Correlation between Thrombin Generation, Standard Coagulation Assays, and Viscoelastic Assays for Hemostatic Assessment in Critically Ill Children

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Background: Accurate assessment of hemostatic function is essential to guide care in critically ill children with acute and acquired coagulopathies. Thrombin generation (TG) provides a global assessment of procoagulant and anticoagulant factors and is commonly used in hemostasis research laboratories. Our objective was to determine the correlation of clinically available hemostasis assays with TG in critically ill children.

Methods: Children (<18 years old, >3 kg in weight) in the intensive care unit were enrolled from March 2016 to December 2019 in a prospective 2-center study. Coagulation tests were prothrombin time, activated thromboplastin time, anti-Xa assay, viscoelastic assays (thromboelastography [TEG], rotational thromboelastometry [ROTEM]), and TG (induced by 20 pM tissue factor in platelet poor plasma and reported as endogenous thrombin potential [ETP; nM*min]). Data are reported as median (interquartile range) or Spearman coefficient (ρ).

Results: Patients (n = 106, age 10.2 years [3.8–15.3]) were divided into 3 groups: (a) no anticoagulation (n = 46), (b) anticoagulation (unfractionated heparin) without extracorporeal life support (n = 34), or (c) with extracorporeal life support (n = 26). ETP was decreased in anticoagulated compared to non-anticoagulated patients (group 1: 902.4 [560.8–1234], group 2: 315.6 [0.0–962.2], group 3: 258.5 [0.0–716.6]; $P < 0.0001$). Across all patients, ETP correlated best with TEG kinetic time (TEG-K), in min ($\rho = -0.639$), followed by TEG reaction time, in min ($\rho = -0.596$). By group, ETP correlated best with international normalized ratio for group 1 ($\rho = -0.469$), TEG-K time for group 2 ($\rho = -0.640$), and anti-Xa for group 3 ($\rho = -0.793$).

Conclusions: Standard and viscoelastic assays have varying correlation with TG in critically ill children. TEG-K time had the most consistent moderate correlation with ETP across all groups.

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IMPACT STATEMENT

Thrombin generation may provide global hemostatic assessment in critically ill children with acute and acquired coagulopathies. The goal of this study was to compare and correlate thrombin generation with standard coagulation and viscoelastic assays used for hemostatic monitoring in critically ill children. Results demonstrated standard and viscoelastic assays had varying correlation with TG. Thromboelastography had the most consistent correlation with thrombin generation, as compared to the rest of the monitoring assays used. These data are useful for improving goal-directed anticoagulation in critically ill children and for informing future trial design focused on multimodel hemostatic monitoring in critically ill populations.

INTRODUCTION

Acquired coagulation abnormalities are frequent and associated with adverse outcomes in critically ill children (1). A myriad of clinical conditions with diverse clinicopathological processes, including trauma, sepsis, surgery, and extracorporeal life support (ECLS), can alter single or multiple components of the hemostatic system in children (2–4). These hemostatic alterations can disturb the equilibrium of a balance between the pro- and anticoagulant milieu in the patient, increasing the risk of bleeding and thrombotic complications (1, 5–7). A prompt and accurate assessment of hemostatic function is essential to determine the phenotype, to estimate the risk of bleeding or thrombosis, and to guide hemostatic therapies to minimize bleeding and thrombotic complications in critically ill children.

Traditionally, prothrombin time (PT) and activated partial thromboplastin time (aPTT) are the most commonly ordered assays for hemostasis monitoring. PT and aPTT measure the functionality of coagulation proteins of the hemostatic system and provide information on the initial 5% of thrombin generation (TG) (8). Abnormal PT or aPTT values reflect a decrease in the quantity or quality of one or more of the coagulation proteins in the extrinsic and intrinsic coagulation system, respectively, involved in initial TG (9). PT and aPTT do not assess alterations in cellular and fibrinolytic components of the hemostatic system. While neither of these assays were designed to estimate TG potential or the effect of heparin on TG potential (10), aPTT has historically and predominantly been used for monitoring unfractionated heparin anticoagulation (11). However, anti-Xa assays measure the inhibition of activated factor X by either unfractionated or low molecular weight heparin, providing a more direct measure of the heparin effect and sensitivity to both heparin concentration and antithrombin activity. Anti-Xa levels have been shown to correlate better with heparin dosing in critically ill children on ECLS or those with deep venous thrombosis (12). Of note, anti-Xa assays do not provide information on TG potential, clot initiation, and clotting formation potential, as they specifically reflect heparin inhibition of factor Xa. Recently, viscoelastic hemostasis assays including thromboelastography (TEG®) and rotational thromboelastometry (ROTEM®) have been increasingly used to assess hemostatic function in critically ill children (13, 14). TEG and ROTEM are global whole blood (WB) based functional assays that measure the time to initial fibrin formation, dynamics of clot formation (fibrin polymerization), clot firmness (platelet and fibrin mesh), and clot lysis (fibrinolysis). Viscoelastic assays have most commonly been used to guide hemostatic transfusions and antifibrinolytic

therapy in critically ill children with trauma or those needing cardiopulmonary bypass or major surgical procedures such as liver transplant (15–17).

The capacity of TG and enzymatic effects of thrombin produced reflect a composite effect of multiple components of hemostasis on blood coagulability (18). TG assays evaluate both TG and its decay over time, thus providing assessment of balance between procoagulant and anticoagulant factors. TG assays have the potential to improve hemostatic monitoring of acquired coagulation disorders in critically ill children (19). TG assays report the endogenous thrombin potential (ETP), which accounts for both the duration of TG and the maximum amount of thrombin generated, thus providing a metric reflective of total TG capacity. Ideally TG should be measured in samples including cells [e.g., WB or platelet-rich plasma] (20). However, the logistics of sample processing, handling, and batching both for clinical assays and research assays can preclude use of cell-containing samples. Platelet-poor plasma (PPP) samples provide a logistically easier sample to assay via TG, and the use of PPP has been validated against TG measurement on WB and platelet-rich plasma samples (20, 21).

The correlation of clinically available hemostasis assays with TG is important to ascertain, especially in critically ill children. Assays that are highly correlated with TG could indicate assays that are able to more accurately reflect global hemostatic function related to TG capacity. More accurate assessment of hemostasis would aid in improving the indication for hemostatic transfusion and goal-directed anticoagulation strategies. In this prospective observational study, intended to inform larger multicenter studies, we evaluated the correlations between TG potential in PPP with clinically used coagulation assays, including PT, aPTT, anti-Xa assays, and viscoelastic assays in critically ill children admitted to the intensive care units (ICUs). We also evaluated these correlations in distinct patient populations to determine whether the use and degree of anticoagulation affected our results.

STUDY DESIGN AND METHODS

Patient Selection and Enrollment

This study was a pilot prospective observational study to analyze TG in pediatric patients monitored by clinically available laboratory measures of hemostasis in ICUs. Patient recruitment and enrollment (target sample size $\geq$ 100 patients) occurred at both Washington University in St. Louis and Nationwide Children's Hospital between March 2016 and December 2019. To reduce bias, any patient in the pediatric ICU, the cardiac ICU, or the cardiac progressive care unit who required clinically indicated hemostasis monitoring was eligible for enrollment. Patients > 18 years old or with a weight of $\leq$3 kg were excluded, and those patients who were enrolled were only enrolled once in the study. Our enrolled patient population resulted in 3 distinct clinical groups: critically ill children with no anticoagulation (group 1; n = 46), anticoagulation (unfractionated heparin) without ECLS (group 2; n = 34), and anticoagulation (unfractionated heparin) for ECLS (group 3; n = 26). In group 3, there were 46% (12/26) on venoarterial and 54% (14/26) on veno-venous ECLS at the time of enrollment in the study. The study was performed in accordance with the Washington University in St. Louis Institutional Review Board (protocol no. 201602005) and Nationwide Children's Hospital Institutional Review Board (protocol no. IRB16-01079), as well as in accordance with the Helsinki Declaration.

Clinical Data Collection

Demographic data collected included age, sex, race, diagnosis (e.g., general medical, cardiac medical, trauma, general surgical, cardiac surgical), current medications, and severity of illness measures

(Pediatric Risk of Mortality III and Pediatric Logistic Organ Dysfunction 2 scores). Clinical variables collected included unfractionated heparin dose (IU/kg/h) at the time of the blood sample collection, length of mechanical ventilation, length of stay in an ICU, presence and duration of indwelling catheter, use and type of extracorporeal device, Pediatric Risk of Mortality III score at time of enrollment, highest Pediatric Logistic Organ Dysfunction 2 score within 7 days prior to enrollment, and mortality. Clinical lab assay results that were simultaneously collected and extracted from the electronic medical record included aPTT (sec), PT (sec), international normalized ratio (INR), anti-Xa level (IU/mL), and TEG with kaolin assay (reaction time [R; min], kinetic time [K; min], alpha angle [°], maximum amplitude [mm], G value [kilodynes/cm^2], and clot lysis at 30 min [%]).

Research Lab Assays

Samples for research lab assays were collected at the same time as samples collected for clinically ordered lab assays to compare tests from the same time in a patient's clinical course. For research lab assays, blood was collected into 1.8-mL vacutainers containing 3.2% sodium citrate, immediately transported to the lab space, and processed and assayed within 1 h of collection. First, WB was used in ROTEM extrinsic (tissue factor [TF]) thromboelastometry (EXTEM) assays (Instrumentation Laboratory Worldwide) according to manufacturer's protocol. The following data were collected: clotting time (sec), clot formation time (sec), alpha angle (°), and maximum clot firmness (mm).

The remaining WB was centrifuged at 2500 *g* for 10 min at room temperature, and then the upper fraction was transferred to a new tube and spun again at 10 000 *g* for 10 min at room temperature. The upper fraction (PPP) was carefully removed, aliquoted, and frozen at −80°C until batched analysis for TG. TG was measured using a calibrated automated thrombogram (Diagnostica Stago Inc.) according to manufacturer's protocol. In brief, frozen PPP aliquots were thawed at 37°C for 10 min, then 80 µL of sample was added to 20 µL of reconstituted PPP-low reagent (1 pM TF) or PPP-high reagent (20 pM TF) and mixed with FluCa buffer and substrate, and TG was measured every 20 sec for 60 min (i.e., fluorescence [390 nm excitation, 460 nm emission]). The fluorescent kinetic curve generated, called a thrombogram (Fig. 1), allows for calculation and extraction for the following parameters: time to initial thrombin formation (lag time; min), maximal TG (peak; nM); time to maximal thrombin formation (time to peak; min), and the area under the curve (ETP; nM*min). The ETP value is representative of the global TG capacity of the plasma sample, as it measures TG in the presence of plasma-based inhibitors.

Statistical Analyses

As the patients in these cohorts were critically ill children, maximum sample volumes required for both clinical and laboratory testing were not always available. This resulted in some patients not having enough sample to perform all of the clinical tests or research lab assays, and differences in clinical tests being ordered and performed may be due to clinical discretion. Analyses were performed using data available for the given parameter; the number of patients included in each analysis is listed in Tables 1 to 3. Pearson chi-square tests were used to compare categorical variables between the 3 groups. Kruskal–Wallis tests were used to compare continuous variables between the 3 groups. For correlations between coagulation test parameters, Spearman correlation coefficients (ρ values) with 95% CIs were obtained. Box-and-whisker plots are graphed as median (interquartile range) with 5% to 95% error bars. *P*-values < 0.05 were considered significant. Heat maps were generated in GraphPad using Spearman's correlation coefficients. All analyses were performed using GraphPad Prism software (v 8.2.0, GraphPad Software) and SPSS (v27, IBM).

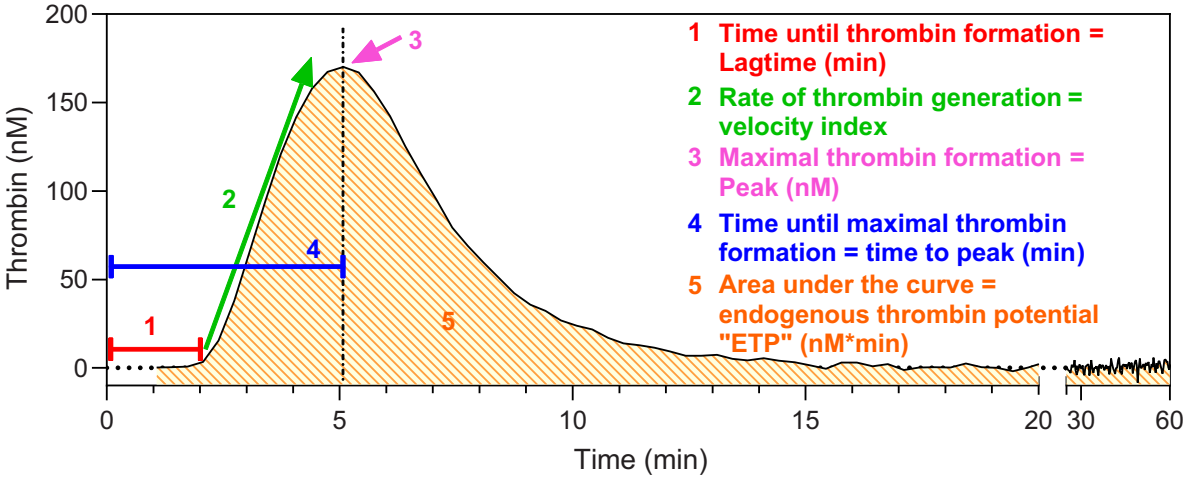

Fig. 1. Anatomy of a thrombogram. Terms used to describe data in a thrombogram, which is the curve derived from a TG assay.

RESULTS

We found no differences in age, sex, or race between the 3 study groups (Table 1). However, we did find significant differences in measures of severity of illness (Pediatric Risk of Mortality III and Pediatric Logistic Organ Dysfunction 2 scores), as well as length of ICU stay and ICU mortality (Table 1). Specifically, anticoagulated patients on ECLS had more severe illness and increased mortality.

Next, we assessed the hemostatic profiles between the study groups based on standard coagulation assays, viscoelastic assays, and the TG assay. We found no differences in PT, INR, and ROTEM EXTEM measures (Table 2). However, anticoagulated ECLS patients (group 3) had significantly longer kaolin TEG-R and TEG-K times, significantly reduced alpha angle values, and no lysis at 30 min when compared to groups 1 and 2 (Table 2). We found significant reduction in TG potential in anticoagulated patients (both groups 2 and 3) compared to non-anticoagulated patients (group 1) when using both low (1 pM) and high (20 pM) concentrations of TF (Fig. 2).

There was significant variability in the degree of correlation between ETP (20 pM TF) and clinically available hemostatic assays (Table 3, Fig. 3; also see Supplemental Figs. 1–3). For the entire cohort, ETP correlated best with TEG-K ($\rho = -0.639$), followed by TEG reaction time ($\rho = -0.596$). In anticoagulated patients without ECLS (group 2), aPTT, kaolin TEG-R, TEG-K time, and anti-Xa were moderately inversely correlated with ETP. In anticoagulated patients on ECLS (group 3), anti-Xa levels had moderate correlation with ETP (Table 3). ETP correlated best with INR for group 1 ($\rho = -0.469$), TEG-K for group 2 ($\rho = -0.640$), and anti-Xa for group 3 ($\rho = -0.793$).

DISCUSSION

In this study, we evaluated TG via ETP in a cohort of critically ill children. Our patient population comprised 3 distinct groups: critically ill

Table 1. Comparison of demographic and clinical characteristics of the 3 clinical groups of critically ill children.

Variables	Group 1 (n = 46)	Group 2 (n = 34)	Group 3 (n = 26)	*P* value
Age, years	10.6 (6.4–15.8)	7.6 (2.7–16.2)	9.9 (4.0–13.1)	0.625
Female	28 (60.9)	15 (44.1)	15 (57.7)	0.311
Race				0.227
Caucasian	38 (82.6)	32 (94.1)	19 (73.1)	
African American	6 (13.0)	1 (2.9)	3 (11.5)	
Asian	0 (0.0)	0 (0.0)	0 (0.0)	
North American Indian/Alaskan Native	0 (0.0)	0 (0.0)	2 (7.7)	
Native Hawaiian/Other Pacific Islander	0 (0.0)	0 (0.0)	0 (0.0)	
Other	1 (2.2)	1 (2.9)	1 (3.8)	
Unknown	1 (2.2)	0 (0.0)	1 (3.8)	
Admission category				0.091
General medical	30 (65.2)	17 (50.0)	21 (80.7)	
Cardiac medical	6 (13.0)	9 (26.5)	2 (7.7)	
Trauma	3 (65.2)	1 (2.9)	0 (0.0)	
General surgery	7 (15.2)	6 (17.6)	1 (3.8)	
Cardiac surgery	0 (0.0)	1 (2.9)	2 (7.7)	
Heparin, units/kg/h	—	19.5 (14.6–22.0)	20.0 (14.0–24.3)	0.686[a]
PRISM III	5 (2–8)	6 (3–9)	12 (9–16)	<0.0001
PELOD-2	4 (2–7)	4 (2–7)	8 (7–10)	<0.0001
ICU LOS, days	8.0 (4.0–19.3)	12.5 (5.8–36.8)	31.0 (16.8–67.0)	0.0002
ICU mortality	4 (8.7)	5 (14.7)	10 (38.5)	0.006

Categorical variables are represented as n (%), and continuous variables are represented as median (interquartile range). Group 1—children without anticoagulation; group 2—children with anticoagulation (unfractionated heparin) but no ECLS; group 3—children with both anticoagulation (unfractionated heparin) and ECLS. PRISM III, Pediatric Risk of Mortality III; PELOD-2, Pediatric Logistic Organ Dysfunction Score 2; LOS, length of stay.
[a]The comparison was made only between groups 2 and 3 (Mann–Whitney test).

children with no anticoagulation (group 1), anticoagulation without ECLS (group 2), or anticoagulation with ECLS (group 3). We found ETP was decreased as expected among anticoagulated patients (groups 2 and 3) compared to non-anticoagulated patients (group 1) in critically ill children. A higher concentration of agonist (20 pM TF) instead of the routine dose (1–5 pM TF) was needed to elicit endogenous TG in children with systemic anticoagulation with or without ECLS. On correlation of ETP with a battery of clinically used standard (plasma-based) coagulation assays and viscoelastic (WB-based) hemostasis assays, we found ETP had varying correlation with standard coagulation assays and viscoelastic hemostasis assays in our cohort.

Some variation was expected based on the use of heparin and assays that do not directly assess inhibition of the intrinsic pathway, for example, INR and ROTEM EXTEM. Kaolin TEG-K was the parameter that had the most consistent moderate correlation with ETP across all groups analyzed ($\rho = -0.639$ all groups combined, $\rho = -0.423$ for group 1, $\rho = -0.640$ for group 2, and $\rho = -0.599$

Table 2. Comparison of hemostatic assays and direct TG among the 3 groups.

Variable	n	Group 1	n	Group 2	n	Group 3	*P* value
Conventional assays							
aPTT, sec	44	35.5 (29.8, 45.5)	31	58.0 (43.3, 45.5)	24	55.9 (47.3, 79.8)	<0.0001
PT, sec	44	15.8 (14.5, 21.5)	18	15.8 (14.7, 16.7)	21	14.8 (13.6, 16.8)	0.160
INR	44	1.21 (1.09, 1.79)	18	1.23 (1.10, 1.28)	21	1.13 (1.01, 1.32)	0.235
Antithrombin, %	36	88 (72, 106)	19	79 (67, 87)	20	67 (50, 94)	0.037
Anti-Xa, IU/mL	—	—	22	0.10 (0.08, 0.40)	23	0.20 (0.10, 0.30)	0.441[a]
TEG kaolin							
R, min	43	4.3 (3.4, 5.8)	31	8.0 (5.1, 14.2)	24	11.4 (6.8, 21.8)	<0.0001
K, min	41	1.4 (0.9, 2.2)	31	2.5 (1.3, 4.8)	22	2.9 (1.8, 6.9)	<0.0001
Angle, °	41	69.2 (60.0, 73.6)	31	52.8 (39.1, 69.5)	22	45.6 (28.1, 63.5)	<0.0001
MA, mm	41	60.1 (53.3, 69.8)	31	56.9 (49.2, 65.3)	23	57.0 (45.1, 62.9)	0.226
G, kilodynes/cm^2	41	7.5 (5.7, 11.6)	31	6.6 (4.8, 9.4)	23	6.6 (4.1, 8.5)	0.214
Lysis at 30 min, %	39	0.3 (0.0, 1.7)	26	0.8 (0.0, 2.1)	16	0.0 (0.0, 0.0)	0.026
EXTEM							
CT, sec	44	80 (72, 95)	25	87 (75, 103)	21	90 (66, 105)	0.894
CFT, sec	44	91 (67, 147)	25	81 (57, 145)	21	110 (86, 132)	0.208
Alpha, °	44	74 (68, 77)	25	74 (63, 79)	21	72 (68, 77)	0.731
A10, mm	44	54 (43, 61)	25	54 (42, 65)	21	49 (46, 56)	0.637
A20, mm	44	60 (51, 65)	25	60 (49, 70)	21	56 (53, 63)	0.771
MCF, mm	44	61 (53, 65)	25	61 (50, 70)	20	59 (56, 64)	0.819
TG[b]							
Lag time, min	43	2.00 (1.67, 2.89)	33	1.89 (0.00, 2.84)	26	2.41 (1.63, 3.87)	0.204
Peak, nM	40	227.6 (145.4, 288.6)	25	53.7 (0.20, 134.1)	25	12.3 (0.9, 69.8)	<0.0001
ttpeak, min	43	4.00 (3.33, 5.05)	33	4.33 (0.00, 8.12)	26	6.92 (4.00, 13.35)	0.031
ETP, nM*min	43	902.4 (560.8, 1234.0)	33	315.6 (0.0, 962.2)	26	258.5 (0.0, 716.6)	<0.0001

Group 1—children without anticoagulation; group 2—children with anticoagulation (unfractionated heparin) but no ECLS; group 3—children with both anticoagulation (unfractionated heparin) and ECLS. Data are represented as median (interquartile range). The Kruskal–Wallis test was used to compare the 3 groups. MA, maximum amplitude; CT, clotting time; CFT, clot formation time; A10, amplitude at 10 min; A20, amplitude at 20 min; MCF, maximum clot firmness; ttpeak, time to peak.

[a]The comparison was made only between groups 2 and 3 (Mann–Whitney test).

[b]Induced by 20 pM TF.

for group 3). TEG-R and TEG-K and ROTEM EXTEM clotting time and clot formation time both reflect time to initial fibrin formation and cross-linking of fibrin, respectively. However, the main difference between the 2 assays are that TEG uses kaolin and ROTEM EXTEM uses TF to accelerate clotting. Interestingly, in children who were not placed on anticoagulation, the TEG-R and TEG-K results (intrinsic activation) appear to have a numerically higher correlation with ETP (extrinsic activation) than the ROTEM assay clotting time and clot formation time results (extrinsic activation). The clinical relevance of this difference is unknown, but it does support the development of trials that compare the 2 assays to determine whether the differences noted affect outcomes when the assays are

Table 3. Correlation of endogenous TG potential with coagulation assays.

	ETP					
Variables	**n**	**Group 1**	**n**	**Group 2**	**n**	**Group 3**
aPTT	41	−0.264 (−0.535, 0.057)	35	−0.539 (−0.744, −0.242)	24	−0.300 (−0.635, 0.130)
INR	41	−0.469 (−0.684, −0.179)	23	−0.195 (−0.571, 0.248)	21	−0.028 (−0.465, 0.420)
TEG-R	40	−0.447 (−0.671, −0.148)	35	−0.567 (−0.761, −0.279)	24	−0.414 (-0.707, 9E-5)
TEG-K	38	−0.423 (−0.660, −0.110)	35	−0.660 (−0.818, −0.411)	22	−0.599 (−0.819, −0.225)
TEG-MA	38	0.230 (−0.106, 0.520)	35	0.558 (0.266, 0.756)	23	0.478 (0.069, 0.749)
EXTEM-CT	41	−0.226 (−0.506, 0.097)	29	−0.275 (−0.590, 0.113)	21	−0.310 (−0.662, 0.154)
EXTEM-CFT	41	−0.358 (−0.606, −0.047)	29	−0.419 (−0.687, −0.051)	21	0.032 (−0.416, 0.468)
EXTEM-MCF	41	0.390 (0.084, 0.628)	29	0.317 (−0.068, 0.619)	20	−0.162 (−0.573, 0.315)
Anti-Xa level	0	—	27	−0.425 (−0.699, −0.042)	23	−0.793 (−0.911, −0.557)

Data are listed as Spearman correlation coefficient (95% CIs). Group 1—children without anticoagulation; group 2—children with anticoagulation (unfractionated heparin) but no ECLS; group 3—children with both anticoagulation (unfractionated heparin) and ECLS. MA, maximum amplitude; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness.

used for direct transfusion or anticoagulation in critically ill children.

Clinicians often provide therapeutic interventions for thrombin function–associated hemostatic dysfunction, such as transfusions of plasma, titration of anticoagulation therapy, or hemostatic adjunct therapeutic agents (e.g., prothrombin complex concentrates) based on the assumption that standard coagulation assays reflect the underlying net TG in a patient. However, our results suggest that standard coagulation assays and viscoelastic assays may not accurately reflect ETP values in critically ill children. The study results were consistent with previously reported adult patient data. Al Dieri et al. reported a low correlation between aPTT and ETP in adult patients on heparin anticoagulation (22). Similarly, Herpers et al. reported that ETP was a more accurate measurement than INR in correcting vitamin K antagonist–induced coagulopathy in adult patients (23). Zekavat et al. reported a low correlation between TG and standard coagulation assays in patients with rare bleeding disorders and found that TG parameters were better predictors of bleeding events compared to standard coagulation assays in this patient population (9).

Hemostasis monitoring in critically ill children remains a challenge. Many factors determine the capacity of TG and resultant blood coagulability in a critically ill patient. No currently available in vitro assay is able to reflect the complex process of hemostasis in vivo in part due to the lack of incorporating flow and biologic surfaces. Consequently, currently used coagulation assays fail to consistently predict clinically relevant outcomes such as thrombosis or bleeding events in critically ill children, especially those needing ECLS (12, 24). Viscoelastic assays have shown some promise in evaluating and managing acquired coagulopathy, especially in postsurgical, liver failure, and sepsis- and trauma-related coagulopathy in critically ill children (17, 25, 26). As of yet, TG assays are not licensed for clinical use (27). Few studies have evaluated ETP in critically ill pediatric patients; those that have are in the clinical research areas of inflammatory bowel diseases (28, 29), leukemia (30), congenital coagulation disorders (31), post-cardiac surgery (32), and catheter-associated thrombosis (33). Many centers have started using

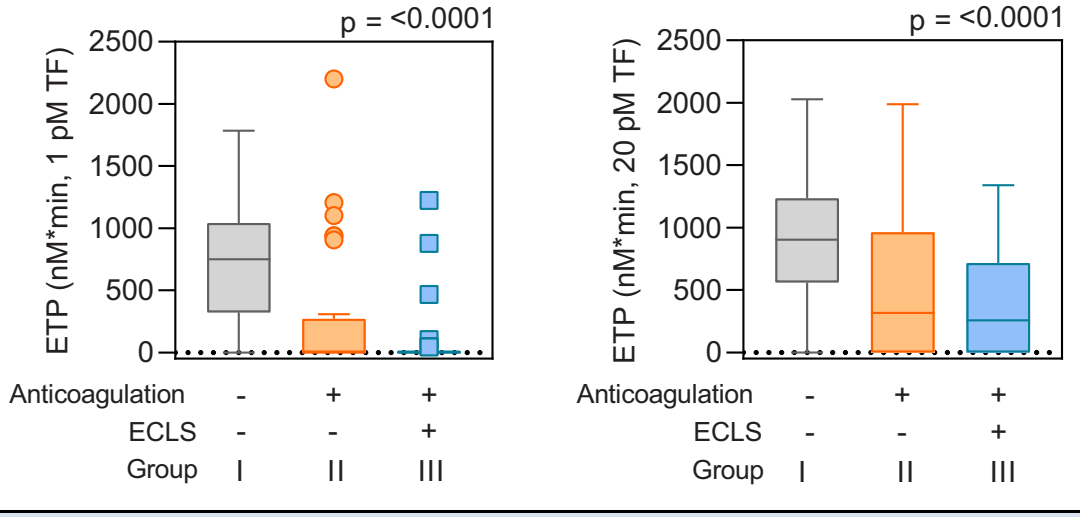

Fig. 2. Comparison of ETP between the 3 groups. ETP (nM*min) induced by 1 (left) or 20 pM (right) TF. Group 1—children without anticoagulation; group 2—children with anticoagulation (unfractionated heparin) but no ECLS; group 3—children with anticoagulation (unfractionated heparin) and ECLS. Data are displayed using box and whisker plots (Tukey representation) and compared via Kruskal–Wallis test.

a combination of coagulation assays for a more comprehensive evaluation of hemostatic function in critically ill children. Such an approach has become routine practice for critically ill children with severe trauma or ECLS (17, 24, 34). However, results of standard coagulation assays and viscoelastic assays can often be divergent. It is unclear which one of these assays most accurately represents endogenous TG in the setting of acquired coagulopathy. In this study, we have provided important correlation data between a TG assay (PPP-based), standard coagulation assays, and viscoelastic hemostasis assays, which may be useful in interpretation of hemostasis monitoring protocols and to guide development of goal-directed anticoagulation trials.

Our study has certain limitations, including the limited generalizability of our results due to our distinct dual-center heterogenous tertiary-care pediatric ICU cohort. We did not collect serial samples, limited by blood draw volume, to assess temporal changes in the correlation between various assays. We did not correlate coagulation assay values with the clinically relevant outcomes, including bleeding complications, clotting complications, and use of hemostatic transfusions. In addition, our institutions do not routinely check antithrombin levels prior to performing anti-Xa assays. Despite these limitations, our study results show that TEG values correlate more strongly with ETP compared to standard coagulation assays in critically ill children. Treating physicians should consider these correlations while interpreting results of coagulation assays.

Standard coagulation and viscoelastic hemostasis assays have varying correlation with TG in critically ill children. Compared to the standard coagulation assays, kaolin TEG values were more consistently correlated with TG in all distinct clinical subgroups. The next step is to determine whether PPP TG assays and WB-based TEG assays are better tools to help guide treatment and superior in discriminating patient outcomes, such as incidence of bleeding and/or clotting. This pilot study both demonstrates feasibility and provides

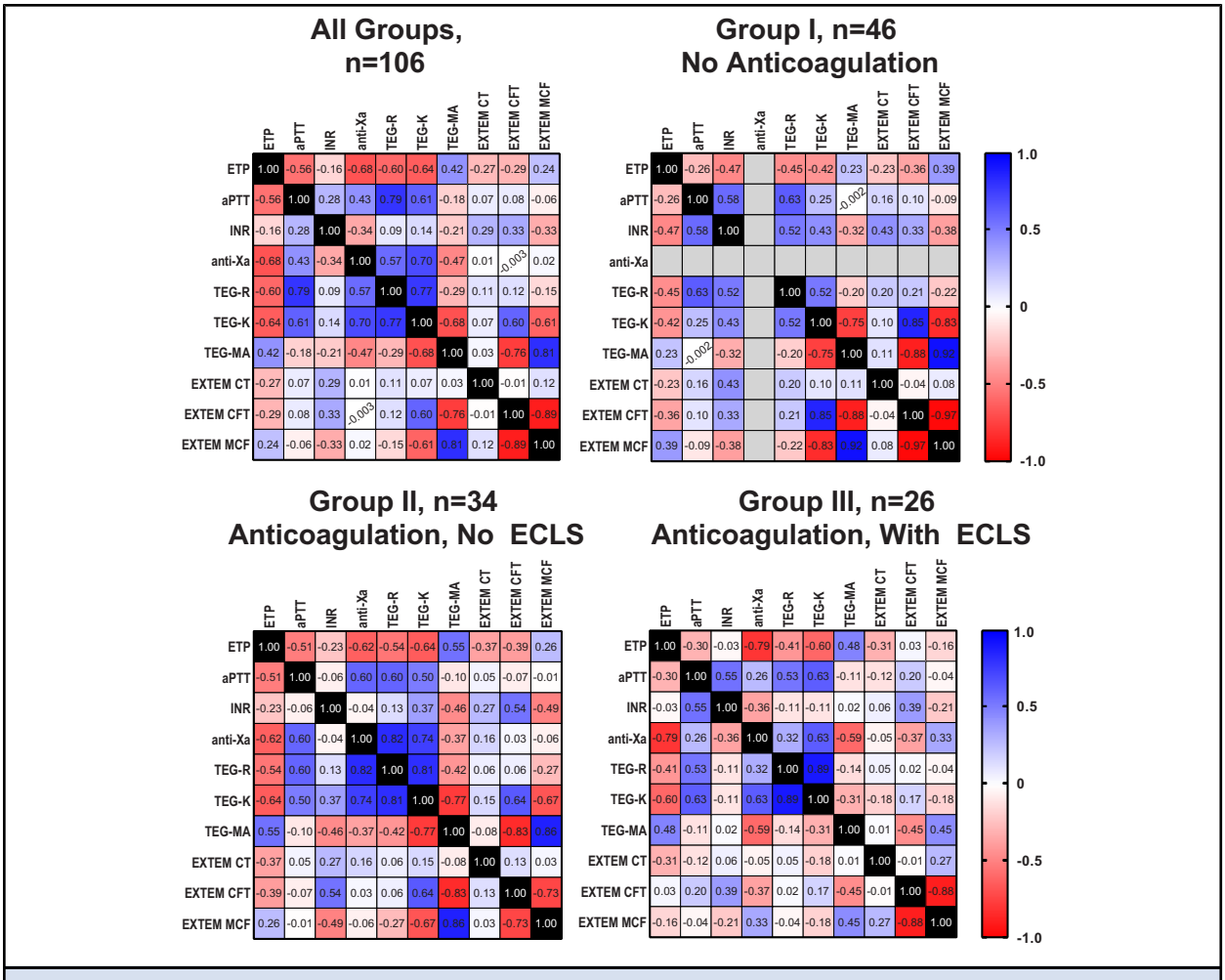

Fig. 3. Correlation of coagulation assays in all 3 patient groups. Spearman correlation coefficients (ρ values) between parameters visualized in matrix form by study group. Spearman correlation coefficients are listed in each box for each comparison. Blue ($\rho = 1.0$) indicates a strong positive correlation between parameters, while red ($\rho = -1.0$) indicates a strong negative correlation.

early data to help design larger multicenter studies to determine the clinical utility of the TG assay and its correlation with existing assays for monitoring hemostatic function.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

Nonstandard Abbreviations: ECLS, extracorporeal life support; PT, prothrombin time; TEG, thromboelastography; aPTT, activated partial thromboplastin time; TG, thrombin generation; ROTEM, rotational thromboelastometry; WB, whole blood; ETP, endogenous thrombin potential; PPP, platelet-poor plasma; ICU, intensive care unit; INR, international normalized ratio; R, reaction time; K, kinetic time; TF, tissue factor; EXTEM, extrinsic (tissue factor) thromboelastometry.

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for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

K.A. Thomas and S.M. Shea performed assays, analyzed data, and wrote the manuscript. A. Saini analyzed the data and wrote the manuscript. J.A. Muszynski helped design the study, edited the manuscript, provided additional funds for the study, and supervised study performance. P.C. Spinella designed, funded, supervised the study, and wrote the manuscript. All authors contributed to the final version of the manuscript.

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