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The coagulation changes associated with acute variceal bleeding in patients with HCV-induced cirrhosis as assessed by rotational thromboelastometry

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Abstract

Background and objectives Alterations of hemostasis in patients with decompensated cirrhosis are complex. Accordingly, standard coagulation tests are not feasible to evaluate bleeding risk in these patients. The aim of this study was to assess the coagulation kinetics in cirrhotic patients with variceal bleeding using rotational thromboelastometry (ROTEM). ROTEM including EXTEM, INTEM, and FIBTEM which represent extrinsic, intrinsic pathways, and fibrinogen activity, respectively, was measured in 60 cirrhotic patients with variceal bleeding who were compared to 60 patients with stable cirrhosis. APTEM was optionally performed to evaluate fibrinolysis.

Results Overall, cirrhosis patients displayed features of hypofibrinolysis, whereas the state of hypocoagulability was significantly higher in cirrhotic patients with variceal bleeding (61.7% versus 30%, $p=0.001$). Values of clot formation time (CFT) by EXTEM and INTEM correlated positively with those of model for end-stage liver disease score ($r=0.529$, $p=0.001$, and $r=0.595$, $p<0.001$, respectively). Furthermore, in a multivariate analysis, values of CFT in both assays were significantly associated with increased risk of 1.9 (95% *CI* = 1.04–2.45, $p=0.02$) and of 1.78 (95% *CI* = 1.02–2.14, $p=0.01$), respectively, for occurrence of variceal bleeding.

Conclusion Cirrhotic patients with variceal bleeding frequently showed a hypocoagulable state that is triggered by thrombocytopenia and/or hypofibrinogenemia. CFT by EXTEM and INTEM seemed to be an extra marker for disease severity and prognosis in cirrhosis patients, in addition to its valuable role in prediction of variceal bleeding in these patients. However, large multicenter studies have yet been required.

Keywords Liver cirrhosis, Variceal bleeding, Coagulation profile, Rotational thromboelastometry

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Introduction

Liver cirrhosis is a global health problem [1]. In spite of the great advancement gained in treatment of hepatitis C virus (HCV) infection in the last decade, it is still a major health dilemma worldwide, predominantly in Egypt [2]. This issue may be due to occult HCV infection that might result in later serologic relapse and continuing liver cell damage, fibrosis, and therefore cirrhosis [3].

Portal hypertension is a substantial complication of cirrhosis that can herald the occurrence of many devastative events including variceal bleeding [4], which is elicited in ~ 50% of cirrhotic patients [5], in particular those with advanced disease, increased severity of portal hypertension, enlarged size of esophageal varices, and excess ethanol intake [6].

Traditionally, coagulopathy is a crucial contributor of bleeding in cirrhosis patients [7]. Under stable conditions, cirrhotic patients display a state of rebalanced hemostasis due to concurrent defective synthesis and clearance of pro- and anticoagulants in addition to changes in fibrinolysis [8]. But this balance is relatively precarious, and bleeding diathesis can be easily induced by hepatic decompensation, bacterial infections, and renal failure [9].

Unluckily, none of the standard coagulation tests (SCTs), including prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin test (aPPT), is suitable enough in precisely characterization of this hemostatic status [10]. Prolongation of PT-INR and aPPT has been frequently observed in cirrhosis patients without actual bleeding episodes and even in those with thrombotic events [11]. Thus, more global hemostatic assays are urgently needed.

Rotational thromboelastometry (ROTEM) is a whole blood hemostasis analyzer which is an enhancement of thrombelastography. It allows a point-of-care monitoring of viscoelastic characteristics of all the dynamic steps of clot formation and lysis [12]. Using multifold and parallel assays, blood samples with different inhibitors and activators permit individual evaluation of each step of hemostasis. Moreover, it could discriminate the role of platelets from that of fibrinogen in final clot strength [13]. The usefulness of ROTEM as a reliable approach to assess coagulopathies has been primarily documented in massive trauma and liver surgery [14, 15].

The aim of this exploratory study was to evaluate alteration in hemostasis using ROTEM in HCV-related patients with acute variceal bleeding and to investigate its clinical correlate.

Materials and methods

Eligible subjects

The current prospective, analytical, hospital-based, case–control study was conducted at the Internal Medicine Department, Minia University Hospital, Egypt, between March 2017 and June 2019. A group of patients with HCV-related liver cirrhosis was enrolled from those who were consecutively referred to the Hepatology and Gastroenterology Units. These patients were categorized according to the presence or absence of variceal bleeding into two groups: group I, which included 60 (48 males, and 12 females) cirrhotic patients with acute variceal bleeding, and group II, which comprised 60 (48 males and 12 females) patients with stable cirrhosis. Cirrhosis was diagnosed by relevant clinical, laboratory, ultrasonographic, and upper endoscopic findings. Chronic HCV infection was defined by the presence of anti-HCV and detectable serum HCV-RNA for 6 months or more.

The exclusion criteria included the following: smoking, regular alcohol consumption, diabetes mellitus, co-infection with hepatitis B virus, human immunodeficiency virus and/or *Schistosoma mansoni*, causes of cirrhosis other than chronic HCV infection, causes of hematemesis other than ruptured esophageal varices, ongoing infections, autoimmune disorders, coagulation disorders, hematological malignancies, hepatic and extrahepatic neoplasms, patients with malignant or nonmalignant portal vein thrombosis, anticoagulant or antineoplastic drugs, and patients who had undergone cardiac bypass.

Clinical and laboratory assessment

Informations on age, sex, special habits of medical importance, current history of type 2 diabetes mellitus, stigmata of liver cirrhosis, and the number of units of transfused blood were obtained.

Venous blood was drawn after a 12-h overnight fast to examine complete blood count, liver and kidney function tests, fasting and 2-h postprandial blood glucose, C-reactive protein, α-fetoprotein, anti-*Schistosoma mansoni* antibodies, and SCTs including PT-INR and aPPT, according to the respective manufacturer's instructions of commercial kits.

- Virological assays: Anti-HCV, hepatitis B surface antigen, and anti-immunodeficiency virus were determined using commercial kits supplied by Ortho Clinical Diagnostic Co. Inc., Tokyo, Japan; Lumi-Plus II hepatitis B surface antigen, Fujirebio Co. Inc., Tokyo, Japan; and Sanofi Diagnostic Pasteur, Marnes-la-Coquette, France, respectively. HCV-RNA was quantified by a standardized autometal qualitative

real-time polymerase chain reaction with a lower detection limit of 12 IU of HCV-RNA/ml.

- The severity of liver cirrhosis: It was evaluated by the means of the model for end-stage liver disease (MELD) scoring system [16].
- Esophagogastroduodenoscopy was performed by videoscope, PENTAX EG-2940, Japan, and fiber-optic endoscope, PENTAX FG-29W, Japan. Esophageal varices were diagnosed and graded according to Paquet's criteria [17] by an expert specialist who was unfamiliar with patients' data.
- ROTEM analysis: It was performed with the ROTEM delta whole blood analyzer (Tem Innovations; GmbH; Munich, Germany) as described by the manufacturer [18]. For each patient, we performed the extrinsically activated (tissue factor) thromboelastometry (EXTEM), the intrinsically activated (ellagic acid) thromboelastometry (INTEM), and fibrinogen thromboelastometry (FIBTEM). FIBTEM measured fibrinogen contribution to clot strength after platelet inhibition by cytochalasin D, and it was compared to EXTEM. APTEM is a modified EXTEM test in which aprotinin is used in the reagent to inhibit plasmin and subsequently correct fibrinolysis. Basically, EXTEM, INTEM, and FIBTEM were done in this study, while APTEM was optionally done. The following parameters were obtained from the outputs of EXTEM and INTEM assays: (1) the clotting time (CT) which was the period in second from the start of the test until the 2-mm amplitude was reached; (2) the clot formation time (CFT), it was the time in second that required to increase the clot firmness from 2 to 20 mm amplitude; and (3) α-angle ^{0} which was the angle between the center line and the tangent to the coagulation curve through a point of 2-mm amplitude, CFT, and α-angle reflected the rate of fibrin polymerization; (4) the maximum clot firmness (MCF), it was the maximum amplitude in millimeter of the curve that measured the clot strength and influenced by platelet count and function, as well as concentrations of fibrinogen and coagulation factor XIII; (5) the amplitude of the curve at 10 min after CT (A10), it gave a sing on the expected MCF which allowed a rapid therapeutic decision; and (6) the maximum lysis (ML), it was the percentage of reduction of clot amplitude 60 min after MCF. Concerning FIBTEM, only MCF was evaluated in this assay (c.f., Fig. 1).

In this study, hypocoagulable state was diagnosed by the presence of ≥ 3 parameters beyond the reference range in EXTEM and INTEM (prolonged CT or CFT, decreased alpha angle, and MCF). A hypercoagulable profile was considered in the presence of at least two of the following: shortened CT or CFT and increased alpha angle and MCF. Hyperfibrinolysis was defined when the clot amplitude decreased > 15% per hour in all tests except APTEM, in the presence of intact other variables [19].

Imaging studies

Abdominal ultrasound with color Doppler analysis was performed by the ultrasound machine, Toshiba Xario 100, Japan, with 3.5-MHz transducer. It was used to explore the stigmata of liver cirrhosis, hepatic focal lesion(s), in addition to the portal vein thrombosis. However, definite diagnosis of portal vein thrombosis [20], and hepatocellular carcinoma [21], was based on

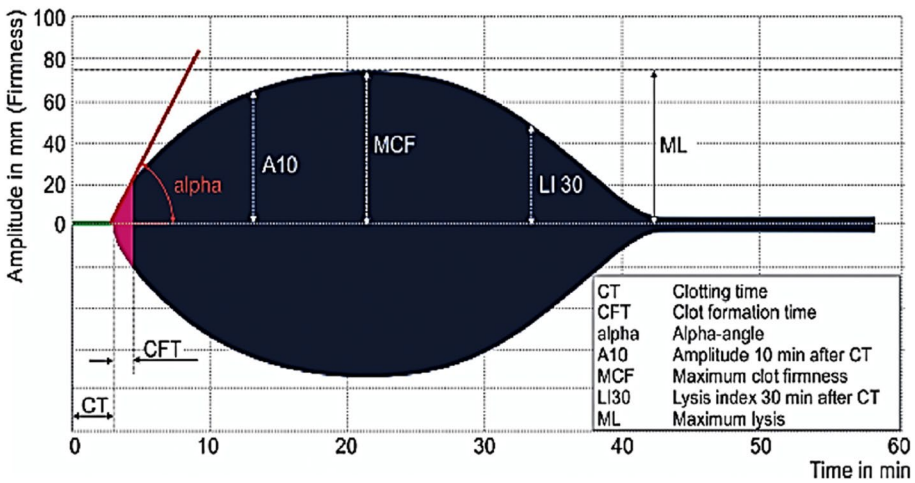


Fig. 1 Graphic representation of rotational thromboelastometry parameters

the imaging characteristics that mentioned by accepted guidelines. Plain chest X-ray was also performed to exclude chest infection as a source of sepsis. Imaging studies were performed by an expert radiologist who was blinded of the patients' data.

Statistical analyses

Data were analyzed using the Statistical Package for Social Science program, version 25, for Windows. According to the normality of distribution which evaluated by one-sample Kolmogorov–Smirnov test, quantitative data were expressed as mean ± standard deviation or median (interquartile range), while qualitative data were presented as number (percentage). Differences between the two groups were tested by Student's *t*-test, Mann–Whitney *U*-test, χ^2 test, or Fisher exact test, when appropriate. Relations between variables were tested using Pearson's correlation for normally distributed data and Spearman's correlation for ordinal variables. A multiple stepwise logistic regression analysis was performed on factors which exhibited a statistical difference between the two study groups, after controlling for other confounders to specify the factors that were independently associated with variceal bleeding in cirrhotic patients. The receiver operating characteristic (ROC) curves were constructed to calculate the area under these curves and to detect the optimal cutoff values with the highest sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy for tested variables. For all the analyses, a *p*-value ≤ 0.05 was considered to be a statistically significant.

Results

Cirrhotic patients with and without acute variceal bleeding

The present study was carried out on 60 cirrhotic patients with variceal bleeding (group 1), 48 (80%) of whom were male. Their ages ranged from 19 to 67 years with mean ± standard deviation of 52.6 ± 12.1 years. This group was compared to another group of patients with stable cirrhosis (group II), which comprised 48 (80%) males and 12 (20%) females. They had ages ranging from 29 to 64 years with mean ± standard deviation of 54.8 ± 9.5 years. Patients of both groups were seropositive for anti-HCV and HCV RNA. Both groups showed no statistically significant differences in age, gender, and the plasma levels of aPTT. However, patients of group I displayed higher levels of MELD score, serum bilirubin, alanine aminotransferase, aspartate transaminase, INR, serum creatinine, portal vein diameter (PVD), and the prevalence of advanced grades of esophageal varices (III and IV) when compared to patients of group II (14.5 ± 3.2 vs. 9.2 ± 2.9, $p < 0.001$; 2.5 ± 1.1 mg/dl vs. 0.9 ± 0.3 mg/dl, $p < 0.001$; 79 (52.8–115) IU/l vs. 22 (17.3–30) I/l, $p < 0.001$; 93 (46.8–180) IU/l vs. 37.5 (23.3–41.5) IU/L, $p < 0.001$; 1.3 (1.2–1.6) vs. 1.2 (1–1.5), $p = 0.049$; 1.1 ± 0.4 mg/dl vs. 0.8 ± 0.2 mg/dl, $p = 0.008$; 14.8 ± 3.9 mm. vs. 11 ± 1.4 mm., $p < 0.001$; and 57 (95%) vs. 0 (0%), $p < 0.001$, respectively). But they had lower levels of blood hemoglobin (9 ± 1.4 g/dl vs. 12 ± 2 g/dl, $p < 0.001$), hematocrit (27.5 ± 4.8% vs. 40.9 ± 6.5%, $p < 0.001$), platelet count (72 (58.3–108.3) 1×10^3/μl vs. 114 (64.3 ± 193) 1×10^3/μl, $p = 0.034$), serum albumin (2.9 ± 0.7 g/dl vs. 4 ± 0.6 g/dl, $p < 0.001$), and peak systolic velocity (12 ± 1.4 cm/s vs. 17 ± 5.3 cm/s, $p < 0.001$) (c.f., Table 1).

ROTEM parameters in cirrhotic patients with and without acute variceal bleeding

Overall, ROTEM profile was hypocoagulable in 37 (61.7%) of patients with variceal bleeding and in 18 (30%) of those with stable cirrhosis ($p = 0.001$). Although both groups showed no significant difference in CT values in either EXTEM or INTEM assay (56.5 (47.3–74.8) s vs. 53.5 (46.3–59.5) s, $p = 0.23$, and 181 (137.5–225) s vs. 174.5 (162.5–205.3) s, $p = 0.99$, respectively), this hypocoagulable state was manifested by a significant prolongation of CFT in EXTEM and INTEM (392 (200.5–796) s vs. 195 (123–309) s, $p = 0.01$, and 251 (170–402) s vs. 156 (100.5–207) s, $p = 0.01$, respectively), with a concomitant significant reduction of α-angle, and MCF in both EXTEM (52.7 ± 17.4^{O} vs. 65.5 ± 9.9^{O}, $p = 0.01$, and 30 (22.5–43) mm vs. 45 (40–58) mm, $p = 0.002$, respectively), and INTEM assays (51.6 ± 14.3^{O} vs. 65.8 ± 13^{O}, $p = 0.01$, and 37.5 (31–46) mm vs. 47 (40–59) mm, $p = 0.01$, respectively). Values of MCF returned to normal levels in FIBTEM assay with no significant difference between the two groups (10 (9–13.8) mm vs. 11.5 (7.3–15.5) mm, $p = 0.45$). Regarding the clot lysis, values of A10 showed a significant reduction in cirrhotic patients with variceal hemorrhage in both EXTEM and INTEM assays ((25.1 ± 11.5) mm, vs. (39.1 ± 12.7) mm, p 0.001, and (31.7 ± 11.9) mm vs. (43.1 ± 15.3) mm, $p = 0.01$, respectively), while ML values were < 15% in both groups (c.f., Table 2).

Correlations of ROTEM parameters

The correlation between ROTEM parameters and various clinical variables was performed to disclose the clinical relevance of these parameters in cirrhosis patients. CFT in EXTEM correlated positively with MELD score ($r = 0.529$, $p = 0.001$), and grades of esophageal varices ($r = 0.450$, $p = 0.006$), while it correlated negatively with platelet counts ($r = -0.340$, $p = 0.043$) and hematocrit % ($r = -0.524$, $p = 0.001$) (c.f. Fig. 2a–d, respectively). Similarly, CFT-INTEM correlated positively with MELD score ($r = 0.595$, $p < 0.001$) and grade of esophageal varices ($r = 0.438$, $p = 0.007$) and negatively with platelet counts

Table 1 Baseline characteristics in the study groups

Variable	Cirrhotic patients with variceal bleeding (G1) (no. = 60)	Patients with stable cirrhosis (G1) (no. = 60)	*p*-value
Age (years) [mean ± SD]	52.6 ± 12.1	54.8 ± 9.5	0.53†
Gender [no. (%)]			
Male	48 (80)	48 (80)	1§
Female	12 (20)	12 (20)	
MELD score [mean ± SD]	14.5 ± 3.2	9.2 ± 2.9	**0.001**†
Hemoglobin (g/dl) [mean ± SD]	9 ± 1.4	12 ± 2	**< 0.001**†
Hematocrit (%) [mean ± SD]	27.5 ± 4.8	40.9 ± 6.5	**< 0.001**†
Platelets (1×10^3/μl) [median (IQR)]	72 (58.3–108.3)	115 (64.3–193)	**0.03**‡
Total bilirubin (mg/dl) [mean ± SD]	2.5 ± 1.1	0.9 ± 0.3	**< 0.001**†
ALT (IU/l) [median (IQR)]	79 (52.8–115)	22(17.3–30)	**< 0.001**‡
AST (IU/l) [median (IQR)]	93 (46.8–180)	37.5 (23.3–41.5)	**< 0.001**‡
Serum albumin (gm/dl) [mean ± SD]	2.9 ± 0.7	4 ± 0.6	**< 0.001**†
INR [median (IQR)]	1.3 (1.2–1.6)	1.2 (1–1.5)	**0.049**–‡
aPPT (s) [mean ± SD]	45 ± 15	37.9 ± 9.4	0.08†
Serum creatinine (mg/dl) [mean ± SD]	1.1 ± 0.4	0.8 ± 0.2	**0.008**†
PVD (mm) [mean ± SD]	14.8 ± 3.9	11 ± 1.4	**< 0.001**†
PSV (cm/s) [mean ± SD]	12 ± 1.4	17 ± 5.3	**< 0.001**†
Grade of EVs [no. (%)]			
0–II	3 (5)	60 (100)	**< 0.001**¶
III–IV	57 (95)	0 (0)	

Bold values donate statistically significant results

G group, *no*. Number, *MELD* Model of end-stage liver disease, *ALT* Alanine aminotransferase, *AST* Aspartate transaminase, *INR* International normalized ratio, *aPPT* activated partial thromboplastin time, *PVD* Portal vein diameter, *PSV* Peak systolic velocity, *SD* Standard deviation, *IQR* Interquartile range

† Student's *t*-test

‡ Mann–Whitney *U*-test

§ χ^2 test

¶ Fisher exact test

($r = -0.377$, $p = 0.021$) and hematocrit% ($r = -0.530$, $p = 0.001$) (c.f. Fig. 3 a – d).

Factors associated with acute variceal bleeding in cirrhotic patients

In a multiple stepwise logistic regression analysis, we found that MELD score (adjusted odds ratio (AOR) = 2.75, 95% *CI* = 1.23 – 3.41, $p = 0.002$), PVD (*AOR* = 2.65, 95% *CI* = 1.16 – 3.35 mm, $p = 0.01$), CFT-EXTEM (*AOR* = 1.9, 95% *CI* = 1.04 – 2.45 s, $p = 0.02$), and CFT-INTEM (*AOR* = 1.78, 95% *CI* = 1.02 – 2.14 s, $p = 0.01$) were independent predictors for variceal bleeding in cirrhotic patients after controlling for other confounders (c.f., Table 3).

Area under the ROC curves of the independent predictors of acute variceal bleeding and their diagnostic performance in cirrhotic patients

The area under ROC curve for MELD score was 0.89 (95% *CI* = 0.74 – 0.96, $p < 0.001$) (c.f. Fig. 4a), for PVD was 0.78 (95% *CI* = 0.62–0.90 mm, $p < 0.001$) (c.f. Fig. 4b), for CFT-EXTEM was 0.76 (95% *CI* = 0.59–0.89 s, $p = 0.002$) (c.f., Fig. 4c), and for CFT-INTEM was 0.75 (95% *CI* = 0.58–0.88 s, $p = 0.002$) (c.f. Fig. 4d).

At a cutoff value > 13, the MELD score yielded a sensitivity of 65%, a specificity of 100%, a positive predictive value of 100%, a negative predictive value of 74.1%, and a diagnostic accuracy of 82.5%. The sensitivity, specificity, positive predictive value, negative predictive value, and the overall diagnostic accuracy of PVD were 65%, 90%, 86.7%, 72%, and 77.5%, respectively, at a cutoff > 13.6 mm, whereas those of CFT-EXTEM were 64.7%, 84.2%, 78.6%, 72.7%, and 75%, respectively, at a cutoff value > 316 s, and those of CFT-INTEM were 78.95%, 61.1%, 68.2%, 73.3%, and 70.3%, respectively, at a cutoff value > 167 s (c.f. Table 4).

Discussion

Variceal bleeding is considered as a crucial determinant of morbidity and mortality in cirrhotic patients. It occurs nearly in 25–40% of those patients. Each attack is associated with more than 20% risk of mortality, in addition

Table 2 Comparison of rotational thromboelastometry (ROTEM) parameters in the study groups

Variable	Cirrhotic patients with variceal bleeding (G1) (no. = 60)	Patients with stable cirrhosis (G1) (no. = 60)	*p*-value
CT (s [median (IQR)]			
EXTEM (38–79)	56.5 (47.3–74.8)	53.5 (46.3–59.5)	0.23‡
INTEM (100–240)	181 (137.5–225)	174.5 (162.5–205.3)	0.99‡
CFT (s) [median (IQR)]			
EXTEM (34–159)	392 (200.5–796)	195 (123–309)	**0.01**‡
INTEM (30–110)	251 (170–402)	156 (100.5–207)	**0.01**‡
α-angle (degree) [mean ± SD]			
EXTEM (63–83)	52.7 ± 17.4	65.5 ± 9.9	**0.01**†
INTEM (70–83)	51.6 ± 14.3	65.8 ± 13	0.01†
MCF (mm) [median (IQR)]			
EXTEM (50–72)	30 (22.5–43)	45 (40–58)	**0.002**‡
INTEM (50–72)	37.5 (31–46)	47 (40–59)	**0.01**‡
FIBTEM (9–25)	10 (9–13.8)	11.5 (7.3–15.5)	**0.45**‡
Clot lysis			
A 10 (mm) [mean ± SD]			
EXTEM (43–65)	25.1 ± 11.5	39.1 ± 12.7	**0.001**†
INTEM (44–66)	31.7 ± 11.9	43.1 ± 15.3	**0.01**†
ML (% of MCF) [median (IQR)]			
EXTEM (< 15)	0 (0–0)	0 (0–3.5)	**0.32**‡
INTEM (< 15)	0 (0–0)	0 (0–2)	**0.61**‡

Values in the parentheses are normal ranges for ROTEM parameters

Bold values denote statistically significant results

G Group, *no*. Number, *CT* Clotting time, *CFT* Clot formation time, *MCF* Maximum clot firmness, *A 10* Clot amplitude at 10 min, *ML* Maximum lysis, *SD* Standard deviation, *IQR* Interquartile range

† Student's *t*-test

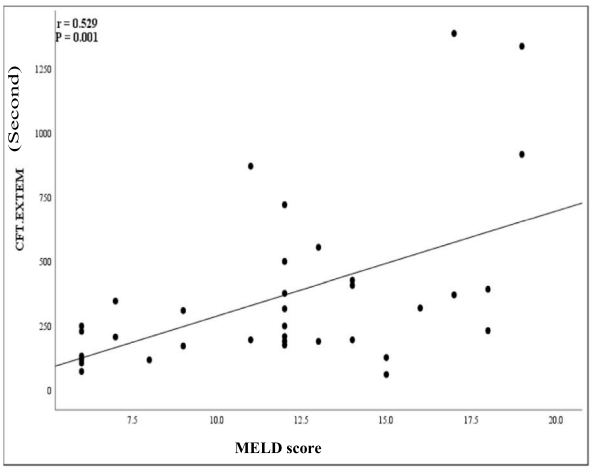
‡ Mann–Whitney *U*-test

to an increased propensity of rebleeding if no preventive aids are used [22]. Although endoscopic band ligation is considered a first-line measure to control bleeding in patients with cirrhosis, it may trigger a profuse bleeding due to post-ligation ulcer [23]. Unfortunately, the SCTs such as PT, INR, and aPTT do not match with procedure-related bleeding in cirrhotic patients [10].

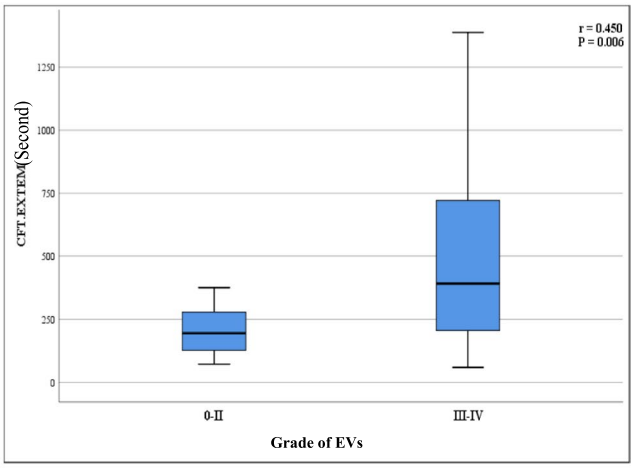
ROTEM profile in the current study showed that cirrhotic patients with bleeding phenotype frequently exhibited hypocoagulable features in the form of delayed clot formation and decreased clot strength in both EXTEM and INTEM assays. Although CT values in both assays were more prolonged in cirrhotic patients who bled compared to those who did not, the differences were not statistically significant. Our findings could be explained by the repeated blood transfusion that might mitigate various coagulation defects [24]. This hypocoagulable state has also been reported in patients with advanced cirrhosis by other investigators [25, 26]. In contrast, studies that were carried out in patients with compensated cirrhosis, and acute hepatic failure, revealed rebalanced coagulation or hypercoagulability [27, 28].

Among our cirrhosis bleeders, the hypocoagulable state was related to thrombocytopenia in 65% of patients. In addition, defective platelet function was probably contributing to hypocoagulability in 15% of patients; however, this diagnosis should be confirmed by platelet mapping assay [29] that was not yet available in our lab. Relying on the results of MCF-FIBTEM that is a good reflection of plasma fibrinogen levels [30], hypofibrinogenemia was observed in 35% of patients. Unfortunately, plasma fibrinogen levels were not measured in this study. Our findings indicated that platelet counts and fibrinogen levels were the main determinants of clot kinetics and clot strength in patients with advanced cirrhosis. Similar results were reported in other study [31].

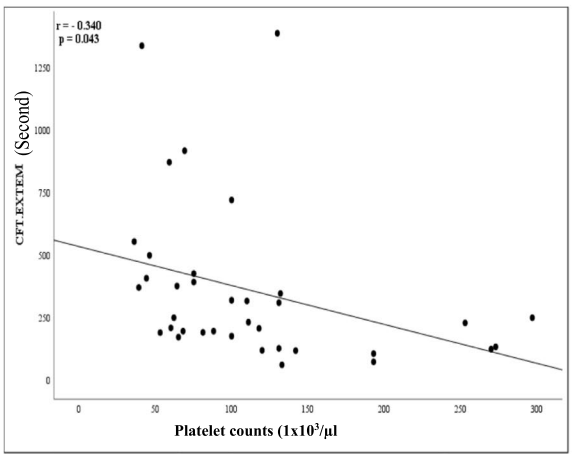
Another relevant finding of this study was the demonstration of hypofibrinolysis in cirrhotic patients. Another study reported similar results, where authors found a decrease in clot lysis in patients with acute-on-chronic liver failure when compared to patients with acute decompensation especially in those with poor short-term prognosis [26]. This hypofibrinolysis state could contribute to organ failure via preservation of microvascular



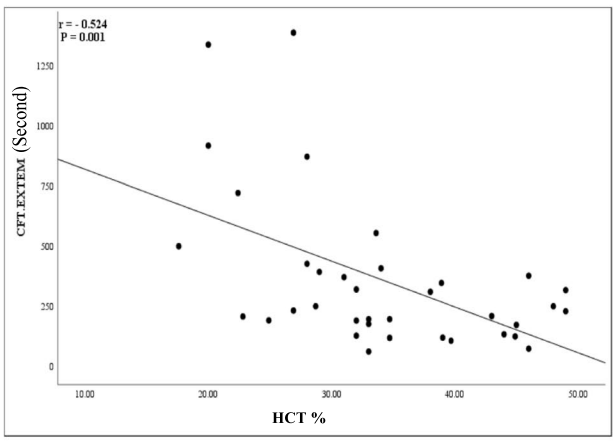
a: The correlation between the clot formation time (CFT) by EXTEM and the Model of End Stage Liver Disease (MELD) score in cirrhotic patients



b: The correlation between the clot formation time (CFT) by EXTEM and the grade of esophageal varices (EVs) in cirrhotic patients



c: The correlation between the clot formation time (CFT) by EXTEM and the platelet counts in cirrhotic patients



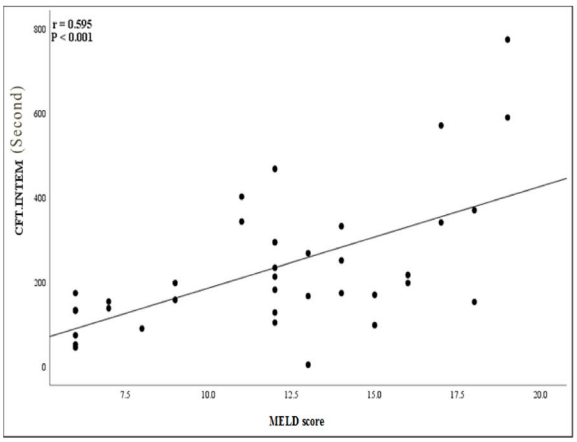
d: The correlation between the clot formation time (CFT) by EXTEM and the percentage of hematocrit(HCT) in cirrhotic patients

Fig. 2 **a** The correlation between the clot formation time (CFT) by EXTEM and the model of end-stage liver disease (MELD) score in cirrhotic patients. **b** The correlation between the clot formation time (CFT) by EXTEM and the grade of esophageal varices (EVs) in cirrhotic patients. **c** The correlation between the clot formation time (CFT) by EXTEM and the platelet counts in cirrhotic patients. **d** The correlation between the clot formation time (CFT) by EXTEM and the percentage of hematocrit (HCT) in cirrhotic patients

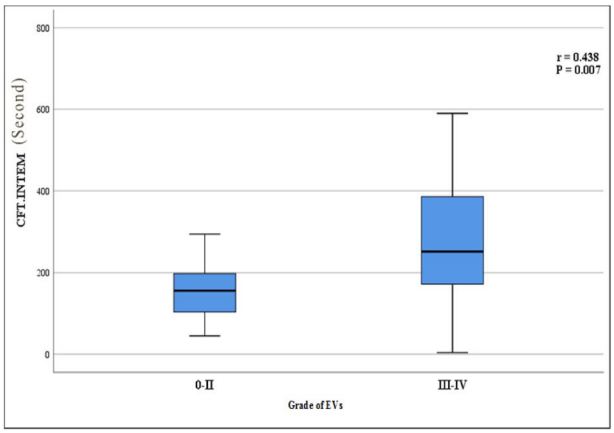
fibrin deposition. Significant association between hypofibrinolysis and severity of organ failure in patients with trauma and sepsis was confirmed by Prakash et al. [32]. In the current study, occurrence of spontaneous bacterial peritonitis as a source of sepsis could not be precisely excluded in cirrhotic bleeders. Spontaneous bacterial peritonitis is considered a risk factor and a consequence of variceal hemorrhage [33].

In the contrary, Kleinegris et al. [25] reported no alteration in clot lysis in patients with decompensated cirrhosis when evaluated by tissue plasminogen activator-ROTEM assay. Using thrombin generation test, Fisher et al. [34] reported variable clot lysis times in patients with acute-on-chronic liver failure compared to those with acute decompensation. These discrepant results might be explained by different patients' characteristics and measurement methods.

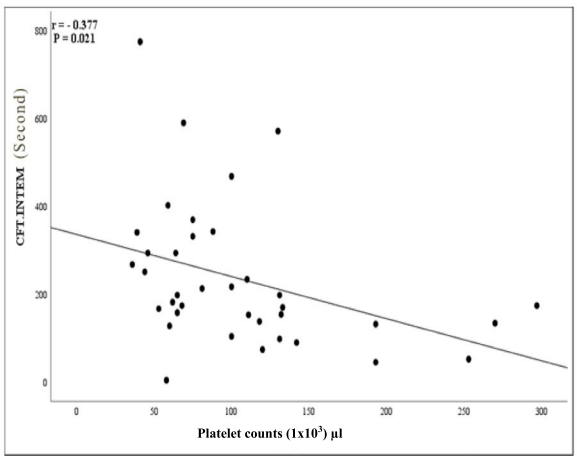
One of the valuable results of the current study was the illustration that prolonged values of CFT in EXTEM and INTEM might institute extra marker of disease severity



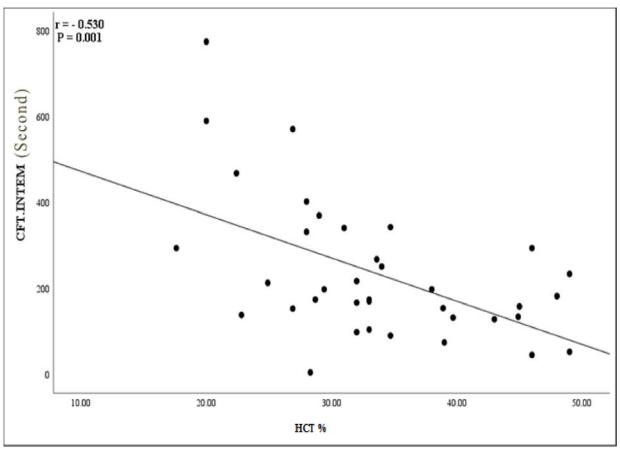
a: The correlation between the clot formation time (CFT) by INTEM and the Model of End Stage Liver Disease (MELD) score in cirrhotic patient



b: The correlation between the clot formation time (CFT) by INTEM and the grade of esophageal varices



c: The correlation between the clot formation time (CFT) by INTEM and the platelet counts in cirrhotic patients



d: The correlation between the clot formation time (CFT) by INTEM and the percentage of hematocrit(HCT) in cirrhotic patients

Fig. 3 **a** The correlation between the clot formation time (CFT) by INTEM and the model of end-stage liver disease (MELD) score in cirrhotic patient. **b** The correlation between the clot formation time (CFT) by INTEM and the grade of esophageal varices (EVs) in cirrhotic patients. **c** The correlation between the clot formation time (CFT) by INTEM and the platelet counts in cirrhotic patients. **d** The correlation between the clot formation time (CFT) by INTEM and the percentage of hematocrit (HCT) in cirrhotic patients

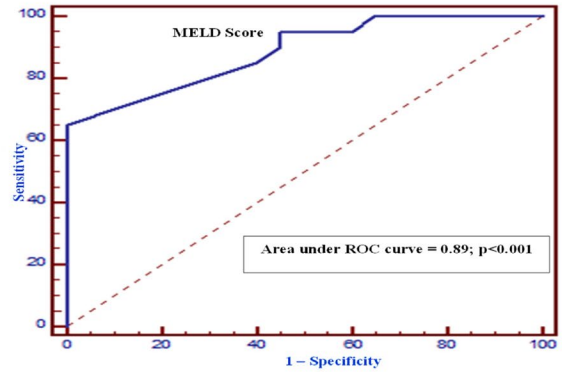
Table 3 Best-fitting multiple stepwise logistic regression predictors of acute variceal bleeding in cirrhotic patients

Variable	AOR	95% confidence interval	*p*-value
MELD score	2.75	1.23–3.41	**0.002**
Portal vein diameter (mm)	2.65	1.16–3.35	**0.01**
CFT-EXTEM (s)	1.9	1.04–2.45	**0.02**
CFT-INTEM (s)	1.78	1.02–2.14	**0.01**

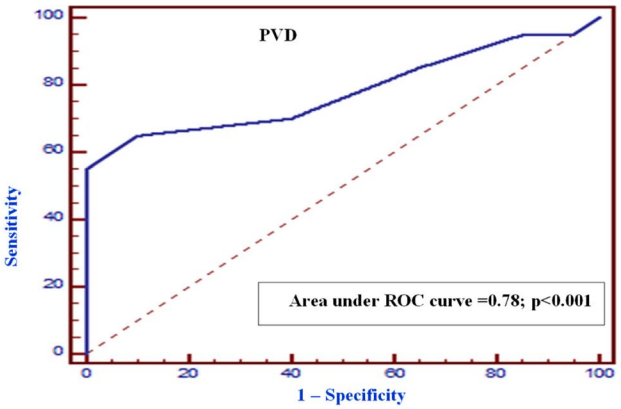
AOR adjusted odds ratio after controlling for other confounders. Bold values denote statistically significant results

MELD Model of end-stage liver disease, *CFT* Clot formation time

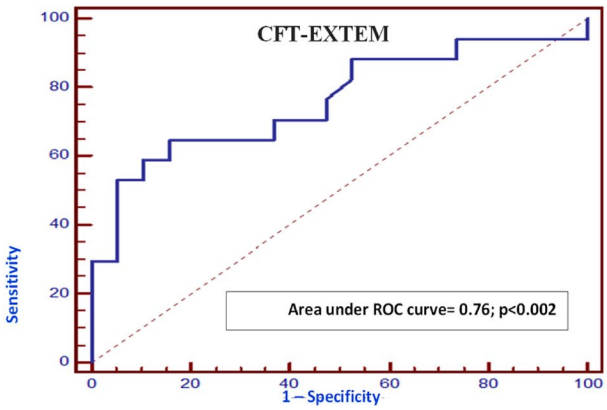
and prognosis in patients with liver cirrhosis. In this study, the prolongation of the aforementioned ROTEM parameter showed a significant association with indices of increased severity of portal hypertension (thrombocytopenia and increased grade of esophageal varices) and deterioration of functional status of the liver as judged by MELD score. MELD is a scoring system that is used to evaluate the disease severity and to predict the short-term survival in cirrhosis patients. Oftentimes, it is used as a robust surrogate of the older Child–Pugh score [16]. By similarity with the increased MELD score and wider PVD, the increased values of CFT in both ROTEM assays



a: Receiver operator characteristic (ROC) curve for Model of End Stage Liver Disease (MELD) score in cirrhotic patients

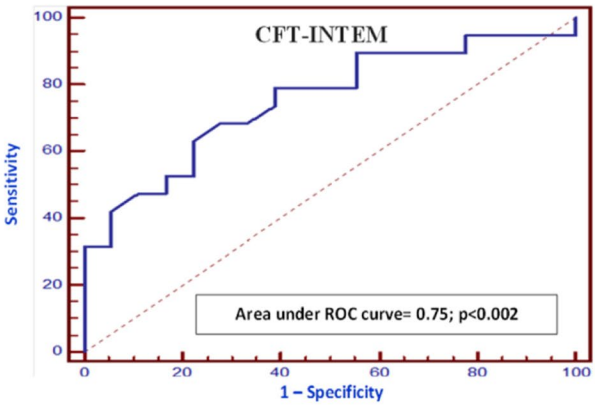


b: Receiver operator characteristic (ROC) curve for portal vein diameter (PVD) in cirrhotic patients



CFT: Clot formation time.

c: Receiver operator characteristic (ROC) curve for CFT-EXTEM in cirrhotic patients



CFT: Clot formation time.

d: Receiver operator characteristic (ROC) curve for CFT-INTEM in cirrhotic patients.

Fig. 4 **a** Receiver operator characteristic (ROC) curve for model of end-stage liver disease (MELD) score in cirrhotic patients. **b** Receiver operator characteristic (ROC) curve for portal vein diameter (PVD) in cirrhotic patients. **c** Receiver operator characteristic (ROC) curve for CFT-EXTEM in cirrhotic patients. CFT, clot formation time. CFT, clot formation time. **d** Receiver operator characteristic (ROC) curve for CFT-INTEM in cirrhotic patients. CFT, clot formation time

Table 4 Diagnostic performance of the best cutoff values of independent predictors of variceal bleeding in cirrhotic patients

Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
MELD:> 13	65	100	**100**	**74.1**	**82.5**
PVD (mm):> 13.6 mm	65	90	**86.7**	**72**	**77.5**
CFT-EXTEM (s):> 316	64.7	84.2	**78.6**	**72.7**	**75**
CFT-INTEM (s):> 167	78.95	61.1	**68.2**	**73.3**	**70.3**

PPV Positive predictive value, *NPV* Negative predictive value, *MELD* Model of end-stage liver disease, *PVD* Portal vein diameter, *CFT* Clot formation time

were identified to be an independent predictor of variceal hemorrhage in a multivariate analysis which revealed that at cutoff values > 316 s for CFT EXTEM and > 167 s for CFT-INTEM, severe coagulopathy could evolve.

It is worth mentioning that the hemoglobin and hematocrit levels were significantly higher in cirrhotic patients with variceal bleeding. It has been reported that red blood cells enhance the release of adenosine diphosphate

under shear flow which facilitates platelet aggregation; therefore, anemia certainly worsens bleeding [35]. It was interesting to demonstrate a substantial negative correlation between hematocrit levels and values of CFT in both EXTEM and INTEM.

Undoubtedly, this study had some limitations. First is the relatively small number of patients. Second, being a case-controlled and hospital-based study, overestimation of the study results could not be completely excluded. Third, neither complete hemostatic profile nor thrombin generation test was evaluated due to financial obstacles. Finally, ROTEM was performed in the absence of thrombomodulin that is responsible for activation of protein C, an issue that could bias the evaluation of the study results.

In conclusion, despite the superior diagnostic performance of MELD score and wide PVD, ROTEM testing may be a reasonable tool to guide transfusion requirements in cirrhosis patients with acute variceal bleeding to avoid massive and unnecessary transfusions that worsen portal hypertension as a result of volume overload. Larger multicenter studies are still required to assess if this test can predict bleeding in those patients and whether targeted correction of those ROTEM abnormalities could improve patients' outcome.

Abbreviations

ROTEM	Rotational thromboelastometry
EXTEM	Extrinsically activated thromboelastometry
INTEM	Intrinsically activated thromboelastometry
FIBTEM	Fibrinogen thromboelastometry
APTEM	Aprotinin thromboelastometry
CT	Clotting time
CFT	Clot formation time
MCF	Maximum clot firmness
HCV	Hepatitis C virus
A10	Amplitude of the curve at 10 min
ML	Maximum lysis
SCTs	Standard coagulation tests
PT	Prothrombin time
INR	International normalized ratio
aPTT	Activated partial thromboplastin test
MELD	Model for end-stage liver disease
PVD	Portal vein diameter

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Authors' contributions
MA, had full access to all of the study data, she conceived and planned the idea of the study, verified the accuracy of the data; contributed to the interpretation of the results, and wrote the manuscript. AA, acquisition of data. OA, performed the ROTEM assays. NI, worked out almost all the laboratory analyses and contributed to the interpretation of ROTEM data. TA, reviewed the results of ROTEM assays. SM, performed the esophagogastroduodenoscopy and evaluated the results. AS, supervised all the findings of this work. All authors have read and approved the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Institutional Review Board, Faculty of Medicine, Minia University, Egypt. The study was conducted according to the guidelines of the 1975 Helsinki Declaration and International Conference on Harmonization Guidelines for Good Clinical Practice. Informed written consent was obtained from all patients.

Consent for publication
Informed written consent for publication was obtained from all participants.

Competing interests
The authors declare that they have no competing interests.

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