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ORIGINAL RESEARCH

TRANSFUSION

Impact of systematic variations in hematocrit and platelet count on thrombelastometry tissue factor activated assay parameters

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Abstract

Background: Thromboelastogram testing is increasingly being used to manage patients with massive bleeding. An earlier study found that the test results were influenced by the hematocrit (Hct) and platelet (PLT) concentrations. This study sought to determine if these factors confounded the results of a different manufacturer's thromboelastography testing.

Methods: Using freshly collected whole blood from volunteers and stored red blood cells (RBC) and plasma, the whole blood was manipulated to achieve different Hct values and PLT concentrations. Each reconstituted whole blood sample was tested in triplicate on the ROTEM Delta device and the ExTEM results were recorded.

Results: Many of the ExTEM results varied according to the Hct and PLT concentration. In particular, the ExTEM clot formation time (CFT) was abnormally long when the Hct was 45% and the PLT concentration was $\leq 75 \times 10^9$/L, normalizing only when the PLT count was $\geq 100 \times 10^9$/L. CFT samples with Hct 25% and 35% were also abnormal with low PLT concentrations but normalized at lower PLT concentrations compared to the Hct 45% samples. The ExTEM angle also demonstrated abnormal results when the Hct was 45% and the PLT concentration was $\leq 50 \times 10^9$/L. The ExTEM A10 and maximum clot firmness (MCF) tests tended to also be abnormal when the Hct was between 25% and 45% and the platelet concentrations were below 75×10^9/L.

Conclusion: While thromboelastogram testing is gaining popularity for managing bleeding patients, clinicians should be aware of these confounding factors when making transfusion decisions based on their results.

KEYWORDS

EXTEM, hematocrit, platelet, ROTEM, testing, thrombelastometry

Abbreviations: A10, clot amplitude at 10 minutes; CFT, clot formation time; CT, clot time; ExTEM, tissue factor activated thrombelastometry; FFP, fresh frozen plasma; FibTEM, fibrinogen thrombelastometry; Hct, hematocrit; MCF, maximum clot firmness; PLT, platelet; RBC, red blood cells; SAGM, sodium chloride, adenine, glucose and mannitol.

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1 | INTRODUCTION

Thromboelastometry is a well-established method for assessing the hemostatic capacity of bleeding patients using whole blood samples, as well as guiding the treatment of patients undergoing massive transfusion.[1–7] In an earlier in vitro mixing study using a TEG 5000 device (Haemonetics, Braintree, MA, USA), successively increased clot strength parameters, such as maximum amplitude and angle, were associated with declining hematocrit (Hct) values and with increasing platelet (PLT) concentrations.[8] In this in vitro study, we aimed to investigate how different Hct levels and PLT concentrations in manipulated whole blood samples affected five different parameters of the ROTEM tissue factor activated (ExTEM) assay that examined the activity of both soluble clotting factors and the contribution of PLTs to the emerging clot.

2 | MATERIALS AND METHODS

The study was designed as a quality improvement project and only volunteer samples and anonymized blood products were used. Hence, according to Danish law an ethics board approval was not necessary. Five fresh 2.7 mL whole blood samples collected in buffered sodium citrate solution (BD Vacutainer® Plastic citrate tube, Buffered sodium citrate [0.109 M, 3.2%], Becton Dickinson, USA) were drawn from each of two healthy volunteers at one time on two separate days (one donor per day). These 10 samples were individually analyzed on a Sysmex XN-1000 Hematology Analyzer (Sysmex Europe, Norderstedt, Germany) immediately after collection to determine their Hct value and PLT concentration.

From the South Danish Transfusion Service Blood Bank at Odense University Hospital, two units of fresh frozen plasma (FFP), and one red blood cell (RBC) unit suspended in sodium chloride, adenine, glucose and mannitol medium (SAGM) were obtained from the transfusable inventory. All units were within their respective shelf lives.

The FFP units were thawed in a 37°C water bath according to the hospital transfusion medicine service's protocol and then made into 50 mL aliquots and centrifuged at high speed (4000× *g* for 10 min; ROTANTA 460 centrifuge, Andreas Hettich GmbH, Tutlingen, Germany) to cause the sedimentation of any residual platelets in the plasma unit. The platelet-depleted supernatant (platelet-depleted FFP) was pipetted into separate, clean containers. The PLT concentration of each of the platelet-reduced FFP aliquots was measured on the Sysmex XN-1000 to verify the complete depletion of PLTs. The RBC unit was made into 50 mL aliquots, then centrifuged using the ROTANTA 460 centrifuge (1800× *g* for 5 min) thus separating the RBCs from the suspension medium. The majority of the suspension medium was manually pipetted and discarded. The RBCs were washed twice; the RBCs were centrifuged and the resulting supernatant was manually removed by pipetting and discarded. Phosphate buffered saline (PBS) was added and the procedure was repeated. After the PBS wash, the supernatant was discarded and the RBCs were washed one last time using platelet-depleted FFP. The end product was a highly concentrated red cell suspension in platelet-depleted FFP. The Hct of all tubes containing highly concentrated RBCs in platelet-depleted FFP were measured using the Sysmex XN-1000.

Different volumes of concentrated RBCs, platelet-depleted FFP, and whole blood (as a source of fresh platelets) from the healthy volunteers were combined in clean plastic tubes. These components were then mixed following an algorithm to produce 21 samples of reconstituted whole blood at seven different PLT concentrations of 0, 15, 30, 50, 75, 100, and 175 × 10^9/L in combination with Hcts of 25%, 35%, and 45%. The target and median platelet concentrations from these seven samples are shown in Table 1. No other diluent was added to any of the reconstituted whole blood mixtures.

To determine the effect of the Hct on the ROTEM ExTEM assay under extreme clinical conditions, an additional three samples were made with a Hct of 0% and PLT concentrations of 15, 50, and 100 × 10^9/L by mixing various amounts of platelet-rich plasma with platelet-depleted FFP using the same algorithm as the reconstituted whole blood mixtures. For these experiments, platelet-rich plasma was created by centrifuging whole blood (drawn from one of the volunteers) at 1800× *g* for 5 min with the ROTANTA 460 centrifuge then decanting the platelet-rich plasma into an empty plastic container using a pipette. An overview of the 24 combinations of PLT concentration and Hct value are given in Table 2.

Before analyzing the reconstituted whole blood samples, the extent to which the ROTEM ExTEM parameters might be affected by delaying the testing after the samples were collected was determined. Three 2.7 mL whole blood samples in buffered sodium citrate solution were drawn from each of three healthy volunteers on three separate days (one volunteer per day). Immediately after sampling, the ROTEM ExTEM (ROTEM Delta, Werfen, GMBH, Munich, Germany) assay was run in triplicate for a minimum of 30 min. The remaining blood was stored at room temperature and ExTEM analysis was repeated at 2, 4, and 6 h post-collection. All the ExTEM parameters were within the normal ranges for samples that were analyzed immediately after procurement, except for 3/27

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TABLE 1 Target and median platelet concentrations for all seven different platelet concentrations tested at various Hct values in this study.

Sample #	1	2	3	4	5	6	7
	Platelet concentration						
Target	0	15	30	50	75	100	175
Median	2	17	28	48	72	100	180
Range	2–3	16–21	28–31	47–56	71–73	94–106	173–181

TABLE 2 ROTEM tissue factor activated clot (ExTEM) parameters (unit) according to hematocrit.

PLT concentration ($\times 10^9$/L)									
ExTEM parameters		Hct	0	15	30	50	75	100	175
CT (s)	<79	0	-	58	-	63	-	61	-
CFT (s)	<159		-	81	-	61	-	42	-
Angle (°)	>63		-	83	-	82	-	83	-
A10 (mm)	>43		-	36	-	47	-	57	-
MCF (mm)	>50		-	39	-	49	-	59	-
CT (s)	<79	25	48	49	52	48	52	51	49
CFT (s)	<159		-	244	167	123	105	80	62
Angle (°)	>63		76	76	77	77	76	79	79
A10 (mm)	>43		15	28	35	40	45	50	58
MCF (mm)	>50		17	37	45	49	54	59	65
CT (s)	<79	35	66	57	55	58	55	56	50
CFT (s)	<159		-	285	216	181	122	102	81
Angle (°)	>63		50	69	66	65	73	69	73
A10 (mm)	>43		9	28	33	38	45	48	54
MCF (mm)	>50		10	38	43	49	53	55	62
CT (s)	<79	45	84	63	59	60	61	63	63
CFT (s)	<159		-	668	407	220	177	151	94
Angle (°)	>63		-	50	53	62	62	63	72
A10 (mm)	>43		6	19	24	33	37	39	49
MCF (mm)	>50		7	28	34	45	47	49	58

Note: Hct and PLT concentration given as median values. ExTEM parameter references are manufacturer's recommendation for reference limits.

(11%) of CT measurements that were slightly prolonged (89–101 s; reference range 38–79 s). None of the ExTEM parameters demonstrated a large change with time. Variation coefficients between the immediately analyzed samples and those analyzed after 2, 4, and 6-h delays were 12.6% for CT and between 1.0% and 2.0% for CFT, angle, A10, and MCF.

Immediately prior to ROTEM analysis of the reconstituted whole blood mixtures, the Hct value and PLT concentration on all mixtures were measured using the Sysmex XN-1000 in order to confirm the expected concentrations of the Table 1.

The effect of modifying the Hct value and PLT concentration in the reconstituted whole blood mixtures was determined using the ROTEM standard ExTEM assay. The samples were aliquoted into three separate cups, which ran simultaneously on the ROTEM device for a minimum of 30 min. Thus, each sample was analyzed in triplicate. The clot time (CT), clot formation time (CFT), angle, clot amplitude at 10 min (A10) and maximum clot firmness (MCF) results were recorded, and the median, minimum, and maximum of each triplicate were calculated in a Microsoft Excel (Microsoft Corporation, Redmond, WA) spreadsheet. All results were transferred to Graphpad Prism 10 for macOS (Graphpad Software, LLC, San Diego, CA) for graphical presentation.

3 | RESULTS

Figure 1 demonstrates the ROTEM CT (1A), CFT (1B), and angle (1C) results after testing the manipulated

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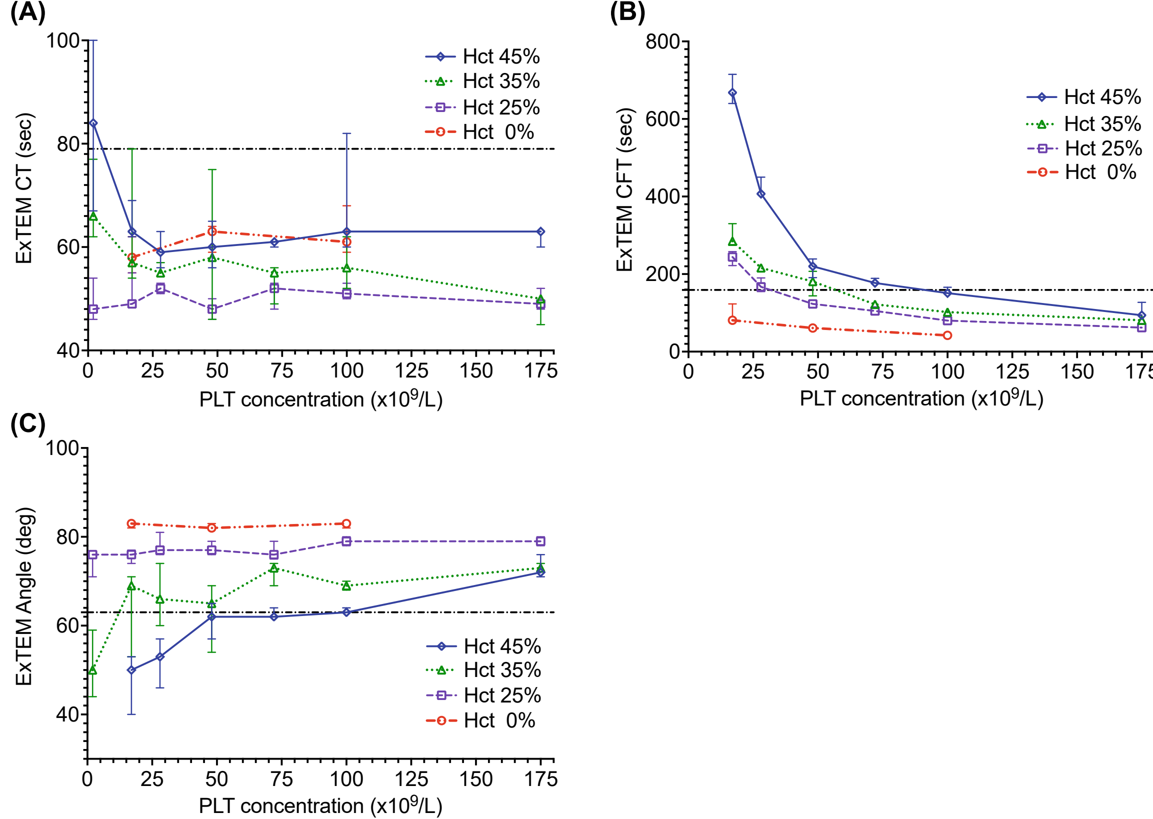

FIGURE 1 ROTEM tissue factor activated clot (ExTEM) formation measured by Clot Time (A), Clot Formation Time (B) and Angle (C). Each point represents median of the triplicate testing with range. The horizontal line represents the manufacturer's recommendation for upper (A and B) or lower (C) reference limit.

whole blood samples at different Hct values and PLT concentrations. The median CT (Figure 1A) measurements demonstrated high variation between the triplicates at low PLT concentrations, which was especially true for the samples with Hct 45%. The amount of inter-sample variation among these samples was reduced as the PLT concentration increased, which also resulted in normalization of the clot times. The CT values for the samples with Hct values between 0% and 35% were always below the upper limit of normal.

The median CFT measurements were more reproducible between the triplicate tests (Figure 1B). Note that none of the manipulated whole blood samples with a PLT concentration of 0×10^9/L achieved a minimum clot firmness of 20 mm, which is the defining point of CFT. At Hct 45% the median CFT value was within the reference range only when the platelet concentration was $\geq 100 \times 10^9$/L, whereas the platelet concentration that was required for the lower Hct samples to be within the reference range was lower than 100×10^9/L.

Figure 1C demonstrates the median ExTEM Angle values. The median value for the Hct 45% samples was below the lower limit until the PLT concentration reached approximately 50×10^9/L; in contrast, the median values for the samples with lower Hcts were above the lower limit at PLT concentrations that were below 50×10^9/L.

Both the median A10 (Figure 2A) and MCF (Figure 2B) values varied systematically and in a reproducible manner with the Hct and PLT concentration. For both parameters, the curves representing the Hct 25% and the Hct 35% samples were practically identical; the curve of the Hct 0% samples demonstrated a higher median A10 value for any given platelet concentration than the samples that contained any RBCs (Figure 2A). The median values for the Hct 45% samples were not within the reference range until the platelet concentration was between 100 and 175×10^9/L for the A10 (Figure 2A) and approximately $\geq 100 \times 10^9$/L for the MCF (Figure 2B). The median MCF for the mixtures containing a platelet concentration of 0×10^9/L were 7, 10, and 17 mm at Hct 45%, 35%, and 25%, respectively. An overview of all results are given in Table 2.

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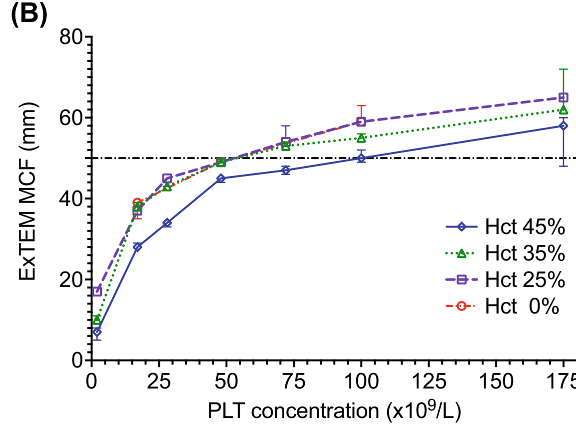

FIGURE 2 ROTEM tissue factor activated clot (ExTEM) strength measured by amplitude at 10 min (A) and maximum clot formation (B). Each point represents median of the triplicate testing with range. The horizontal line represents the manufacturer's recommendation for lower reference limit.

4 | DISCUSSION

These in vitro dilution experiments revealed that the clot characteristics as determined by the ROTEM ExTEM assays improved as the Hct decreased and as the PLT concentration increased. These are important findings because clinicians who base their transfusion decisions on thromboelastographic results need to be aware that the results of these tests are dependent on the patient's physiological status, that is, the potential for anemia and thrombocytopenia to confound the results of these tests.

Noorman and Hess,[8] in their experiments using the thromboelastogram (TEG) assay found that the TEG MA (maximum amplitude, which represents the maximum clot strength and is affected by the PLT and fibrinogen concentrations) was logarithmically related to the PLT concentration for a fixed Hct. Using a different instrument, the findings in this study were quite similar for the ROTEM CFT; for a fixed PLT concentration, especially in markedly thrombocytopenic samples with a PLT concentration as low as 30×10^9/L, the result of the test depended on the Hct, with lower values producing faster CFT times for a given PLT concentration. Thus, clinicians need to be cognizant of the Hct when deciding if transfusion is appropriate—a patient with a CFT that is above normal might not actually require a PLT or fibrinogen containing plasma product (or concentrate) if they have a relatively high Hct because of the effect of the relatively increased quantity of RBCs on the performance of the test. Thus, while these assays might help in deciding which patients should be transfused, clinical correlation, and correlation of the assay value with other laboratory test results, is required. As Noorman and Hess suggest,[8] thromboelastic tests might function best as general markers of derangement in coagulation rather than indicating the specific nature of the defect, and the findings of this study support that suggestion by adding another layer of complexity to the interpretation of the thromboelastogram test results.

This study has several limitations. It was performed using donated blood products, some of which were stored under routine blood bank conditions, which might not have the same composition as the blood from injured or massively bleeding patients due to the increased concentration of acute phase reactants that occurs in bleeding patients, medications administered during the resuscitation, and so forth. A range of PLT concentrations and Hct values were studied and so it is not necessarily possible to determine exactly at which combination of concentration and value, respectively, the changes in the ROTEM ExTEM occurred. Thus, clinicians interpreting these tests might not necessarily know at which combination of concentration and value to expect the changes in ROTEM test output will start occurring, but rather they should be aware that the output of the test can be affected by both the PLT concentration and Hct.

Although the concentration of coagulation factors should have remained consistent across all reconstituted blood samples (all added platelet-depleted plasma came from two donated FFP units), the manipulated samples with a lower Hct contain a proportionately greater plasma fraction, and thus they had a higher absolute amount of coagulation factors, including fibrinogen, per units of reconstituted whole blood. This could potentially explain the normalization of ExTEM parameters in samples containing a low Hct value and low PLT concentration. While all samples were performed on the ExTEM assay, the thromboelastograms for samples containing a PLT concentration of 0×10^9/L were a proxy for the FibTEM assay (in FibTEM, the addition of cytochalasin D

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blocks PLTs). These samples showed a variation in MCF that was dependent on the Hct value, where a lower Hct value correlated with a higher MCF. The FibTEM MCF parameter is associated with fibrinogen levels and is used by clinicians to evaluate the need for fibrinogen administration in bleeding patients. A higher FibTEM MCF is associated with a higher concentration of fibrinogen and vice versa. The observed inverse association of the Hct value and MCF values in the mixtures with a PLT concentration of 0×10^9/L in this study was likely the result of the fibrinogen containing plasma fraction in each sample. In addition, the delayed testing experiments validated our experimental approach in that there was minimal variation observed in the samples that were tested 6 h after collection compared to those tested immediately. It took approximately 330 min from the blood sample were collected to the last ROTEM analysis of the reconstituted whole blood begun, which was within the 6 h of the validation experiment. Thus, the delay in preparing the samples did not likely affect the results.

Lastly, although the principle of thrombelastography measurement is similar between different manufacturers and models, it is not clear if the magnitude of the changes in test output based on the PLT concentration and Hct described in this study apply to tests performed on machines other than the specific machine used in this study (ROTEM Delta). Similarly, it is not clear if the PLT concentration and Hct values that produced the changes in this study would be expected to cause the same degree of result variation on other machines.

Thromboelastogram test results are commonly used to manage massively bleeding patients with a variety of different bleeding etiologies. Clinicians who base their transfusion decisions on these results should be aware of the limitations of the tests, including the confounding effect of different Hct values and PLT concentrations.

CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

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