Early Prediction of Coagulopathy in Acquired Bleeding Disorders - Revisited Using Rotational Thromboelastometry

Soonam John[1], Nitty Skariah Mathews[2], Sukesh Chandran Nair[3]

ABSTRACT

Introduction: Acquired bleeding disorders are a major cause of mortality, both in the developed and developing countries. An acute haemorrhage should be managed immediately with blood products, factor concentrates or anti-fibrinolytics. Investigations to detect coagulopathies typically include baseline screening tests like prothrombin time, activated partial thromboplastin time, platelet count and fibrinogen level. These tests have a long turn around time which frequently lead to a blinded approach towards blood product support leading to under or over transfusion. In contrast, rotational thromboelastometry (ROTEM) which assesses haemostasis from the start of clot formation to fibrinolysis gives earliest results within ten minutes. This study was done to establish a correlation between ROTEM parameters and standard coagulation profile in the context of acquired bleeding disorders.

Material and Methods: A total of 138 subjects - 70 patients who presented with acquired bleeding disorders and 68 subjects diagnosed to be normal on the basis of a complete coagulation work up were included as the cases and controls respectively. All samples were subjected to standard coagulation profile and ROTEM analysis which included Clotting Time, Clot Formation Time, Alpha Angle, Maximum Clot Firmness and Maximum Lysis.

Results: The Maximum Clot Firmness had a very good co relation with serum fibrinogen levels (k value - 0.807; p<0.000; Sensitivity - 88%; Specificity - 92%), and good correlation with platelet count (k value - 0.793; p< 0.000; Sensitivity - 86%, Specificity-92%), whereas Clot Formation Time showed moderate correlation with aPTT. Clotting time had a poor correlation with prothrombin time and activated partial thromboplastin time.

Conclusion: The achievement of haemostasis is a crucial factor for determining patient outcomes in acquired bleeding disorders. The gold standard test to diagnose coagulopathy is the standard coagulation profile. Rotational thromboelastometry correlates well with standard coagulation parameters. This test which is performed on whole blood showed interpretable results within 10 minutes, whereas standard coagulation profile required an average of 45 – 75 minutes. In view of the good correlation to the standard coagulation profile, it appears that Rotational Thromboelastometry results can be safely used to implement early transfusion therapy for haemorrhage.

Keywords: Blood, Haemostasis, Coagulation, Thromboelastometry, Transfusion.

INTRODUCTION

Bleeding disorders comprise a varied group of diseases due to the malfunction of blood vessels, coagulation proteins and platelets. The clinical spectrum varies from a mild bleeding diathesis to severe hemorrhage which can lead to death. It can be due to either quantitative or qualitative abnormalities of coagulation proteins or platelets. Bleeding-history assessment tools can be used to assess the patient. A high bleeding score indicates a significant bleeding disorder but there can be an overlap of scores for individuals with mild bleeding disorder and without any bleeding symptoms.[1]

The bleeding disorders are broadly classified into inherited bleeding disorders and acquired bleeding disorders. Inherited bleeding disorders are diseases due to hereditary abnormalities of proteins or platelets which are necessary for blood clotting. Acquired bleeding disorders occur due to depletion, reduced synthesis, or inhibition of platelets and coagulation factors. The causes include disseminated intravascular coagulation, Vitamin K deficiency or antagonists, acquired inhibitors to coagulation factors, liver transplantation, renal causes, use of anticoagulants and traumatic bleeding. The acquired platelet function disorders include drug usage (Aspirin, Clopidogrel, Prasugrel) food additives, myeloproliferative neoplasms, Acquired Platelet Dysfunction with Eosinophilia and Paroxysmal Nocturnal Hemoglobinuria. The clinical picture ranges from mild bruises to life-threatening hemorrhage. The location, frequency and, severity of bleeding depends on the cause of bleeding disorder. A complete medical, surgical, family, social, and medication history is necessary in understanding the etiology.

The basic laboratory tests prothrombin time (PT), activated partial thromboplastin time (aPTT), serum fibrinogen and platelet count, help in exposing the cause of bleeding. The PT and aPTT tests identify only partially if any coagulation defects are present. These tests also give a high number of false positive and false negative results.[2] The management of clinical condition depends on the history and laboratory

[1]Assistant Professor, Department of Transfusion Medicine, Government Medical College, Thiruvananthapuram, Kerala, [2]Assistant Professor, Department of Transfusion Medicine, Christian Medical College Hospital, Vellore, Tamil Nadu, [3]Professor, Department of Transfusion Medicine, Christian Medical College Hospital, Vellore, Tamil Nadu, India

Corresponding author: Dr. Nitty Skariah Mathews, Assistant Professor, Department of Transfusion Medicine, Christian Medical College Hospital, Vellore, Tamil Nadu, India

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tests. The treatment decision includes administration of vitamin K, packed red cell concentrate, fresh frozen plasma, cryoprecipitate or platelet concentrate transfusion and blood derivatives like factor concentrates.

Rotational thromboelastometry

Background

Rotational thromboelastometry (ROTEM) is a point of care (POC) viscoelastic tests of hemostasis which detects clot formation and dissolution. This test provides a visual evaluation of clot formation, propagation, stabilization and lysis under low shear conditions that is similar to those present in the vena cava, large veins, and the arterial system.[3] This process is analyzed by a software. This system has four separate measurement channels which allow the independent tests like EXTEM, INTEM, APTEM AND HEPTEM (Table 1.) and a computer for automatic analysis.

Technology

Rotational thromboelastometry evaluates the clot formation kinetics and strength by measuring the amount of a rotational force that is transmitted to an electromechanical transduction system by developing clot. In this system, a cylindrical cup which is fixed and containing a 340 µl whole blood sample and a pin suspended on a ball bearing mechanism initially oscillates through 4° 75′ every 6 sec through application of a constant force. As the strength of the clot increases the rotation of the pin is hindered which is detected optically using a image sensor system. ROTEM device is capable of analyzing 4 samples simultaneously. ROTEM provides automated pipetting.[4]

Parameters

Clotting time (CT) - It is the time from the beginning of the test by adding the clot activator until the time when amplitude of 2 mm is achieved.

Clot Formation Time (CFT)- It is the time between 2 mm amplitude and 20 mm amplitude of the clotting signal.

Alpha Angle (α)- It is defined as the angle between the middle axis and the tangent to the clotting curve through the 2 mm amplitude point. It describes the kinetics of clotting.

Maximum Clot Firmness (MCF)- It is the measure for the firmness of the clot and therefore the clot quality. It is the maximum amplitude that is reached before the clot is dissolved by fibrinolysis and the clot firmness falls again.

Maximum Lysis (ML)- It is the degree of fibrinolysis relative to maximum clot firmness achieved during the measurement.

The use of rotational thromboelastometry in transfusion support assessment allows targeted hemostatic approach. This strategy reduces blood component usage as compared to a blinded approach to transfusion on the basis of laboratory tests with long turn-around times. The usual turnaround time for PT, aPTT, fibrinogen concentration and platelet count is generally long (nearly 45- 60 min) to determine the appropriate therapy in severely bleeding patients. The initial variables of ROTEM are available within 15–20 min.[5] This study was done to assess the efficiency of rotational thromboelastometry in comparison to the standard coagulation profile in assessing coagulopathy in acquired bleeding disorders.

MATERIAL AND METHODS

This was a case control study conducted at the Department of Transfusion Medicine and Immunohaematology, Christian Medical College, Vellore, India for a period of two years from 01 January 2013 to 31 December 2014. The study was approved by the institutional research and ethics committee and was conducted on seventy newly admitted consecutive bleeding patients in the wards, the emergency and intensive care units of Christian Medical College Hospital. A detailed history, diagnosis, complete blood count profile, transfusion requirements and, condition at discharge were collected with the help of a standardized proforma.

Seventy patient samples of acquired bleeding disorder cases received in the Haemostasis Laboratory, Department of Transfusion Medicine and Immuno hematology that have shown abnormal coagulation profile were considered as the test samples. The samples were collected on the basis of requests for blood products received in the blood bank from emergency, intensive care units and all surgical/ medical units including super specialty departments. A detailed bleeding history was obtained taken from the treating physician/ surgeon. The blood samples for the tests were taken before transfusing blood products.

The control group consisted of subjects who had no bleeding symptoms at presentation and did not require any medical, surgical or transfusion interventions. On the basis of a complete coagulation work up, which included, complete blood count profile, standard coagulation profile, coagulation factor assays, Ristocetin cofactor activity (RiCof) levels and Platelet aggregometry studies, this category was diagnosed to have a normal coagulation profile. The first 68 consecutive samples during the study period were selected as the control samples.

All paediatric patients were excluded as the normal reference range for prothrombin time, activated partial thromboplastin time, platelet count and serum fibrinogen levels are different from the adult ranges and there are no well- defined pediatric reference ranges for ROTEM variables.[6] The patients who had received transfusion and medication were also excluded as it will affect the coagulation parameters especially stable coagulation factors like factor XIII and fibrinogen.[7]

Detailed history including history of risk factors if any was taken. It includes the presenting symptoms with duration, significant medication history especially use of antiplatelet drugs or, vitamin K antagonists. Treatment details like transfusion of blood, platelets, fresh frozen plasma, cryoprecipitate, factor concentrates, use of antifibrinolytics like tranexamic acid were also taken in detail.

Two citrated 5 ml blood samples were obtained and transported immediately to Haemostasis lab, and one tube was analysed after centrifugation at 3000g for ten minutes on coagulation analyser Sysmex CS 2000i (Siemens Healthcare Diagnostics, Erlangen, Germany) for investigations like Prothrombin time, INR, Activated partial thromboplastin time and Serum Fibrinogen. The thromboelastometry tests

were done by using the second 5 ml citrated sample in the ROTEM device. The technical details of the device have been described elsewhere.[3] The platelet count was performed using a K2 EDTA sample in the Beckman Coulter DXH 800 automated haematology analyzer (Beckman Coulter, Inc, Miami, FL,US). All samples including cases and controls were subjected to thromboelastometric analysis using the ROTEM machine in INTEM mode.

To compare the standard coagulation tests the following ROTEM tests were defined. CT and CFT were compared to PT and aPTT and MCF compared to Serum Fibrinogen and platelet count. The automated devices were set up according to quality standards and underwent periodic quality checks including calibration and daily quality control runs.

STATISTICAL ANALYSIS

The GraphPad Prism 7 (GraphPad Software Inc,San Diego, CA) software package was used for statistical analysis. The sensitivity and specificity, positive predictive value and negative predictive value were calculated using 2x2 table (Table 2). To explain the agreement between ROTEM and standard coagulation profile the k (kappa) value was calculated for concordance of these two findings. A k coefficient of <0.20 was poor, 0.21-0.40 Fair, 0.41-0.60 Moderate, 0.61-0.80 Good, 0.81-1.0 Very Good Almost perfect agreement.

RESULTS

We studied 70 cases of acquired bleeding disorders (case group) and 68 normal adults who were non-bleeders as the control group. In the seventy cases, certain cases had one, two or three altered tests (Prothrombin time, activated Partial thromboplastin Time, Platelet count and Serum Fibrinogen levels), but none had all the four coagulation parameters altered.

The mean age of cases was 41.3±16.2 years and that of controls was 33.3 ±13.7 years. Females accounted for 34.3% of the cases and 54.4% of the controls. In both cases and control group, a majority of cases were seen in the age group 21 -30 years.

The bleeding manifestations ranged from minor mucosal bleeds like epistaxis to life threatening operative hemorrhages requiring transfusion support. A major proportion of our cases had severe intraoperative and postoperative bleeds leading to coagulopathy. Twelve percent of the total cases had more than one bleeding manifestation. Among the cases, nearly 52% of the patients were not on any medications that cause bleeding while the remaining were on Heparin,

INTEM	Contact activation. Reagent - phospholipid and ellagic acid as activators Provides information similar to that of the APTT.
EXTEM	Tissue factor activation. Reagent contains tissue factor as an activator. Provides information similar to that of the PT.
APTEM	Aprotinin for inhibiting fibrinolysis Used in conjunction with EXTEM FOR assessing fibrinolysis.
HEPTEM	Contains lyophilized heparinase for neutralizing unfractionated heparin Used in conjunction with INTEM to assess heparin effect.
FIBTEM	Utilizes cytochalasin D, an actin polymerization inhibitor to block the platelet contribution to clot formation. Used in conjunction with EXTEM reagent for qualitative analysis of the fibrinogen contribution to clot strength independent of platelets.
NATEM	Native whole blood sample analyzed following only recalcification. Impractical for clinical use given long CFT time

Table-1: ROTEM Assays

2X2 Table for analysis		**Standard coagulation profile (gold standard)**		**Total**
		Positive	**Negative**	
ROTEM (Test)	Positive	a	b	a + b
	Negative	c	d	c + d
Total		a+c	b+d	a+b+c+d
Sensitivity = a/a+c; Specificity=d/b+d				

Table-2: Statistical analysis plan for calculating sensitivity and specificity of Rotational Thromboelastometry compared to Standard coagulation profile.

ROTEM Parameter	**Coagulation Test**	**Sensitivity**	**Specificity**	**K value**	**Agreement**
Clotting Time	Prothrombin Time	18.8%	91.2%	0.101	Poor
Clotting Time	Activated Partial Thromboplastin Time	22.2%	92.7%	0.160	Poor
Clot Formation Time	Prothrombin Time	42.2%	95.6%	0.384	Fair
Clot Formation Time	Activated Partial Thromboplastin Time	44.4%	95.6%	0.422	Moderate
Maximum Clot Firmness	Platelet Count	86.3%	92.7%	0.793	Good
Maximum Clot Firmness	Serum Fibrinogen	88.6%	92.7%	0.807	Very Good

Table-3: Correlation between Rotem parameters and Standard coagulation profile

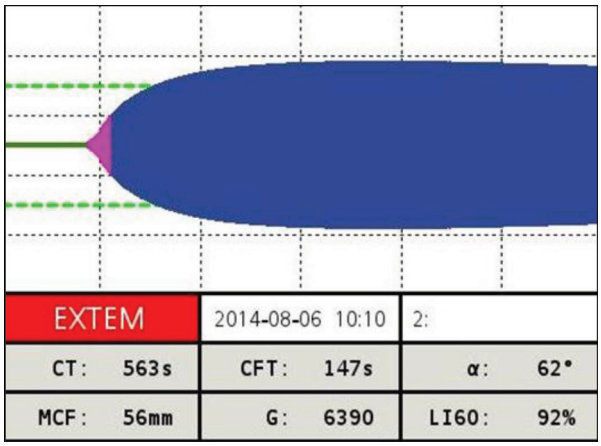

Figure-1: A normal thromboelastogram

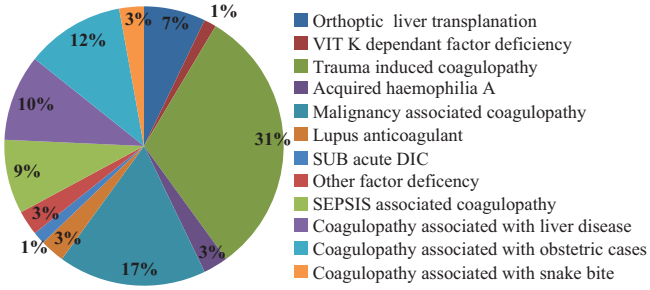

Figure-2: Distribution of diagnosis in cases

Aspirin and/or Clopidogrel therapy. In the control group all 100% were free of medications. The diagnosis of acquired bleeding disorder is depicted in figure 2.

Out of the sixty four cases which had a prolonged prothrombin time, eleven cases did not show any correction with pooled normal plasma and the clinical condition associated with this scenario included orthoptic liver transplantation, trauma induced coagulopathy, coagulopathy associated with liver disease, sepsis associated coagulopathy, coagulopathy associated with obstetric cases. In five out of fifty four cases with prolonged aPTT, correction with normal plasma was not achieved and these included cases of Trauma induced coagulopathy, Acquired haemophila A, Lupus anticoagulant and sepsis associated coagulopathy. Low fibrinogen levels of less than 150 mg/dL were noted in 53.7% of cases.

Comparison of ROTEM - Clotting Time (CT) with the Prothrombin time (PT) (table-3)

Sensitivity - 18.75% (95% C.I. =10.09% to 30.47%)

Specificity- 91.18% (95% C.I. =81.77% to 96.67%).

The Kappa Statistic for agreement between ROTEM – CT and PT in detection of coagulopathy is K = 0.101 (-0.019 to 0.221) indicating a poor agreement between the ROTEM - CT and PT. (p<0.129)

Comparison of ROTEM Clotting time (CT) with Activated partial thromboplastin time (APTT)

Sensitivity - 22.22% (95% C.I. =12.04%-35.60%)

Specificity - 92.65% (95% C.I. =83.67% – 97.57%).

The Kappa Statistic for agreement between CT and APTT in detection of coagulopathy is

K =0.160 (0.023-0.297) indicating a poor agreement between the CT and APTT (p<0.033).

Comparison of ROTEM Clot Formation Time (CFT) with the Prothrombin time (PT)

Sensitivity - 42.19% (95% C.I. =29.94-55.18)

Specificity - 95.59% (95% C.I. =87.63-99.03).

The Kappa Statistic for agreement between ROTEM CFT and APTT in detection of coagulopathy is K = 0.384 (0.248-0.519) indicating a FAIR agreement between the CFT and APTT (p<0.000).

Comparison of ROTEM Clot Formation Time with the activated Partial Thromboplastin Time

Sensitivity - 44.44% (95% C.I. =30.92 -58.60.)

Specificity -95.59% (95% C.I. =87.63 -99.03).

The Kappa Statistic for agreement between ROTEM CFT and APTT in detection of coagulopathy is K =0.422(0.275-0.570) indicating a moderate agreement.

Comparison of ROTEM MCF with the Platelet count

Sensitivity- 86.36% (95% C.I. =72.64%-94.79%)

Specificity - 92.65% (95% C.I. =83.66%-97.54%.).

The Kappa Statistic for agreement between ROTEM MCF and Platelet count in detection of coagulopathy is K =0.793 (0.677-0.909) indicating a good agreement between the MCF and PC - p<0.000).

Comparison of ROTEM MCF with the serum fibrinogen

Sensitivity - 88.57% (95% C.I. = 73.24%-96.73%)

Specificity - 92.65% (95% C.I. =83.66%-97.54%).

The Kappa Statistic for agreement between ROTEM MCF and fibrinogen in detection of coagulopathy is K = 0.807 (0.686 -0.927) indicating a very good agreement (p<0.000).

DISCUSSION

The management of acquired bleeding disorders depends on the clinical diagnosis that causes the bleeding disorder. In our institution any patient admitted with traumatic injury, severe blood loss or coagulopathies of any aetiology are given blood product support. The transfusion medicine physician or the clinical hematologist is informed regarding the patient's clinical condition, who suggests the necessary blood product(s) to be transfused. The samples are drawn for preliminary investigations which include the standard coagulation tests and complete blood counts. The transfusion therapy is given empirically, starting with cryoprecipitate followed by other products like red cell concentrate, fresh frozen plasma and platelets. Further transfusion requirements are based on the coagulation parameters of the patient, the results of which take about 45 minutes to be available on the institutional database. In case of postoperative bleeding in cardio thoracic and liver transplant cases, coagulopathy monitoring is done by standard coagulation tests and thromboelastometry, as per the surgeon's request.

Our study aimed to compare the thromboelastometry method to standard coagulation profile in detecting coagulopathies. The current study is a case control study which included 70 patients with acquired bleeding disorders and 68 controls who had normal coagulation profile. In all samples the standard coagulation tests (gold standard) and thromboelastometry

were performed.

The role of thromboelastometry in assessing coagulopathy has not been extensively studied in India. There are several observational studies showing poor correlation between Prothrombin Time and activated Partial Thromboplastin Time to Clotting Time, a good correlation between Prothrombin Time and activated Partial Thromboplastin Time to Clot Formation Time, and a very good correlation between Fibrinogen level and Maximum Clot Firmness[8] Platelet count and fibrinogen levels correlated significantly with clot strength, and fibrinogen levels correlated with fibrin polymerization.[9] A significant correlation was found between activated partial thromboplastin time and Clot Formation Time.

From our study we found that ROTEM MCF has a high sensitivity and specificity of 88.57% and 92.65% respectively. Similar results were obtained by a study done by Tauber et al.[9] We evaluated the cases with fibrinogen levels less than 100 mg/dl as this was considered as a threshold of cryoprecipitate transfusion in our institution. The fibrinogen levels ranged from undetectable levels to 148.9 mg/d. The clinical conditions which led to decreased fibrinogen levels that correlated well with MCF values were coagulopathy due to trauma and obstetric causes. There are several studies on obstetric conditions leading to coagulopathy and diagnosis on the basis of thromboelastometry.[10,11,12]

The sensitivity of ROTEM maximum clot firmness to detect low platelet count was high. This finding was similar to data from liver transplantation in adults, where thromboelastometry was used to assess the coagulation status[13] Thus, MCF values might serve as surrogate parameters to estimate platelet function.

The sensitivity of CFT to detect low aPTT was 44.44% This is in contrast to a study by Haas et al and Rugeri et al which showed higher correlation between of activated partial thromboplastin time (aPTT) and CFT[8,13]

The study showed a poor correlation between Clotting Time and Prothrombin Time. This is contradictory to the study by Haas et al where there was good correlation between PT and CT.[8]

LIMITATIONS

There were some limitations in our study. Predictive values could not be ascertained as it is not a prevalence study. The data was analyzed in the binary or dichotomous mode which could have been processed in the ordinal mode. We were not able to correlate maximum lysis with any factor, since all patients did not have a D Dimer or factor XIII levels done. All the ROTEM tests were done in INTEM channel only.

CONCLUSION

The achievement of haemostasis is a crucial factor for determining patient outcome in acquired bleeding disorders. The standard coagulation tests are still the gold standard for the diagnosis of acquired coagulation factor deficiencies. These tests are designed in such a way that it is incapable of representing the balance of coagulation, as it is an in vitro test. Rotational Thromboelastometry analysis can be performed as bedside tests but standard coagulation profile testing is done in a central laboratory and this can cause a delay in sample transport and analysis. The turnaround time for standard coagulation tests are long (average- 45 minutes) compared to the earlier results of thromboelastometry which are obtained within ten minutes. Therefore thromboelastometry can be used as a screening method for detecting thrombocytopenia and hypofibrinogenemia in hemorrhagic conditions and can help in early transfusion support of these patients.

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