.0)	0.22
Alpha Angle	71 (66, 73)	70 (64, 75)	68 (63, 72)	0.10
MA	61 (57, 64)	60 (54, 66)	59 (46, 64)	0.15
G Value	8.0 (6.7, 9.1)	7.6 (5.9, 9.4)	7.2 (4.2, 9.3)	0.24
LY30	0 (0, 0.5)	0.2 (0, 1.6)[*]	4.6 (0.8, 51)[*,#]	<0.01
LY60	0.6 (0.1, 3.2)	2.1 (0.4, 5.0)[*]	11.4 (4.4, 69.8)[*,#]	<0.01
Standard Coag Tests				
APTT (s)	25.1 (23.3, 29.5)	26.4 (23.6, 32.5)	26.5 (22.9, 35.5)	0.47
PT (s)	12.4 (11.1, 14)	12.7 (11.2, 15.2)	12.5 (10.8, 13.9)	0.34
PT INR	1.12 (1.01, 1.26)	1.15 (1.02, 1.36)	1.13 (0.98, 1.25)	0.34
Factor Levels				
Fibrinogen (mg/dL)	157 (133, 192)	163 (119, 225)	147 (112, 213)	0.16
D-Dimer (ng/mL)	509 (151, 1440)	6112 (1249, 16460)[*]	21956 (6413, 35354)[*,#]	<0.01
PAP (µg/L)	1098 (685, 1282)	6791 (3805, 11093)[*]	25088 (22487, 30289)[*,#]	<0.01
tPA (ng/mL)	166 (102, 321)	251 (132, 496)[*]	1307 (619, 1610)[*,#]	<0.01
PAI-1 (ng/mL)	29.1 (16.9, 59.1)	14.9 (8.2, 33.2)[*]	16.7 (10.6, 25.7)[*]	<0.01
Alpha 2 AP (ng/mL)	62.1 (51.9, 76.7)	59.2 (44.6, 74.3)	40.5 (19.6, 56.0)[*,#]	<0.01

[*] p<0.05 compared to Low

[#] p<0.05 compared to Moderate

TABLE 5

Patient demographics, injury, resuscitation volumes and outcomes within the low LY30 population comparing those with low (PAP <1,500 μg/L), moderate (PAP 1,500-20,000 μg/L), and high (PAP >20,000 μg/L) enzymatic fibrinolysis. Median and IQR values are reported. Significance between groups was determined using Kruskal-Wallis rank sum tests using a Dunn's posthoc test with a Bonferroni correction to determine which groups were significantly different

	Low LY30, Low PAP (N=37)	Low LY30, Moderate PAP (N=243)	Low LY30, High PAP (N=53)	p-value
Demographics				
Age	28 (26, 36)	34 (25, 53)	42 (26, 57)	0.07
Male	31 (84%)	196 (81%)	44 (83%)	0.85
Injury				
Blunt	13 (35%)	125 (51%)	31 (58%)	0.19
Systolic BP	100 (85, 133)	97 (78, 120)	109 (88, 123)	0.13
RTS	7.1 (4.1, 7.8)	6.4 (4.1, 7.6)	6.9 (4.1, 7.6)	0.18
Head AIS	0 (0, 0)	0 (0, 2)	0 (0, 2.5)	0.11
ISS	22 (16, 26)	26 (17, 38)[*]	25 (17, 41)	0.03
Base Excess	−6.6 (−10.0, −3.0)	−7.9 (−12.0, −4.8)	−8.1 (−12.2, −3.6)	0.35
Resuscitation Volumes				
Pre-hospital Crystalloid	0.5 (0, 1.1)	0.5 (0.1, 1.0)	0.2 (0, 0.5)[*,#]	<0.01
24 Hour RBC	8 (4, 11)	9 (5, 14)	8 (5, 16)	0.46
24 Hour Plasma	4 (2, 9)	6 (3, 10)	5 (2, 11)	0.56
24 Hour Platelets	6 (6, 12)	6 (6, 12)	6 (0, 18)	0.39
24 Hour Cryoprecipitate	0 (0, 0)	0 (0, 6)	0 (0, 5)	0.16
Outcomes				
Vent-free days	28 (26, 29)	25 (7, 28)[*]	26 (1, 29)[*]	<0.01
ICU-free days	26 (23, 28)	19 (0, 26)[*]	17 (0, 25)[*]	<0.01
Hospital-free days	18 (0, 22)	1 (0, 15)[*]	0 (0, 13)[*]	<0.01
24 Hr Mortality, n (%)	1 (2.7%)	25 (10.3%)	4 (7.5%)	0.30
30 day Mortality, n (%)	2 (5.4%)	46 (18.9%)	10 (18.9%)	0.12

[*] p<0.05 compared to Low

[#] p<0.05 compared to Moderate