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Post-injury Hyperfibrinogenemia Compromises Efficacy of Heparin-Based VTE Prophylaxis

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Abstract

Background—Venous thromboembolism (VTE) prophylaxis remains debated following trauma, and recommendations have not been established. Although hyperfibrinogenemia is a marker of pro-inflammatory states, it is also contributes to thrombus formation. Post-injury hyperfibrinogenemia is common, but the effect of hypefibrinogenemia on VTE prophylaxis has not been fully elucidated. Therefore, we hypothesized that heparin is less effective for VTE prophylaxis following severe injury due to hyperfibrinogenemia.

Methods—In vitro studies evaluated TEG parameters in 10 healthy volunteers after the addition of fibrinogen concentrate and heparin. Data from a recent randomized controlled trial, conducted at an academic level-1 trauma center surgical intensive care unit, were reviewed. Critically injured patients were randomized to standard VTE prophylaxis (5,000 Units LMWH daily) or TEG-guided prophylaxis (up to 10,000 Units LMWH daily), and were followed for 5 days. Analysis was performed to evaluate the relationship between fibrinogen levels, measures of anticoagulation, and TEG parameters.

Results—In vitro studies revealed increased fibrinogen reversed the effects of heparin as measured by TEG. Fifty patients were enrolled in the clinical study with 25 in each arm. TEG parameters, fibrinogen, platelet count, and anti-Xa levels did not differ between groups despite treatment provided. Fibrinogen levels increased over the 5-day study period (597±24.0 to 689.3±25.0), as well as clot strength (9.8±0.4 to 14.5±0.6), which had a significant correlation coefficient ($p<0.01$). Moreover, there was a moderate inverse correlation between fibrinogen level and the effect of heparin (R_F), which was significant on study days 1 and 3, implicating hyperfibrinogenemia in heparin resistance.

Conclusion—Hypercoagulablity and heparin resistance are common following trauma. The preclinical and clinical relationships between fibrinogen levels and hypercoagulability implicate hyperfibrinogenemia as a potential factor in heparin resistance.

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Conflicts of Interest

There are no conflicts of interest reported by any authors.

Keywords

Post-injury Hypercoagulabilty; Trauma; Functional Fibrinogen; Hyperfibrinogenemia; Thrombelastography (TEG); Venous Thromboembolism (VTE)

INTRODUCTION

The incidence of symptomatic venous thromboembolic events following trauma is approximately 6–7% despite receiving recommended chemical and/or mechanical prophylaxis.[1–3] However, the true incidence of all VTEs, symptomatic and asymptomatic, are suspected to be substantially higher.[4,5] Even with the overwhelming adoption of mechanical/chemical prophylaxis in post-injury patients, little evidence exists showing efficacy of VTE prophylaxis in this patient population. In fact, recent review concluded that there is no evidence that any existing method of VTE prophylaxis is superior to other methods, or even to no prophylaxis in trauma patients.[6] Failure to find differences in outcomes in the critically injured patient may be due to inadequate dosing of heparin, since some studies have shown anti-factor Xa (anti-Xa) levels to be below recommended thresholds for prophylaxis.[7,8] Thrombeslastography (TEG) has been proposed to be the optimal tool in measuring hypercoagulability, which is more sensitive than plasma-based tests, and has been suggested to be more sensitive than anti-Xa levels, which may help guide chemical prophylaxis in the prevention of VTEs.[9,10]

We therefore completed a phase II randomized controlled trial to compare TEG-guided chemical VTE prophylaxis, which included anti-platelet therapy, to the standard-of-care, as well as to examine the study design, evaluate endpoints, and to ensure safety to prepare for a larger study. The initial results of this study, along with some *in vitro* data, have been previously presented, and demonstrated that platelets contribute significantly to clot strength, that LMWH may increase platelet activation, and that platelets themselves increase thrombus generation and fibrin production.[11] In addition, it was observed that LMWH, as well as increased doses of LMWH, had little, if any, effect on TEG parameters. Currently, the lack of LMWH efficacy in trauma patients has been largely attributed to decreased bioavailability due to peripheral edema, vasoconstriction, decreased cardiac output, or even obesity.[8] However, our patient population was quite heterogeneous, and the lack of LMWH efficacy was consistent, raising additional questions about other factors affecting the pharmacokinetics of LMWH.

Interestingly, our study also noted a significant increase in fibrinogen over the 5-day study period, which was consistent throughout this population, and moreover, the role of fibrinogen in thrombus formation is clinically gaining recognition. Currently, there is a European emphasis to address fibrinogen levels early in trauma patients to adequately achieve hemostasis, and fibrinogen has also been shown to be a key component in thrombus generation and clot integrity.[12,13] However, the role of fibrinogen has been largely ignored regarding its effect on LMWH. Moreover, recent evidence has shown that hyperfibrinogenemia, itself, increases thrombosis and resists thrombolysis.[14] Therefore, we tested the effect of hyperfibrinogenemia on heparin in an *in vitro* model, then re-examined our data from our phase II trial with the hypothesis that hyperfibrinogenemia would compromise the efficacy of LMWH.

MATERIALS AND METHODS

In vitro studies were performed on citrated whole-blood samples obtained from healthy volunteers (n=10). Venipuncture was performed with a 21-guage needle in an antecubital

vein, and blood was collected into two separate 3.5 mL plastic Vacutainers® containing 3.2% citrate. In one citrated whole-blood sample, 20 mg of lyophilized human fibrinogen concentrate (Sigma-Aldrich Co., St. Louis, MO, Product F3879) was slowly added directly to the Vacutainer® and gently inverted until the powder was completely dissolved. This method limited the volume change as well as the change in concentration of citrate in the Vacutainer®. Pre-study experiments were performed to determine the optimal addition of fibrinogen to roughly double the functional fibrinogen concentration. Both Kaolin and Functional Fibrinogen (FF) TEGs were performed within 30 minutes of collection on each sample, and all TEG parameters were recorded. In addition, 5.0 μL of a 0.1 units/mL concentration of unfractionated heparin was added to 1.0 mL of blood from the Vacutainer® containing unaltered blood, as well as to blood containing excess fibrinogen (as described above), and both Kaolin TEG and FF TEG were performed with all TEG parameters recorded. Normal TEG parameters for our clinical laboratory include: R-time (2–8 min), k-time (1.1–3.5 min), α-angle (55.0–78.0 degrees), MA (55.8–73.3 mm), and FLEV (200–445 mg/dL).

The methods from our phase II randomized, controlled study have been described previously.[11] This study was conducted at the Denver Health Medical Center, the academic level-1 trauma center for the University of Colorado Denver, and was approved by the Colorado Multiple Institutional Review Board. This study was also registered with the NIH (#NCT01050153). All patients considered for inclusion in this study were trauma patients admitted to the surgical intensive care unit in which VTE prophylaxis with low molecular weight heparin (LMWH) was indicated. Inclusion criteria were patients with age greater than or equal to 18 years, who experienced blunt or penetrating trauma requiring admission to the SICU, who would normally receive LMWH therapy for prophylaxis of VTE as standard-of-care, and for whom informed consent by the patient, legally authorized representative or proxy decision maker could be obtained and documented. Exclusion criteria included the presence of any absolute contraindication to LMWH therapy (heparin hypersensitivity, heparin-induced thrombocytopenia, end-stage chronic liver disease (MELD>12), ongoing resuscitation for hemorrhagic shock, known persisting bleeding disorder or ongoing post-injury coagulopathy, and subdural or epidural hematoma), any relative contraindication to LMWH therapy (new intracranial lesions, neoplasm or monitoring devices, extravascular thrombolytic therapy, severe uncontrolled hypertension, arterial dissection, recent intraocular surgery, recent intracranial or spine surgery, or conditions associated with increased risk of hemorrhage), presence or removal within the last 12 hours, of an indwelling epidural or spinal catheter or recent neuroaxial anesthesia or spinal puncture, or patient history with concomitant or known use within one week prior to hospitalization of drugs affecting hemostasis such as NSAIDS, platelet inhibitors or other anticoagulants.

Patients were enrolled and randomized into a control group, or a TEG-guided treatment algorithm group based upon a pre-designed randomization table and implemented by a trauma research coordinator. Neither research staff nor participants were blinded to the intervention. For patients in the control group, dalteparin 5000 IU was administered subcutaneously once daily. For patients in the TEG-guided group (Figure 1), VTE prophylaxis was guided by the difference in R-times obtained from simultaneously running citrated whole blood samples with a standard Kaolin TEG assay (R_{Kaolin}) and a Kaolin TEG with heparinase ($R_{Heparinase}$). Dalteparin was initiated if the TEG R_F (R_{Kaolin}-$R_{Heparinase}$) was less than 1 minute ($R_F < 1.0$) with a starting dose of 5000 IU subcutaneously once daily. The dalteparin dose was then adjusted based on the TEG results four hours post-dose, with the goal treatment being a R_F value at or between 1.0 and 1.4 minutes. For R_F values less than 1 minute, dalteparin was increased by 2500 IU, to a maximum dose of 10,000 IU subcutaneously per day, divided and given every 12 hours. The dalteparin dose could not be

increased more than once in a 24-hour period. For R_F values greater than 1.4 minutes, the dalteparin dose was decreased by 2500 IU if receiving greater than 5000 IU daily, or held if receiving only 5000 IU once daily. Anti-platelet therapy was started once the maximal dose of LMWH was reached in the TEG-guided prophylaxis group and who also had a G-value > 10.9. TEG Platelet Mapping was also performed to ensure the percent inhibition of the arachidonic acid (AA) and adenosine diphosphate (ADP) pathways did not exceed 50% with anti-platelet therapy. If exceeding 50% inhibition, the aspirin dose was held. Aspirin was initiated at a dose of 81 mg, and was increased daily to 162 mg, and ultimately 325 mg, until inhibition exceeded 50%. Patients were followed for 5 days in the SICU.

In addition, conventional plasma-based coagulation tests were measured including aPTT, INR, as well as platelet count, peak and trough anti-factor Xa, antithrombin III, and functional fibrinogen levels. Peak anti-factor Xa levels were measured four hours following LMWH administration, and trough levels were measured just prior to LMWH administration. The TEG-based Functional Fibrinogen assay assesses the fibrinogen component to clot strength, and strongly correlates to the clinical standard von Clauss fibrinogen levels.[13] The fibrinogen contribution to clot strength was calculated by $MA_{Fibrinogen}/MA_{Kaolin}$.

Statistical analysis

Patient data were analyzed on an “intent-to-treat” basis. Randomization effectiveness was assessed by comparing demographic and injury severity variables between the two groups. Continuous variables were reported as mean and standard error of the mean (SEM) when normally distributed and as median and interquartile range when not-normally distributed. In comparing the two study groups, we used Chi-square tests or Fisher Exact tests for proportions, t-test for normally distributed variables, and the Wilcoxon test for continuous non-normally distributed variables, as indicated. Variables measured over time were compared using repeated measures ANOVA, and post-hoc pairwise comparisons at individual times adjusted using Tukey’s method. In vitro studies were analyzed using a paired t-test. All tests were two-tailed and overall experiment error significance set at $p<0.05$.

The Pearson correlation statistics were used to evaluate the association of fibrinogen level and clot strength (as measured by the G-value) as well as R_F, and the correlation coefficient with correspondent 95% confidence intervals (calculated using the Fisher’s z transformation and bias adjustment) and p-values reported.

RESULTS

To examine the effect of hyperfibrinogenemia on TEG parameters, as well as on the efficacy of heparin, *in vitro* studies were conducted. Human fibrinogen concentrate was added to blood from healthy volunteers (n=10) with a mean age of 33±7 years, with 50% being male. With the addition of a standard amount of fibrinogen concentrate, functional fibrinogen levels significantly increased (261.5±18.98 to 469.52±29.64; $p<0.0001$), as well as clot strength (7.81±0.45 to 10.58±0.52 dynes/cm^2). In addition, the increase in fibrinogen levels significantly correlated with an increase in clot strength ($R^2=0.43$; $p<0.0001$). Fibrinogen concentrate also increased the percent fibrinogen contribution to clot strength from 23.7±1.8% to 38.1±2.4% ($p=0.002$). This 14.4±3.3% change in fibrinogen contribution to clot strength was remarkably similar to the 12.2±3.2% change in clot strength suggesting a direct effect of fibrinogen on clot strength.

As expected, the addition of human fibrinogen concentrate also significantly decreased the R and k-time, and increased α-angle, MA, and thrombus generation. Furthermore, the addition

of heparin to normal blood significantly prolonged R and k-time, and decreased α-angle, MA, G, and thrombus generation (Table 1). However, with the addition of the identical concentration of heparin to blood containing fibrinogen concentrate, the hyperfibrinogenemia negated the effect of heparin. This was noted in all TEG parameters, with R-time, k-time, α-angle, MA, G, and thrombus generation all returning to their baseline values (Table 1). Moreover, the mean R_F value of the heparinized blood was 7.02±1.23 minutes, but after the addition of fibrinogen concentrate, the mean R_F value significantly decreased to 3.64±0.44 minutes (p=0.023).

To determine if these effects were also seen clinically, data from our phase II clinical trial were re-examined. A total of 627 patients were screened over a 21-month period between 2010 and 2012, and 66 met inclusion criteria. Of those, 50 patients gave consent and were enrolled in the study. The control group (n=25) and TEG-guided prophylaxis group (n=25) were similar in demographics, including age, BMI, and gender. In addition, patients had similar injury severity regarding ISS, base deficit, and APACHE scores (Table 2). Initial coagulation parameters of aPTT, INR, fibrinogen levels, antithrombin III levels, Anti-Xa levels, platelet count, and hemoglobin did not differ between groups. Furthermore, initial citrated Kaolin TEG parameters were similar between groups (Table 2). The median time from injury to study enrollment was 3.0 days (IQR:2–3) for the control group and 2.0 days (IQR:2–3) for the TEG-guided group (Wilcoxon p=0.98). The first dose of dalteparin was administered on the day of enrollment. The doses of LMWH for each group, as well as the doses of aspirin, are shown in Table 3.

The proposed TEG-guided VTE prophylaxis algorithm did not affect standard TEG parameters including R-time, K-time, α-angle, MA, G, and LY30 compared to controls through study day 5. In addition, there was no difference in aPTT, INR, fibrinogen levels, platelet count, anti-Xa, and antithrombin III levels between the control and TEG-guided prophylaxis groups over the 5-day study period (Table 4). Anti-Xa peak and trough values were recorded, which demonstrated that Anti-Xa levels increased 4-hours following LMWH administration, but remained below recommended prophylaxis values (0.2–0.4 IU/ml) for both the control and TEG-guided prophylaxis groups (Figure 2). To determine if the maximal dosing of LMWH affected TEG parameters, patients receiving 10,000 IU of LMWH were compared to those in the control group who received 5,000 IU. No differences were observed up through study day 5. The mean TEG parameters for patients in the control group receiving 5,000 IU of LMWH and those in the TEG-guided group receiving 10,000 IU are: SP (4.55±0.24 vs 4.14±0.52); p=0.41, R (5.03±0.26 vs 6.16±0.82); p=0.21, K (1.14±0.06 vs 1.52±0.40); p=0.37, Angle (74.15±0.91 vs 68.75±4.75); p=0.29, MA (73.72±1.11 vs 72.77±1.40); p=0.61, G (14.72±0.87 vs 13.67±0.93); p=0.45, and LY30 (0.01±0.00 vs 0.01±0.00); p=0.42.

However, fibrinogen levels significantly correlated with overall clot strength for each study day (Table 5). Moreover, there was a moderate inverse correlation between fibrinogen level and R_F, which was significant on study days 1 and 3, implicating hyperfibrinogenemia as a potential factor in heparin resistance (Table 5). Therefore, we further examined the fibrinogen contribution of clot strength between groups. Although not significant by repeated measures ANOVA, there was a trend toward a decreased fibrinogen contribution to clot strength in the TEG-guided prophylaxis group on study days 2 through 4, suggesting that additional LMWH may decrease the fibrinogen contribution to clot strength (Figure 3).

Within the 5-day study period, no patients in either group developed a VTE, and there were no bleeding complications as a result of increased VTE prophylaxis. Following the study period and upon transfer out of the surgical ICU, patients were given the standard-of-care, which consisted of 5000 IU of LMWH once daily until discharge. Although outside the

study period, it was noted that one patient in the control group developed a PE prior to discharge. No patients in the TEG-guided group had clinical symptoms of VTE after transfer out of the surgical ICU. During the study, no changes to the methods were made after trial commencement, and there were no changes in outcomes being measured.

DISCUSSION

These clinical data further bring into question the effectiveness of heparin-based VTE prophylaxis in trauma patients. Although no VTE events were observed in this study, these data demonstrate that both standard doses and TEG-guided doses of LMWH are ineffective based on both anti-Xa levels as well as by thrombelastography parameters. Although TEG R_F values have been proposed as a more sensitive parameter to guide prophylaxis, rarely did R_F values exceed 1.0 minute, even in the setting of giving 10,000 IU of LMWH. Therefore, it still remains unclear which assay (anti-Xa or TEG) is the optimal tool to measure the efficacy of LMWH; however, both are comparable in showing that current recommendations for VTE prophylaxis are inadequate in the critically injured trauma patient. Despite the inability of increased LMWH to change outcomes in this study, TEG has provided significant insight into post-injury hypercoagulability and the role of fibrinogen in this phenomenon. First, fibrinogen has a significant role in clot stability/integrity, and in post-injury patients, both fibrinogen levels as well as clot strength continued to rise despite LMWH use in the control and TEG-guided prophylaxis groups. Second, the rise in fibrinogen levels significantly correlated with the rise in clot strength for each study day suggesting hyperfibrinogenemia substantially contributes to hypercoagulability. Third, there was an inverse correlation with fibrinogen level and R_F, implicating hyperfibrinogenemia in decreasing the efficacy of LMWH. Lastly, there was a trend, although non-significant, showing a decreased fibrinogen contribution to clot strength in the TEG-guided group, suggesting that increased doses of LMWH may ultimately reduce the fibrinogen contribution to clot strength.

Similar results were evidenced in the *in vitro* studies conducted, which demonstrated increased fibrinogen levels in whole blood directly increased clot strength, increased the fibrinogen contribution to clot strength, decreased R_F values, and negated the anticoagulant properties of heparin. The alteration of just one variable in this *in vitro* setting, the addition of fibrinogen, resulted in many of the findings observed in the clinical study. Moreover, the addition of heparin decreased the fibrinogen contribution to clot strength, providing some evidence that a more aggressive escalation of LMWH prophylaxis may in fact reduce the fibrinogen contribution to clot strength and prevent VTE events.

There have been several studies in the medical literature that have associated hyperfibrinogenemia with myocardial infarctions, strokes, arterial thrombosis, and VTE.[15–23] However, literature showing a causal relationship between hyperfibrinogenemia and hypercoagulability remained lacking. It was not until recently that hyperfibrinogenemia has been shown to induce thrombosis and oppose fibrinolysis.[14] Despite this, our understanding of post-injury hypercoagulability and effective VTE prophylaxis are still evolving. Although many factors following serious injuries have been associated with the decreased bioavailability and/or pharmacokinetics of LMWH, decreased bioavailability has not been established, and other factors are likely responsible for the decreased pharmacokinetics of LMWH.[8] These *in vitro* data clearly demonstrate hyperfibrinogenemia decreases the pharmacokinetics of heparin in the setting of standardized doses, but this still raises questions regarding bioavailability.

Mechanistically, heparin is a highly negatively charged molecule, which has many known interactions with plasma-based proteins.[24–26] Fibrin(ogen), is one such protein, which has

known heparin binding sites.[27,28] These proteins have been shown to sequester heparin in *in vitro* studies, limiting its catalytic activity, and is reversed once displaced from plasma proteins.[29] Also, the gamma prime chain of fibrinogen, has been shown to bind thrombin, preventing its inhibition by antithrombin III.[30] Despite this, thrombin still retains its catalytic activity, and is resistant to the heparin-potentiated effects of antithrombin III. Thus, the hypercoagulability seen in post-injury patients despite heparin therapy is likely due to the decreased bioavailabilty of heparin, as well as to thrombin's resistance to antithrombin III from hyperfibrinogenemia.

In addition, we have previously shown that thrombocytosis is common following severe injury, and directly promotes thrombin generation and fibrin production.[11] This phenomenon can be explained through the cell-based model of hemostasis, which proposes that hemostasis occurs in a step-wise process, but is highly regulated by tissue factor-bearing cells and platelets.[32] Once coagulation is initiated on tissue factor-bearing cells, activated platelets are recruited, and thrombin generation is propagated on the platelet surface. Cofactors Va and VIIIa, as well as IXa and XIa, rapidly localize on the platelet surface converting factor X to Xa, and promote thrombin generation.[31,32] Since a majority of thrombin generation takes place on the surface of the platelet, one may deduce that by increasing the platelet number and surface area, it is likely to promote thrombin generation and fibrin formation.

Consequently, severe injury and hypoperfusion results in endothelial injury and induces hyperfibrinogenemia, a known acute phase protein. Although initially inhibited following trauma, new platelets are subsequently formed, resulting in thrombocytosis and subsequent return of platelet function.[33,34] These platelets then have the optimal milieu for activation, as well as substrate to promote fibrin production. This, in combination with other thrombogenic stimuli (immobility, decreased concentrations of antithrombin, protein C and S, and a delay in the initiation of prophylaxis), places post-injury patients at high risk for venous thrombus formation. Therefore, optimal post-injury VTE prophylaxis likely requires a comprehensive approach to inhibit both platelet function and fibrin production.

Since the clinical portion of this study was designed as a small, phase II clinical trial to evaluate our study design, ensure safety, and to detect differences between standard VTE prophylaxis and a TEG-based algorithm, there are several limitations to this study. At the time of trial design, there was minimal data confirming the safety of higher-dose LMWH and antiplatelet therapy in high-risk trauma patients. Therefore, the escalation in LMWH dosing and administration of anti-platelet therapy was prolonged to ensure safety. Consequently, there were no adverse bleeding events in this study; however, standard plasma-based measures of anticoagulation, as well as TEG parameters, did not reflect significant changes between groups, which may have been the result of the slow escalation. On the other hand, anti-Xa levels and TEG parameters in this study demonstrated that post-injury patients did not receive adequate prophylaxis within the first week following the onset of injury. Therefore, escalation of LMWH dosing and anti-platelet therapy may occur more rapidly.

Additionally, doses of LMWH where held in both groups either secondary to procedures, or elevated R_F value greater than 1.4, which could have a role in why no difference was seen in coagulation parameters. Regarding procedures performed during the study period for each group, there was no statistical difference between groups, which was 1 (IQR 0:2) for the control group and 2 (IQR 1:2) for the TEG-guided group (Wilcoxon p=0.12).[11] With regards to R_F values, it is important to note that very few patients achieved a goal R_F value between 1 and 1.4. However, 1 patient in the control group, and 2 patients in the TEG-guided group achieved R_F values greater than 1.4, which resulted in holding a dose of heparin. This goal

was established based on retrospective studies, and it remains unclear if achieving an R_F value between 1.0 and 1.4 is efficacious or even safe. Also, since patients were not enrolled into the study until post-injury day 2 or 3, when hyperfibrinogenemia was already established (normal levels 200–400 mg/dL), the effects of LMWH on lower levels of fibrinogen are unknown. However, enrolling patients earlier may have affected patient safety with increased bleeding risks. Although Platelet Mapping was used to guide aspirin therapy, only 6 patients received aspirin late in the study period due to the slow escalation of VTE prophylaxis. Therefore, no conclusions could be drawn from these data. Therefore, subsequent clinical trials are required, and should evaluate a more rapid escalation of LMWH, and earlier initiation of antiplatelet therapy.

A limitation to note in the *in vitro* portion of the study is that the addition of fibrinogen concentrate to the blood of healthy volunteers may not have the same functional effects as hyperfibrinogenemia in the trauma patient. Also, it is difficult to correlate the concentrations of fibrinogen and heparin used in our *in vitro* studies to clinical scenarios. Both these concentrations were determined in pre-study experiments to detect measurable differences. Subsequently, higher doses of fibrinogen concentrate were needed to provide an adequate amount of functional fibrinogen, and lower doses of heparin, compared to what we use clinically, were adequate to provoke changes in the TEG tracings.

As post-injury hypercoagulability is further explored, thrombelastography continues to play a crucial role in elucidating mechanisms, and may ultimately prove to be valuable in guiding VTE prophylaxis. Despite current methods of prophylaxis, the incidence of VTE remains high following trauma due to multiple risk factors specific to this patient population. Therefore, the standard-of-care in other patient populations, may not directly translate to trauma patients, as evidenced by the historical lack of effect of both chemical and mechanical VTE prophylaxis. Multiple hypotheses have been proposed without strong evidence. However, the role of hyperfibrinogenemia, likely secondary to thrombocytosis, has been overlooked, which affects the bioavailability and activity of heparin. Basic studies seem intuitive, but have remained lacking, likely due to limitations in assays to sensitively measure hypercoagulability. Thrombelastography has now made this possible using whole blood. Although this study has several limitations, it is the first to characterize the role of hyperfibrinogenemia in post-injury hypercoagulability, and provide *in vitro* and clinical evidence demonstrating that hyperfibrinogenemia may compromise heparin-based VTE prophylaxis. Therefore, further basic research, and larger clinical trials with more aggressive LMWH and antiplatelet VTE prophylaxis regimens should be performed.

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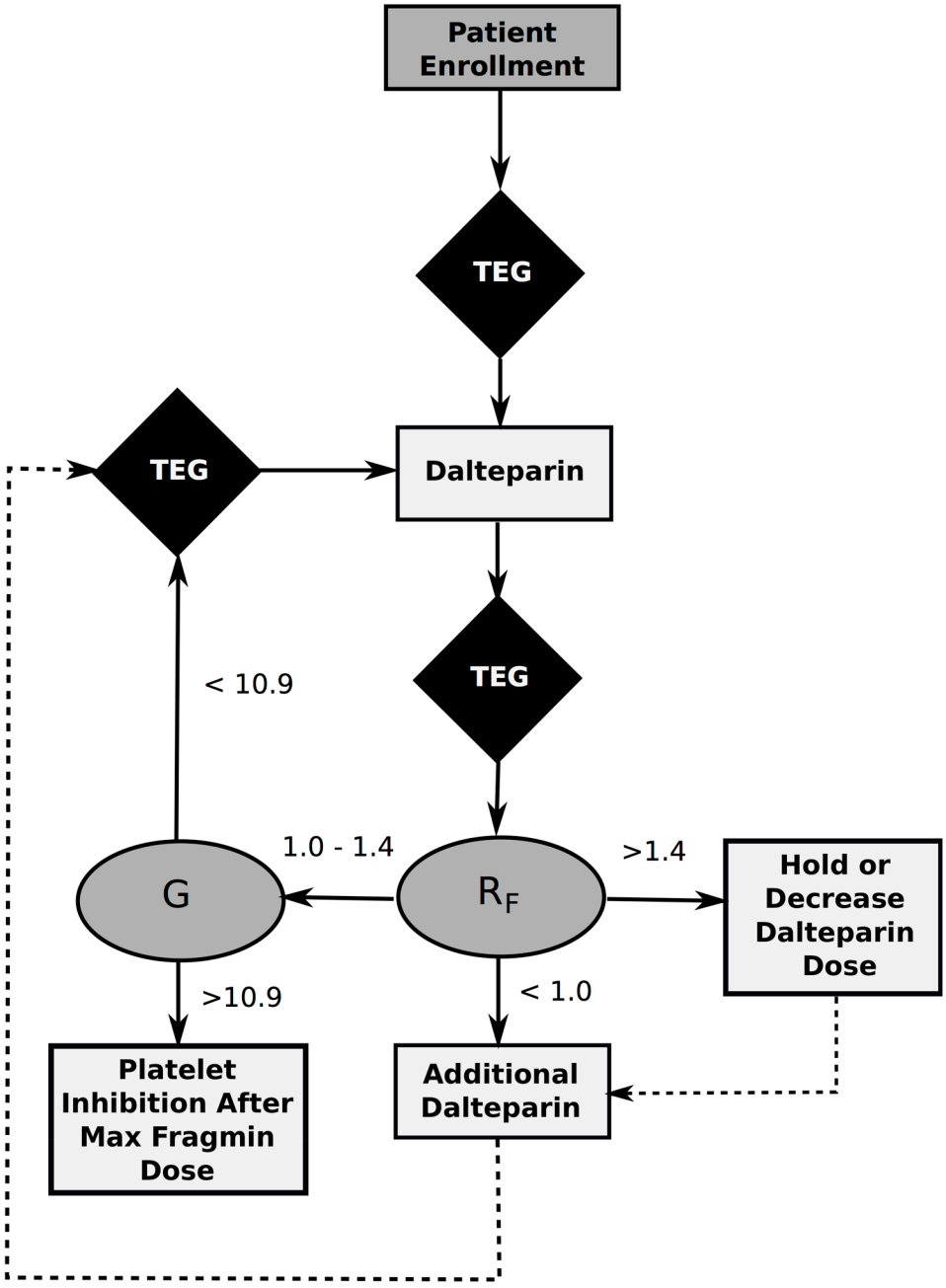

Figure 1.
VTE prophylaxis algorithm for patients randomized to the TEG-guided prophylaxis group.

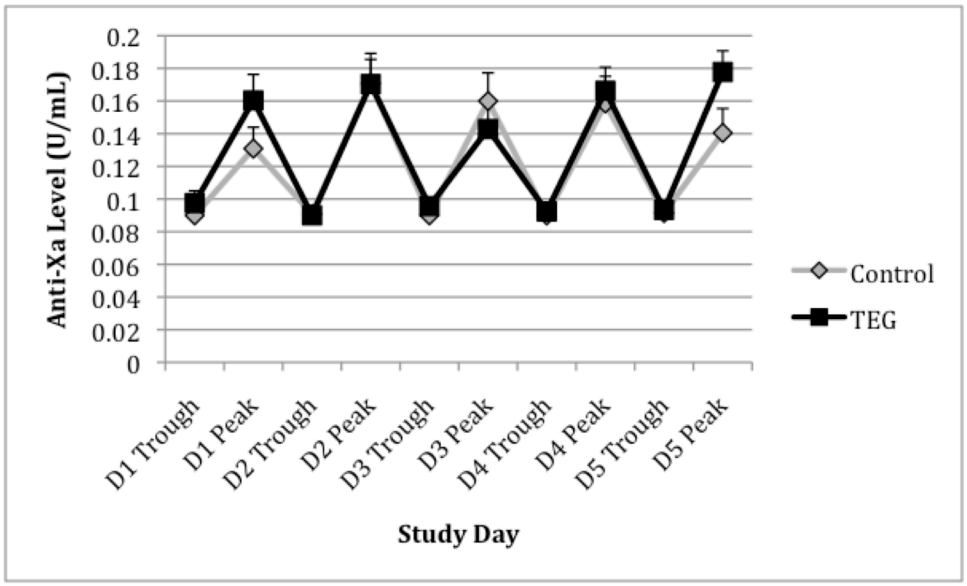

Figure 2.

Anti-Xa peak and trough values for each study day demonstrate that Anti-Xa levels increased following LMWH administration, but remained below recommended prophylaxis values (0.2–0.4 IU/ml) for both the control and TEG-guided prophylaxis groups.

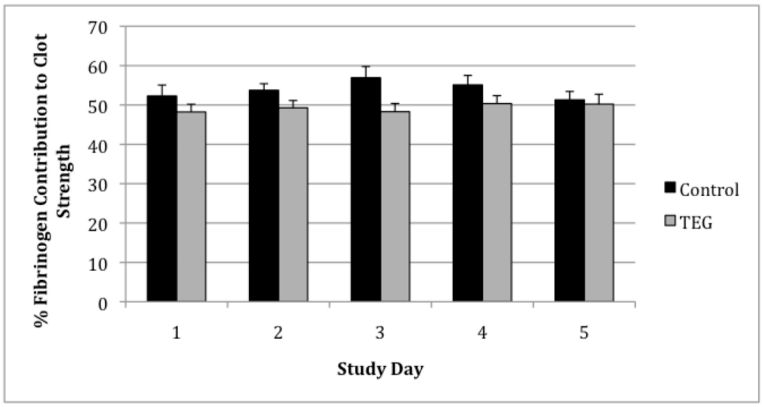

Figure 3.

Fibrinogen contribution of clot strength between control and TEG-guided prophylaxis groups. There is a trend toward a decreased fibrinogen contribution to clot strength in the TEG-guided prophylaxis group on study days 2 through 4, suggesting that additional LMWH may decrease the fibrinogen contribution to clot strength. The overall difference between groups was not significant using repeated measures ANOVA.

Table 1

Mean TEG parameters of normal blood obtained from healthy volunteers, blood with addition of heparin, blood with addition of fibrinogen, and blood with addition of both heparin and fibrinogen. Heparin significantly increased R-time and K-time, and reduced α-angle, MA, G, and thrombus generation. Fibrinogen significantly decreased R-time and K-time, and increased α-angle, MA, G, and thrombus generation. The addition of fibrinogen to heparinized blood negated the anticoagulant effects of heparin.

	Normal (n=10)	Heparin (n=10)	Fibrinogen (n=10)	Heparin + Fibrinogen (n=10)
R-time (min)	8.51±0.69	15.53±1.48*	5.93±0.19*	9.57±0.52
K-time (min)	3.16±0.3	6.99±0.51*	2.22±0.16*	4.92±0.75
α-angle (degrees)	51.74±2.1	30.27±1.78*	58.01±2.18*	41.03±3.14*
MA (mm)	60.48±1.43	52.79±1.5*	67.53±1.19*	61.39±1.29
G (dynes/cm^2)	7.81±0.45	5.68±0.32*	10.58±0.52*	8.08±0.41
Thrombus Generation (mm/min)	736.2±18.1	651.7±18.4*	825.9±14.7*	736.2±19.2

*$p < 0.05$ compared to normal blood.

Table 2

Baseline demographics and coagulation parameters

	Control (n=25)	TEG (n=25)	p-value
Demographics			
Male gender, N (%)	19 (76%)	17 (68%)	0.4*
Age (years), Mean (SEM)	40 (2.44)	38 (2.87)	0.67
BMI (kg/m^2), Mean (SEM)	29.23 (1.30)	28.37 (1.39)	0.65
Injury Severity			
Injury Severity Score, Median (IQR)	27 (16–36)	27 (19–33)	0.92#
APACHE, Median (IQR)	20 (17–26)	23 (17–26)	0.99#
Lactate (mmol/L), Mean (SEM)	3.59 (0.43)	3.97 (0.32)	0.47
Base Deficit (mEq/L), Mean (SEM)	7.336 (0.77)	8.04 (0.76)	0.52
Hemoglobin (g/dL), Mean (SEM)	9.00 (0.33)	8.53 (0.25)	0.26
Coagulation Parameters			
Platelet Count (10^9/L), Mean (SEM)	139.16 (9.78)	120.32 (8.20)	0.15
Fibrinogen (mg/dL), Mean (SEM)	636.00 (39.60)	562.64 (27.18)	0.13
Antithrombin III (%), Mean (SEM)	72.45 (3.53)	62.60 (3.64)	0.06
aPTT (sec), Mean (SEM)	34.17 (1.65)	35.53 (1.09)	0.5
INR, Mean (SEM)	1.35 (0.07)	1.34 (0.03)	0.91
Anti-Xa, Mean (SEM)	0.09 (0.00)	0.09 (0.01)	0.33
Kaolin TEG			
SP (min), Mean (SEM)	4.63 (0.35)	4.13 (0.44)	0.38
R (min), Mean (SEM)	5.31 (0.32)	5.26 (0.31)	0.9
K (min), Mean (SEM)	1.43 (0.06)	1.51 (0.08)	0.41
Angle (degrees), Mean (SEM)	68.54 (1.06)	66.40 (1.82)	0.31
MA (mm), Mean (SEM)	65.84 (1.35)	64.37 (1.10)	0.4
G (dynes/cm^2), Mean (SEM)	10.26 (0.76)	9.36 (0.49)	0.32
LY30 (%), Mean (SEM)	0.01 (0.00)	0.01 (0.00)	0.76

*X^2 test

#Wilcoxon nonparametric test

All other *p* values were from *t* test for continuous variables

APACHE Acute Physiology and Chronic Health Evaluation; SP, split point; R, reaction time; K, kinetic time; MA, maximum amplitude; G, clot strength; LY30, lysis 30 minutes following MA.

Table 3

Number of patients in each group receiving low-molecular weight heparin (LMWH) and aspirin (ASA), the dose given, and reason for holding doses if indicated.

	Day 1	Day 2	Day 3	Day 4	Day 5
Control (n=25)					
LMWH Dose-1					
5000 Units	25 (100%)	23 (92%)	21 (84%)	21 (84%)	23 (92%)
Dose Held for $R_F > 1.4$	0 (0%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)
Dose Held Procedure	0 (0%)	1 (4%)	4 (16%)	3 (12%)	2 (8%)
TEG-Guided (n=25)					
LMWH Dose 1					
5000 Units	25 (100%)	24 (96%)	22 (88%)	19 (76%)	17 (68%)
LMWH Dose 2					
2500 Units	2 (8%)	12 (48%)	4 (16%)	2 (8%)	1 (4%)
5000 Units	0 (0%)	0 (0%)	0 (0%)	15 (60%)	13 (52%)
ASA					
81 mg	0 (0%)	0 (0%)	0 (0%)	2 (8%)	5 (20%)
162 mg	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)
325 mg	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Dose Held for $R_F > 1.4$	0 (0%)	1 (4%)	2 (8%)	0 (0%)	2 (8%)
Dose Held Procedure	0 (0%)	0 (0%)	1 (4%)	6 (24%)	6 (24%)

Shock. Author manuscript; available in PMC 2015 January 01.

Table 4

Mean and standard error of the mean (SEM) for R-time, G, fibrinogen level, platelet count, activated partial thromboplastin time (aPTT), international normalized ratio (INR), and antithrombin III (ATIII) levels of the control and TEG-guided groups for each study day. There was no statistical difference between groups.

	Day 1	Day 2	Day 3	Day 4	Day 5
Control (n=25)					
R-time (min)	5.32±0.32	6.08±0.52	4.96±0.21	4.70±0.29	5.03±0.26
G (dynes/cm^2)	10.26±0.76	11.29±0.76	12.25±0.91	13.83±0.85	14.72±0.87
Fibrinogen (mg/dL)	636±137	671±117	715±120	716±159	691±147
Platelets (1000/μL)	139±49	154±83	183±135	216±185	262±172
aPTT (sec)	34.18±1.69	34.03±1.38	32.15±1.12	32.89±0.83	32.27±1.40
INR	1.35±0.07	1.28±0.05	1.22±0.04	1.23±0.04	1.23±0.04
ATIII (%)	72.45±3.53	77.17±3.69	81.90±5.17	78.40±5.52	79.30±4.31
TEG-Guided (n=25)					
R-time (min)	5.26±0.31	5.75±0.34	4.98±0.23	5.31±0.43	5.68±0.55
G (dynes/cm^2)	9.36±0.49	10.47±0.48	12.35±0.64	14.16±0.85	14.17±0.74
Fibrinogen (mg/dL)	563±102	610±136	624±154	652±161	687±158
Platelets (1000/μL)	120±41	128±35	148±54	199±75	268±83
aPTT (sec)	35.54±1.05	35.83±1.01	33.97±1.09	32.84±1.07	33.82±1.20
INR	1.35±0.03	1.75±0.48	1.20±0.03	1.16±0.04	1.13±0.02
ATIII (%)	62.61±3.63	66.00±4.34	75.14±5.07	80.70±4.99	80.00±4.31

Table 5

Correlation coefficients comparing the association of fibrinogen levels to clot strength and R_F. Fibrinogen has a significant correlation to clot strength on each study day. Moreover, fibrinogen has an overall trend toward an inverse correlation to R_F, which is significant on study days 1 and 3, and approached significance on study day 5.

	Correlation Coefficient	95% Confidence Limits	p-value
Clot Strength (G)			
Day 1	0.68	(0.38 to 0.84)	<0.0001
Day 2	0.66	(0.44 to 0.79)	<0.0001
Day 3	0.43	(0.13 to 0.64)	0.0052
Day 4	0.52	(0.23 to 0.72)	0.0007
Day 5	0.42	(0.11 to 0.65)	0.0079
R_F (min)			
Day 1	−0.54595	(−0.77 to −0.19)	0.0033
Day 2	0.07344	(−0.23 to 0.36)	0.6376
Day 3	−0.38775	(−0.62 to −0.08)	0.0128
Day 4	−0.09852	(−0.41 to 0.23)	0.5644
Day 5	−0.30651	(−0.57 to 0.02)	0.0648