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Serial rotational thromboelastometry measurements show worsening hypocoagulability in acute-on-chronic liver failure and are associated with the severity of liver disease

George Kampelos[a], Larisa Vasilieva[b], Theodoros Alexopoulos[c], Iliana Mani[a], Emilia Hadziyannis[a], Stavroula Giannouli[a], Sofia Manioudaki[d], Efrosyni Nomikou[e], Alexandra Alexopoulou[a]

Medical School, National and Kapodistrian University of Athens, Hippokration General Hospital; Alexandra General Hospital; National and Kapodistrian University of Athens, Medical School, Laiko General Hospital; Sismanogleio General Hospital of Athens; Hippokratio General Hospital of Athens, Athens, Greece

Abstract

Background Viscoelastic tests are used to better understand the complex picture of hemostasis in cirrhosis. Limited data exist regarding the clinical relevance of rotational thromboelastometry (ROTEM) in acute-on-chronic liver failure (ACLF) or acute decompensation (AD). We examined the pattern and role of sequential observations of 9 ROTEM components in both ACLF and AD groups.

Method ROTEM measurements were compared within and between groups at 3 time points: on admission (T1), at 24 h (T2) and 48 h post-admission (T3).

Results Forty-two consecutive patients (22 ACLF, 20 AD) were included. ROTEM determinants exhibited significant hypocoagulable deterioration in ACLF but not in AD over the 3 time points in clot formation time $(CFT)_{EXTEM}$ (P=0.01), maximum clot $firmness_{EXTEM}$ (P=0.014), CFT_{INTEM} (P<0.001), and $alpha_{INTEM}$ (P=0.028). The sum of hypocoagulable determinants increased from T1 to T3 in ACLF (P=0.029), but remained stable in AD. Five ROTEM variables showed significant differences towards hypocoagulability in ACLF compared to AD at T3. A "hypocoagulable" profile was associated with more severe liver disease (P<0.001 for model for end-stage liver disease [MELD] or Child-Pugh scores) and higher 30- and 90-day mortality (log-rank P=0.001 and P=0.013, respectively) but no more bleeding episodes or transfusions. Two ROTEM variables displayed strong correlations with MELD at T1 and 7 at T3 (|r coefficient|>0.5).

Conclusions ROTEM measurements indicated worsening hypocoagulability shortly post-admission compared to baseline in ACLF, but remained stable in AD. The hypocoagulable derangement was mostly correlated with the severity of liver disease and higher short-term mortality, but not more bleeding episodes.

Keywords Acute-on-chronic liver failure, acute decompensation, rotational thromboelastometry "hypocoagulable" profile

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Conflict of Interest: None

Correspondence to: Alexandra Alexopoulou, 2nd Department of Internal Medicine and Research Laboratory, Medical School, National & Kapodistrian University of Athens, Hippokration General Hospital, 114 Vas. Sofias Av, 11527 Athens, Greece,
e-mail alexopou@ath.forthnet.gr

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Introduction

Coagulopathy plays a pivotal role in morbidity and mortality in critically ill patients with cirrhosis, namely those with acute-on-chronic liver failure (ACLF), a syndrome characterized by extrahepatic multi-organ failures and high mortality [1,2], and those with acute decompensation (AD), who exhibit an elevated systemic inflammatory response without multi-organ failures [3].

Conventional coagulation tests (CCTs) are routine hemostatic assays that cannot accurately identify patients with coagulopathy. They may be able to evaluate the procoagulant defects—mainly due to reduced coagulation factors, fibrinogen and platelets—but are unable to reveal the anticoagulant aberrations in liver cirrhosis [4,5].

These alterations are evidenced in liver cirrhosis as proteins (S and C) are decreased, factors favoring platelet adhesion and clot formation are increased (von Willebrand factor-vVF and factor VIII) and levels of ADAMTS-13 (a metalloproteinase that cleaves large multimers of vVF) levels are decreased, leading to enhanced procoagulant activity [6]. Consequently, a coagulation rebalance is achieved in cirrhotics. This rebalance may be abrogated in end-stage liver disease, when a precipitating event triggers a severe complication [6,13].

To better characterize the complex picture of hemostasis, viscoelastic tests (VCAs), i.e., rotational thromboelastometry (ROTEM), thromboelastography [7], and Sonoclot [8], have been used to display both procoagulant and anticoagulant abnormalities of liver cirrhosis [9]. VCAs may better predict the risk of invasive procedure-related bleeding compared to CCTs in decompensated cirrhosis [10-13], and have been applied to study the fast and dynamic development of coagulation defects during an acute event [14]. Specifically, ROTEM, which assesses the stability and time-properties of clot formation in non-centrifuged, citrated whole blood, might be better than CCTs for detecting coagulation disturbances *in vivo* [9,14].

ROTEM kinetics were recently studied in critically ill patients in the intensive care unit, with severe trauma [15], sepsis [16], or COVID-19 [17,18], to explore whether serial ROTEM measurements could identify persons at high risk of complications or death. However, limited data exist regarding the clinical relevance of ROTEM kinetics in ACLF or AD [19]. The aim of the current study was to examine the pattern of serial ROTEM changes within and between ACLF and AD groups and to unravel whether ROTEM kinetics were associated with the severity of liver disease and outcome.

Patients and methods

Study population

A prospective, observational study, investigating the hemostatic changes tested by ROTEM, was performed in a single tertiary care hospital from June 2019 to March 2021. The diagnosis of cirrhosis was based on previous liver histology and/or imaging, laboratory, endoscopic or clinical findings. Consecutively admitted patients with ACLF or AD according to previously defined criteria [3,20] were included.

Patients with malignancies, anticoagulant or antiplatelet treatment, recent surgical intervention, those transfused with red blood cells, blood products or coagulation factor concentrates within 7 days prior to admission or within 48 h post-admission, and patients with known coagulation disorders or thrombotic events were excluded.

The study protocol was approved by the Hospital Ethics Committee. All participants or their relatives provided written informed consent prior to study enrolment.

[a]2nd Department of Internal Medicine and Research Laboratory, Medical School, National and Kapodistrian University of Athens, Hippokration General Hospital (George Kampelos, Iliana Mani, Emilia Hadziyannis, Stavroula Giannouli, Alexandra Alexopoulou); [b]Gastroenterology Department, Alexandra General Hospital (Larisa Vasilieva); [c]Gastroenterology Department, National and Kapodistrian University of Athens, Medical School, Laiko General Hospital (Theodoros Alexopoulos); [d]Intensive Care Unit, Sismanogleio General Hospital of Athens (Sofia Manioudaki); [e]Blood Bank and Haemophilia Unit, Hippokratio General Hospital of Athens (Efrosyni Nomikou), Athens, Greece

Collection of data

Demographic, clinical and laboratory data were obtained on admission. The severity of liver disease was assessed by the model for end-stage liver disease (MELD) and Child-Pugh scores. Components of MELD and Child-Pugh scores were also examined separately. All infections on admission and during hospitalization were recorded. Systemic inflammation was assessed by measuring white blood cell and neutrophil counts, C-reactive protein and neutrophil-to-lymphocyte ratio (NLR). An absolute increase in serum creatinine of ≥0.3 mg/dL in less than 48 h, or a 1.5-fold increase from baseline in less than 7 days, was used as the definition of acute kidney injury (AKI) [21]. All patients were hospitalized and treated with standard of care for hepatic encephalopathy, variceal bleeding, AKI and bacterial infections [21].

The study was not designed to guide the transfusion of red blood cells, blood products or coagulation factor concentrates to minimize the bleeding risk before any invasive procedures. The transfusion of the above products remained at the discretion of the attending physician, who was not aware of the ROTEM measurements.

Patients were prospectively followed-up during hospitalization and, if discharged, at the outpatient clinic, using electronic medical records and telephone communication every 30 days. The outcome was assessed at 30 and 90 days.

ROTEM

All included patients were tested with the following ROTEM assays: extrinsic coagulation pathway thromboelastometry (EXTEM); intrinsic coagulation pathway thromboelastometry (INTEM); and fibrin polymerization thromboelastometry (FIBTEM), according to the standard protocols supplied by the manufacturer [22-24]. For ROTEM analysis (ROTEM, Delta device, TEM innovations, Munich Germany), citrated blood was analyzed at 37°C within 1 h of blood being drawn. The following variables were measured in the curves generated by EXTEM and INTEM assays: clotting time (CT), clot formation time (CFT), alpha-angle, and maximum clot firmness (MCF). MCF was the only parameter analyzed from the FIBTEM test. Reference ranges were defined in accordance with previously published recommendations (Reference Ranges 2012-02 Ver0007.Doc Copyright © 2012 Tem Innovations GmbH Edition 2012-02-07).

The above 9 ROTEM variables were assessed at 3 different time points: at baseline (on admission) (time point 1, T1), at 24 h (time point 2, T2) and at 48 h post-admission (time point 3, T3). A sum score from 0 to 9 was also calculated over

the 3 time points: 1 point for each parameter out of range towards hypocoagulability; minimum=0, if no hypocoagulable parameter, to maximum=9, if all the above 9 ROTEM parameters were out of range, indicating hypocoagulability.

A "hypocoagulable" profile by ROTEM (definite) was defined if all 4 (CT, CFT, MCF, alpha angle) variables evaluating clot formation and stability in EXTEM, INTEM or FIBTEM, were indicative of hypocoagulation (or below normal range) according to Guvea *et al* [25] and Campello *et al* [26]. All the remaining patients were considered "non-hypocoagulable" (including "likely hypocoagulable" and normal profile) [25,26].

The "hypocoagulable" profile was determined at T1 and T3 for all patients, and the results were compared.

Statistical analysis

Categorical variables were expressed as count (percentage), and differences were compared using the chi-square test. Quantitative variables were expressed as median and interquartile ranges or mean (standard deviation), according to the Kolmogorov-Smirnov test for the normality of the distribution. Mann-Whitney *U* and Student's *t*-tests were used according to the normality of data distribution to identify differences between 2 groups. Kruskal-Wallis or 1-way analysis of variance (ANOVA) tests for non-normally or normally distributed variables, respectively, were conducted to determine differences among 3 groups. Bonferroni's method was used to adjust for multiple comparisons. The Friedman test (followed by a Wilcoxon signed-rank test) and repeated measures analysis of variance (ANOVA) for non-normally or normally distributed variables, respectively, were used to investigate changes in ROTEM variables in the same subjects within groups (ACLF or AD, separately) over the 3 time points. For the repeated measures ANOVA, Mauchly's test of sphericity was used to test the assumption of sphericity; when it yielded statistically significant results, the Greenhouse-Geisser correction was used to adjust for violations of sphericity. The cumulative probability of survival was evaluated using the Kaplan-Meier method and the differences were tested using the log-rank test. All statistical analyses were performed using the statistical package SPSS (version 21; SPSS Inc., Chicago, Ill., USA).

Results

Patients' characteristics

Forty-two patients (22 ACLF and 20 AD) were consecutively admitted for an episode of acute decompensation. The causes of admission were the following: bacterial infection (N=16), hepatic encephalopathy (N=9), alcoholic hepatitis (N=6), gastrointestinal (GI) bleeding (N=6), and AKI (N=5).

Table 1 contains the baseline demographic and clinical characteristics of the patients. Liver disease was more severe (as assessed by MELD, international normalized ratio [INR], and total bilirubin) in ACLF compared to AD, and AKI (accompanied by high creatinine) was more common in ACLF. In addition, white blood cell count, neutrophil count and NLR representing a high inflammatory response were higher in ACLF patients. Activated partial thromboplastin time (aPTT) levels were more elevated in ACLF compared to AD. No significant differences were found in relation to age, sex, the etiology of liver disease, or other laboratory characteristics.

Twenty healthy subjects matched for age and sex with the patients (80% male; median age 63 years [55.5-68]; P=0.737, P=0.2, respectively) were used as controls and ROTEM variables were measured.

Dynamic ROTEM variable changes in the same subjects over 3 time points (ACLF or AD, separately)

Based on the results of Friedman or repeated-measures ANOVA tests, significant variations over time in CFT_{EXTEM}, MCF_{EXTEM}, CFT_{INTEM}, and $alpha_{INTEM}$ were revealed in ACLF patients (P=0.01, P=0.014, P<0.001 and P=0.028, respectively) (Table 2). Wilcoxon signed-rank or multiple comparison tests showed significant differences between time points T1 vs. T3 (P=0.002), and T2 vs. T3 (P=0.001) for CFT_{EXTEM}; T1 vs. T3 (P=0.041) for MCF_{EXTEM}; T1 vs. T3 (P=0.004) and T2 vs. T3 (P=0.001) for CFT_{INTEM} and time points T2 vs. T3 (P=0.05) for alpha $angle_{INTEM}$, in ACLF patients. No changes in individual ROTEM determinants over time were observed within the AD group (Table 2, Fig. 1).

The mean total score (Sum) of hypocoagulable ROTEM measurements exhibited a statistically significant increase from 1.5 (0-8) at T1 to 3 (0.8-7) at T2, and finally to 5.5 (2-9) at T3, in ACLF patients (P=0.029) (Table 2, Fig. 1). A Wilcoxon signed-rank test showed significant changes between time points T1 and T3 (P=0.011) and between T2 and T3 (P=0.02). No difference in the medians of Sum score over the 3 time points were observed in AD patients (Table 2, Fig. 1).

ROTEM variable differences between ACLF and AD

Statistically significant differences between ACLF, AD and controls were demonstrated in 5 ROTEM values at baseline (CFT_{EXTEM}, MCF_{EXTEM}, $alpha_{EXTEM}$, CFT_{INTEM}, and MCF_{INTEM}) (Table 3). A significant difference between ACLF and AD at baseline was demonstrated only in $alpha_{EXTEM}$. However, 48 h post-admission, the variations between ACLF and AD became statistically significant for 5 parameters, CFT_{EXTEM}, MCF_{EXTEM}, $alpha_{EXTEM}$, CFT_{INTEM} and $alpha_{INTEM}$, and borderline in CT_{EXTEM}, as a result of the worsening of coagulation measurements towards hypocoagulability in ACLF patients (Table 3).

"Hypocoagulable" vs. "non-hypocoagulable" profile

Patients with a "hypocoagulable" profile (N=11) compared with those without (N=31) had more severe liver disease

Table 1 Comparison of baseline clinical characteristics, laboratory, conventional coagulation 3 tests and mortality rates, between ACLF and AD groups

Clinical characteristics and demographics	Total N=42	AD N=20	ACLF N=22	P-value
Age	58.8 (13.3)	58.1 (13.2)	59.6 (13.7)	0.722
Male sex, n (%)	32 (76.2%)	14 (70%)	18 (81.8%)	0.369
Etiology				
Alcoholic	17 (40.5%)	9 (45%)	8 (36%)	
Viral	9 (21.4%)	(25%)	4 (18%)	0.584
Other	16 (38.1%)	(30%)	10 (45.5%)	
Severity of liver disease				
MELD score	22.8 (9.9)	16.1 (5.4)	28.9 (9.2)	<0.001
Child-Pugh score (points)	11.2 (2.4)	10.6 (2.5)	11.8 (2.2)	0.109
Child-Pugh class (N%)				
A	1 (2.4%)	51 (5%)	0 (0%)	0.347
B	10 (23.8%)	6 (30%)	4 (18.2%)	
C	31 (73.8%)	13 (65%)	18 (81.8%)	
Acute kidney injury	19 (45.2%)	2 (10%)	17 (77.3%)	<0.001
Laboratory values				
Hemoglobin (g/dL)	9.1 (2.3)	9.6 (1.8)	8.6 (2.7)	0.183
Total bilirubin (mg/dL)	5.8 (1.8-11.5)	4.8 (1.6-6.4)	9.9 (2.8-20.5)	0.02
White blood cells ($\times 10^9$ /L)	5.6 (4.1-8.6)	4.7 (3.6-7.6)	6.4 (5.0-12.4)	0.022
Neutrophils ($\times 10^9$ /L)	3.9 (2.7-7.4)	3.1 (2.5-5.7)	5.1 (3.0-10.7)	0.023
Neutrophil-to-lymphocyte ratio	8.5 (7.4)	5.7 (6.4)	11 (7.5)	0.019
C-reactive protein	46.7 (40.5)	48.7 (44)	44.9 (38)	0.768
Creatinine (mg/dL)	1.0 (0.8-2.2)	0.8 (0.6-0.9)	2.2 (1.8-2.8)	<0.001
Albumin (g/dL)	3.0 (1.0)	2.8 (1.1)	3.1 (1.0)	0.481
Conventional coagulation tests				
INR	1.7 (1.3-2.2)	1.5 (1.2-1.9)	1.9 (1.5-3.6)	0.009
aPTT (sec)	48.2 (21.6)	38.4 (7.2)	57.0 (26.3)	0.006
Platelets ($\times 10^9$/L)	93.1 (55.0)	101.6 (86.2)	57.5 (53.2)	0.383
Fibrinogen (mg/dL)	218.3 (110.0)	235.2 (99.0)	203.0 (119.3)	0.349
Mortality				
30-day	9 (21.4%)	0 (0%)	9 (40.9%)	0.001
90-day	14 (33.3%)	2 (10%)	12 (54.5%)	0.001

Values as mean (standard deviation) or median (interquartile range) reported according to normality of distribution and Mann Whitney U or t-test were used accordingly; the difference in survival was computed in Kaplan-Meier curves (log-rank)

ACLF, acute-on-chronic liver failure; AD, acute decompensation; ROTEM, rotational thromboelastometry; MELD, model for end-stage liver disease; INR, international normalized ratio; aPTT, activated partial thromboplastin time

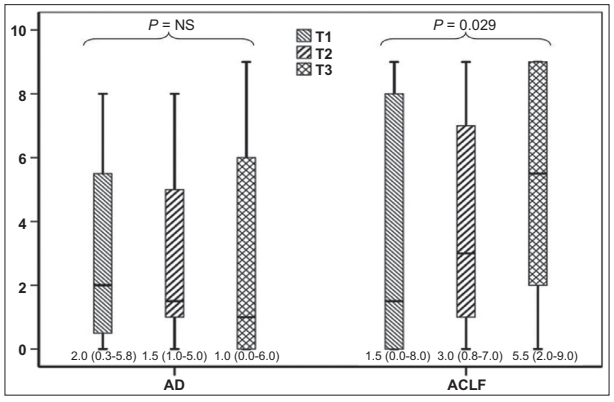

Figure 1 The mean total score (Sum) of hypocoagulable ROTEM parameters exhibited a statistically significant gradual increase from T1 (baseline) to T2 (24 h) to T3 (48 h post-admission) in ACLF but stable or improving values in AD patients

ROTEM, rotational thromboelastometry; ACLF, acute-on chronic liver failure; AD, acute decompensation

according to MELD (P<0.001) and Child-Pugh scores (P<0.001). Infection episodes were more prevalent (P=0.005) and NLR higher (P=0.022) in patients with a "hypocoagulable" phenotype. No significant variations were demonstrated in the etiology of liver disease, AKI or inflammatory reaction markers.

Regarding patients meeting the criteria for a "hypocoagulable" profile, variability was noted over time in percentage (N=11 [26.2%] at T1 and N=12 [28.5%] at T3) and in status; namely, 2 patients who were "hypocoagulable" at T1 became "non-hypocoagulable" at T3, while the reverse was found for 3 patients. ACLF was more prevalent than AD among "hypocoagulable" patients (72.7% vs. 27.3% at T1 and 75% vs. 25% at T3, respectively) (Table 4).

No spontaneous major bleeding events apart from GI bleeding (massive spontaneous deep hematomas, spontaneous intracranial hemorrhage, spontaneous hemoperitoneum, orbital hemorrhage) [13] were recorded on admission or

Table 2 Changes in mean values of individual ROTEM variables within groups (ACLF or AD) over 3 time points (T1, T2, T3). The changes in the total of 9 ROTEM parameters indicating hypocoagulability 3 (sum score) were also assessed

ROTEM measures	Time	ACLF			AD		
				P-value			P-value
CT_{EXTEM} Median (IQR)	T1	75.5 (69.8-108)		0.135	75.5 (60-86)		0.368
	T2	84.5 (66-103)			73 (66.3-80)		
	T3	86 (73-114.3)			73 (60.5-90)		
CFT_{EXTEM} Median (IQR)	T1	141 (104.3-288.3)		0.01	146 (94.5-195.3)		0.638
	T2	138 (93.8-301.5)			119 (96-194)		
	T3	224 (128.5-396)			124.5 (84.3-193.3)		
MCF_{EXTEM} Mean (SD)	T1	47.1 (14.1)	F=5.966	0.014	52.1 (9.5)	F=0.055	0.894
	T2	47.2 (14.3)			52.4 (10.7)		
	T3	42.6 (13.7)			52.05 (12)		
$alpha_{EXTEM}$ Mean (SD)	T1	59.7 (17.4)	F=2.250	0.139	67.70 (9.2)	F=1.025	0.368
	T2	60.1 (15.4)			67.2 (10.8)		
	T3	55.3 (18.7)			65.5 (11.6)		
CT_{INTEM} Mean (SD)	T1	225.6 (135.4)	F=0.168	0.846	205.6 (84.5)	F=0.010	0.990
	T2	219.1 (99.8)			204.9 (91.1)		
	T3	232.4 (62.7)			207.1 (113.7)		
CFT_{INTEM} Median (IQR)	T1	112 (81.5-312)		<0.001	111 (77-183.5)		0.462
	T2	117 (78-312.3)			113 (82.5-164.8)		
	T3	164 (123.8-375.3)			121 (79.5-245.5)		
MCF_{INTEM} Mean (SD)	T1	45.9 (15.9)	F=2.417	0.104	49.4 (14.3)	F=0.563	0.574
	T2	46.86 (15.1)			51.9 (10.4)		
	T3	43.00 (12.3)			49.4 (13.7)		
$alpha_{INTEM}$ Median (IQR)	T1	70 (48.5-74)		0.028	72 (65-76)		0.133
	T2	69 (51.25-75.3)			70 (65-74.5)		
	T3	62 (45.5-69.0)			71.5 (63-74)		
MCF_{FIBTEM} Mean (SD)	T1	14.9 (7.7)	F=2.375	0.105	17.2 (8.2)	F=0.489	0.563
	T2	13.2 (7.4)			18.30 (9.5)		
	T3	12.2 (7.1)			16.90 (9.5)		
Sum of hypocoagulable parameters Median (IQR)	T1	1.5 (0-8)		0.029	2 (0.3-5.8)		0.983
	T2	3 (0.8-7)			1.5 (1-5)		
	T3	5.5 (2-9)			1 (0-6)		

Mean values (SD) or median (IQR) were reported and repeated measures ANOVA or Friedman test were used according to the normality of the distribution; Bonferroni's method was used to adjust for multiple comparisons

ACLF, acute-on-chronic liver failure; AD, acute decompensation; ROTEM, rotational thromboelastometry; CT, clotting time; CFT, clotting formation time; MCF, maximum clot firmness; SD, standard deviation; IQR, interquartile range

during hospitalization. GI bleeding was observed in 11 patients (variceal bleeding, N=4; gastric ulcer, N=2; ligation-related ulcer, N=1; esophagitis D, N=1; ectopic varices, N=1; portal gastropathy, N=2). No differences in GI bleeding events or red blood cell transfusion requirements were observed between patients with a "hypocoagulable" profile and those without (Table 4).

Similar associations between the "hypocoagulable" and "non-hypocoagulable" profile were observed at T3. Patients with a "hypocoagulable" profile (N=12) vs. those without (N=30) had more severe liver disease according to MELD (P<0.001) and Child-Pugh scores (P<0.001) and more frequent infection episodes (P=0.014). No further differences between the 2 profiles were observed.

No patient had a "hypercoagulable profile" [25]. Four patients were diagnosed with portal vein thrombosis (3 had normal and 1 "hypocoagulable" profile).

Table 3 Mean or median values in individual thromboelastometry variables between ACLF and AD groups at baseline, T2 and T3

Variables	Controls	AD	Baseline (T1)				24 h post-admission (T2)		48 h post-admission (T3)		
			ACLF	P1	P2	AD	ACLF	P3	AD	ACLF	P4
EXTEM											
CT (38-79) sec	69.5 (60.5-76.8)	75.5 (60- 86)	75.5 (69.8- 108)	0.119	0.372	73 (66.3-80)	84.5 (66-103)		73 (60.5-90)	86 (73-114.3)	0.051
CFT (34-159) sec	94 (76.5-114.5)	146 (94.5- 195.3)	141 (104.3-288.3)	<0.001	0.320	119 (96-194)	138 (93.8-301.5)		124.5 (84.3-193.3)	224 (128.5-396)	0.019
MCF (50-72) mm	63 (2.6)	52.0 (9.5)	47.0 (14.1)	<0.001	0.114	52.4 (10.7)	47.2 (14.3)		52.0 (12.0)	42.6 (13.7)	0.024
alpha-angle (63-83)^0	73.4 (2.9)	67.7 (9.2)	59.7 (17.3)	0.002	0.032	67.2 (10.8)	60.1 (15.4)		65.5 (11.6)	55.3 (18.7)	0.039
INTEM											
CT (100-240) sec	182.6 (26.9)	205.6 (84.5)	225.7 (135.4)	0.349	0.498	204.9 (91.1)	219.1 (99.8)		207.1 (113.7)	232.4 (62.7)	0.371
CFT (30-110) sec	76.5 (63.3-83.3)	111 (77-183.5)	112 (81.5-312)	<0.001	0.413	113 (82.5-164.8)	117 (78-312.3)		121 (79.5-245.5)	164 (123.8-375.3)	0.03
MCF (50-72) mm	63.1 (4)	49.4 (14.3)	45.9 (15.9)	<0.001	0.364	51.9 (10.4)	46.86 (15.1)		49.4 (13.7)	43 (12.2)	0.119
alpha-angle (70-73)^0	73.5 (71-75)	72 (65-76)	70 (48.5-74)	0.097	0.165	70 (65-74.5)	69 (51.25-75.3		71.5 (63-74)	62 (45.5-69)	0.02
FIBTEM											
MCF (9-25) mm	16.6 (3.1)	17.2 (8.2)	14.9 (7.7)	0.505	0.287	18.30 (9.5)	13.2 (7.4)		16.9 (9.5)	12.2 (7.1)	0.074

Mean values (SD) or median (IQR) reported in parenthesis

P1, Comparison among controls, ACLF and AD at baseline (1-way ANOVA or Kruskal-Wallis); P2, ACLF vs. AD (Mann-Whitney-U or t-test) at baseline; P3, ACLF vs. AD at 24 h post-admission; P4 ACLF vs. AD at 48 h post-admission. All P-values were adjusted according to Bonferroni's method

ACLF, acute-on-chronic liver failure; AD, acute decompensation; CT, clotting time; CFT, clotting formation time; MCF, maximum clot firmness; SD, standard deviation; IQR, interquartile range

Table 4 Comparison of clinical and laboratory characteristics between patients with "hypocoagulable" profile vs. those without at T1

Characteristics	Patients with "hypocoagulable" profile	Patients without "hypocoagulable" profile	P-value
Etiology			
Alcoholic	6 (54.5%)	11 (35.5%)	0.073
Viral	3 (27.3%)	6 (19.4%)	
Other	2 (18.2%)	14 (45.2%)	
MELD score	32.6 (8.3)	19.3 (8.0)	<0.001
Child-Pugh score (points)	13.4 (1.1)	10.5 (2.4)	<0.001
Acute-on-chronic liver failure	8 (72.7%)	14 (45.2%)	0.116
Acute decompensation	3 (27.3%)	17 (54.8)	
Acute kidney injury	7 (83.6%)	12 (38.7%)	0.154
Bacterial infections	9 (81.8%)	10 (32.3%)	0.005
Gastrointestinal bleeding	2 (18.2%)	9 (29%)	0.482
Transfusions of red blood cells	0 (0-3)	0 (0-1)	0.173
Neutrophil-to-lymphocyte ratio	12.8 (6.8)	6.9 (7.1)	0.022
White blood cell count $\times 10^9$/L	6.2 (3.6-7.6)	5.4 (4.1-9.6)	0.875
Neutrophil count $\times 10^9$/L	4.9 (2.7-6.7)	3.5 (2.7-7.5)	0.520
C-reactive protein (mg/dL)	52.8 (30.6)	44.6 (43.8)	0.568
Creatinine (mg/dL)	2.1 (0.8-3.1)	0.9 (0.8-2.0)	0.093
*Mortality at 30 days	6 (54.5%)	3 (9.7%)	0.001
*Mortality at 90 days	6 (54.5%)	8 (25.8%)	0.013

A 'hypocoagulable' profile was defined if all 4 ROTEM parameters (CT, CFT, a angle, MCF) were 'hypocoagulable' (definite hypocoagulability) according to the definition by Gouvea *et al* [25] and Campello *et al* [26]. Continuous variables are presented as mean (standard deviation) or median (interquartile range) according to normality of distribution; *the difference in survival was computed by Kaplan-Meier curves (log-rank)

ROTEM, rotational thromboelastometry; MELD, model for end-stage liver disease; CT, clotting time; CFT, clotting formation time; MCF, maximum clot firmness

Spearman correlations between ROTEM observations and MELD score

The ROTEM determinants showing the strongest correlation with MELD at T1 ($|r|>0.5$) were MCF_{EXTEM} (r=-0.512, P=0.001) and MCF_{INTEM} (r=-0.510, P=0.001). More ROTEM components were correlated with MELD at T3, including CT_{EXTEM} (r=0.530, P<0.001), CFT_{EXTEM} (r=0.511, P=0.001), MCF_{EXTEM} (r=-0.620, P<0.001), CFT_{INTEM} (r=0.546, P<0.001), MCF_{INTEM} (r=-0.512, P=0.001), $alpha_{INTEM}$ (r=0.531, P<0.001), and MCF_{FIBTEM} (r=-0.526, P<0.001).

Survival analysis

The causes of mortality at 30 days were liver-related: infections and complications, N=4; hepatic encephalopathy, N=3; AKI=1, variceal bleeding and complications, N=1.

As expected, Kaplan-Meier curve analysis showed a higher 30- and 90-day mortality rate in ACLF patients compared to the AD group. Mortality in the ACLF group was 40.9% and 54.5% at 30- and 90-days, respectively, vs. 0% and 10% in the AD group (log-rank P=0.001 for both) (Table 1). The causes of mortality at 90 days were also liver-related.

"Hypocoagulable" patients (T1 time point) displayed a higher mortality rate compared to "non-hypocoagulable" patients at 30 days (54.5% vs. 9.7%; log-rank P=0.001; Fig. 2) and 90 days (54.5% vs. 25.8%; log-rank P=0.013) (Table 4). The deaths at 30 days in the "hypocoagulable" phenotype concerned exclusively ACLF, as no AD patient died within 30 days. Similarly, patients characterized as "hypocoagulable" at the T3 time point had lower survival rates at 30-days compared to "non-hypocoagulable" patients (log-rank P=0.039).

Discussion

In the current study, the ROTEM profile more often included hypocoagulable characteristics in ACLF patients, compared to healthy controls and AD patients. These hypocoagulable alterations deteriorated further at 48 h post-admission in ACLF, whereas they were rather stable in the AD group. The profound hypocoagulable characteristics in the ACLF group were not associated with major bleeding episodes, but with the severity of liver disease and outcome.

Specifically, the ACLF group demonstrated deterioration in individual hypocoagulable ROTEM components, as well as in their sum, implying a complex defect of initiation, speed of clot formation and stability of the clot. This worsening occurred over a short time period, i.e., within 48 h post-admission. Namely,

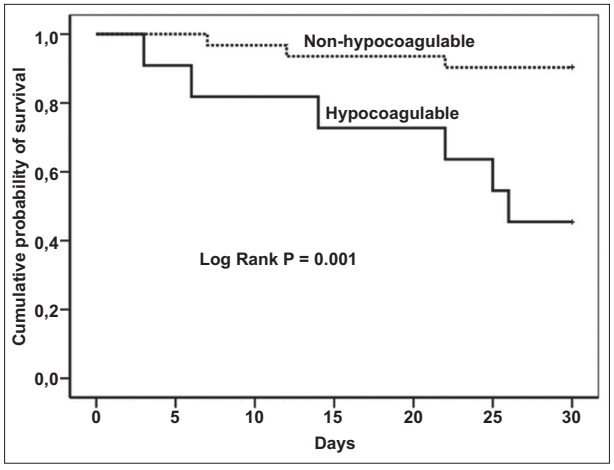

Figure 2 Kaplan-Meier curves in whole series. Observed non-adjusted probability of 30-day survival of patients with "hypocoagulable" vs. those with "non-hypocoagulable" profile (all the remainder)

aggravation in ROTEM values towards hypocoagulability was displayed within ACLF group in both a quantitative and a semi-quantitative way. More specifically, 4 out of 9 distinctive TEM components assessed at baseline further deteriorated 48 h post-admission, while no difference was observed in the AD group. These results demonstrated that serial measurements of clot formation and stability might capture the dynamic process of complex coagulation changes towards hypocoagulability in critically ill patients (ACLF) with cirrhosis. In addition, a considerable variability in coagulation phenotype was also observed, as 12% (N=5) of total patients changed from "hypocoagulable" to "non-hypocoagulable" profile or *vice versa* within 48 h.

A rebalanced hemostatic state is common in cirrhosis, roughly because of the simultaneous decline of both procoagulant and anticoagulant factors [6]. According to the ROTEM output results in our study, this dynamic hemostatic state was rather stable (or improving) in the short-term in AD, but worsened in ACLF patients. Despite this finding, we did not observe any association between the degree of ROTEM alterations, as expressed by the "hypocoagulable" profile, and the occurrence of bleeding episodes or transfusion requirements. The lack of correlation between VCAs alterations and bleeding risk was previously reported by other researchers [19,26,27], who did not identify patients at high risk of bleeding based on ROTEM assessments. This finding may be explained by the fact that the severity of portal hypertension, and not hemostatic failure *per se*, is mainly involved in bleeding events in cirrhosis [9,14,19,26,28]. In addition, ROTEM may not be able to predict bleeding, but it can be used as a tool to guide the need for transfusions or coagulation factors in case of bleeding or before high-risk invasive procedures [13,14,29]. It has been reported that ROTEM's diagnostic accuracy could be improved by adding Protac, a protein C activator. However, this assumption was not confirmed by previous investigators [30]. Our study was not designed to guide transfusion or coagulation factor requirements and no high-risk invasive procedures were applied, except for a few variceal ligations.

We have shown that the ROTEM "hypocoagulable phenotype" detected on admission and at 48 h post-admission was associated with the severity of liver disease, as indicated by higher MELD and Child-Pugh scores and infection episodes. Furthermore, individual ROTEM values, mainly those determined at 48 days post-admission, exhibited high correlation with the MELD score. Previous investigators who evaluated the importance of ROTEM measurements have also reported that VCA determinants may correlate with disease severity markers such as INR, total bilirubin or creatinine [26,31-33].

Regarding the association of infection episodes and neutrophil-to-lymphocyte ratio with the "hypocoagulable", profile it is known that ACLF and AD could be triggered by the presence of an infection [3,20], and that bacterial infections are accompanied by reduced coagulation factors VII and XII, lower levels of all natural anticoagulants and reduced platelet aggregation, but also hypo- and hyper-fibrinolytic changes [34] that may destabilize the rebalanced state of hemostasis in cirrhosis.

To the best of our knowledge, only Blasi *et al* [19] evaluated serial ROTEM observations at baseline and during the following 3 days, reporting that the "hypocoagulable" phenotype remained steady or worsened in ACLF, but improved in AD. However, no further information about sequentially measured separate ROTEM components or the dynamic variation over time was given.

Our study had some strengths and limitations. A control group of healthy individuals matched for age and sex was used. The principal strength, though, is the originality of outcomes, as only one study so far has dealt with the dynamic changes in ROTEM measurements in ACLF or AD. Moreover, the use of ROTEM output as an important tool for assessing the severity and prognosis of liver disease has not been well established.

On the other hand, the findings were based on data from a single institution and the sample is small. In addition, ROTEM assessments beyond 48 h post-admission were not available and a thrombin generation test, with and without thrombomodulin, as a reference test for the global evaluation of hemostasis, was not performed [35].

In conclusion, follow-up ROTEM measurements shortly after admission exhibited worsening hypocoagulability compared to baseline in ACLF, but stable values in AD. The alterations displayed both delayed clot formation and decreased clot firmness. However, this hypocoagulable derangement was mostly correlated with the severity of liver disease and bacterial infection, but not with bleeding episodes. A "hypocoagulable" phenotype was associated with poor outcomes in ACLF patients. More studies are further required on the role of dynamic changes in viscoelastic tests in critically ill patients with cirrhosis.

Summary Box

What is already known:

- Viscoelastic tests have been used to display both procoagulant and anticoagulant abnormalities of liver cirrhosis
- Viscoelastic tests can predict the risk of invasive procedure-related bleeding compared to conventional coagulation tests in decompensated cirrhosis
- Rotational thromboelastometry (ROTEM) kinetics were recently studied in critically ill patients in intensive care units but not in the setting of acute-on-chronic liver failure (ACLF) or acute decompensation of cirrhosis (AD)

What the new findings are:

- Serial ROTEM measurements shortly after admission displayed a more pronounced hypocoagulability in ACLF but not in AD patients
- Worsened hypocoagulability was characterized by both delayed clot formation and decreased clot firmness in ACLF
- Hypocoagulable derangement was mostly correlated with the severity of liver disease and bacterial infection
- A "hypocoagulable" profile was associated with poor outcomes in ACLF patients

References

1. Silva PE, Fayad L, Lazzarotto C, et al. Single-centre validation of the EASL-CLIF consortium definition of acute-on-chronic liver failure and CLIF-SOFA for prediction of mortality in cirrhosis. *Liver Int* 2015;**35**:1516-1523.
2. Fisher C, Patel VC, Stoy SH, et al. Balanced haemostasis with both hypo- and hyper-coagulable features in critically ill patients with acute-on-chronic-liver failure. *J Crit Care* 2018;**43**:54-60.
3. Jalan R, Pavesi M, Saliba F, et al; CANONIC Study Investigators; EASL-CLIF Consortium. The CLIF Consortium Acute Decompensation score (CLIF-C ADs) for prognosis of hospitalised cirrhotic patients without acute-on-chronic liver failure. *J Hepatol* 2015;**62**:831-840.
4. La Mura V, Bitto N, Tripodi A. Rational hemostatic management in cirrhosis: from old paradigms to new clinical challenges. *Expert Rev Hematol* 2022;**15**:1031-1044.
5. Roberts LN, Lisman T, Stanworth S, et al. Periprocedural management of abnormal coagulation parameters and thrombocytopenia in patients with cirrhosis: Guidance from the SSC of the ISTH. *J Thromb Haemost* 2022;**20**:39-47.
6. Valla DC, Rautou PE. The coagulation system in patients with end-stage liver disease. *Liver Int* 2015;**35**(Suppl 1):139-144.
7. Zanetto A, Rinder HM, Senzolo M, Simioni P, Garcia-Tsao G. Reduced clot stability by thromboelastography as a potential indicator of procedure-related bleeding in decompensated cirrhosis. *Hepatol Commun* 2021;**5**:272-282.
8. Premkumar M, Sarin SK. Current concepts in coagulation profile in cirrhosis and acute-on-chronic liver failure. *Clin Liver Dis (Hoboken)* 2020;**16**:158-167.
9. Premkumar M, Kulkarni AV, Kajal K, Divyaveer S. Principles, interpretation, and evidence-based role of viscoelastic point-of-care coagulation assays in cirrhosis and liver failure. *J Clin Exp Hepatol* 2022;**12**:533-543.
10. Kumar M, Ahmad J, Maiwall R, et al. Thromboelastography-guided blood component use in patients with cirrhosis with nonvariceal bleeding: a randomized controlled trial. *Hepatology* 2020;**71**: 235-246.
11. Fayed N, Mourad W, Yassen K, Görlinger K. Preoperative thromboelastometry as a predictor of transfusion requirements during adult living donor liver transplantation. *Transfus Med Hemother* 2015;**42**:99-108.
12. Maria A, Lal BB, Khanna R, et al. Rotational thromboelastometry-guided blood component use in cirrhotic children undergoing invasive procedures: randomized controlled trial. *Liver Int* 2022;**42**:2492-2500.
13. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on prevention and management of bleeding and thrombosis in patients with cirrhosis. *J Hepatol* 2022;**76**: 1151-1184.
14. Zanetto A, Senzolo M, Blasi A. Perioperative management of antithrombotic treatment. *Best Pract Res Clin Anaesthesiol* 2020;**34**:35-50.
15. Rimaitis M, Cechanovičiūtė V, Bilskienė D, et al. Dynamic changes of hemostasis in patients with traumatic brain injury undergoing craniotomy: association with in-hospital mortality. *Neurocrit Care* 2023;**38**:714-725.
16. Scărlătescu E, Lancé MD, White NJ, Tomescu DR. Thromboelastometric prediction of mortality using the kinetics of clot growth in critically ill septic patients. *Blood Coagul Fibrinolysis* 2018;**29**:533-539.
17. Hulshof AM, Brüggemann RAG, Mulder MMG, et al. Serial EXTEM, FIBTEM, and tPA rotational thromboelastometry observations in the Maastricht intensive care COVID cohort-persistence of hypercoagulability and hypofibrinolysis despite anticoagulation. *Front Cardiovasc Med* 2021;**8**:654174.
18. Kamal M, Hariprasad R, Bhatia PK, et al. Use of rotational thromboelastometry to predict the outcome of COVID-19 patients: A retrospective observational study. *J Anaesthesiol Clin Pharmacol* 2022;**38**:S89-S95.
19. Blasi A, Calvo A, Prado V, et al. Coagulation failure in patients with acute-on-chronic liver failure and decompensated cirrhosis: beyond the international normalized ratio. *Hepatology* 2018;**68**:2325-2337.
20. Jalan R, Saliba F, Pavesi M, et al; CANONIC study investigators of the EASL-CLIF Consortium. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol* 2014;**61**:1038-1047.
21. Angeli P, Bernardi M, Villanueva C, et al.; European Association for the Study of the Liver. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018;**69**:406-460.
22. Spiezia L, Radu C, Marchioro P, et al. Peculiar whole blood rotation thromboelastometry (Rotem) profile in 40 sideropenic anaemia patients. *Thromb Haemost* 2008;**100**:1106-1110.
23. Spiezia L, Bertini D, Boldrin M, et al. Reference values for thromboelastometry (ROTEM®) in cynomolgus monkeys (Macaca fascicularis). *Thromb Res* 2010;**126**:e294-e297.
24. Shalaby S, Simioni P, Campello E, et al. Endothelial damage of the portal vein is associated with heparin-like effect in advanced stages of cirrhosis. *Thromb Haemost* 2020;**120**:1173-1181.
25. Gouvêa G, Diaz R, Auler L, et al. Perioperative coagulation profile

in living liver donors as assessed by rotational thromboelastometry. *Liver Transpl* 2010;**16**:387-392.
26. Campello E, Zanetto A, Bulato C, et al. Coagulopathy is not predictive of bleeding in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure. *Liver Int* 2021;**41**: 2455-2466.
27. Hugenholtz GCG, Lisman T, Stravitz RT. Thromboelastography does not predict outcome in different etiologies of cirrhosis. *Res Pract Thromb Haemost* 2017;**1**:275-285.
28. Raeven P, Baron-Stefaniak J, Simbrunner B, et al. Thromboelastometry in patients with advanced chronic liver disease stratified by severity of portal hypertension. *Hepatol Int* 2020;**14**:1083-1092.
29. Blasi A, Patel VC, Spanke ENHE, et al. Fibrin clot quality in acutely ill cirrhosis patients: Relation with outcome and improvement with coagulation factor concentrates. *Liver Int* 2022;**42**:435-443.
30. Calvo A, Torrente MA, Görlinger K, et al. Haemostasis patterns in patients with acute-on-chronic liver failure and acute decompensation of cirrhosis including thromboelastometric tests with and without the addition of Protac: a pilot study. *Thromb J* 2022;**20**:75.
31. Seeßle J, Löhr J, Kirchner M, Michaelis J, Merle U. Rotational thrombelastometry (ROTEM) improves hemostasis assessment compared to conventional coagulation test in ACLF and Non-ACLF patients. *BMC Gastroenterol* 2020;**20**:271.
32. Kohli R, Shingina A, New S, et al. Thromboelastography parameters are associated with cirrhosis severity. *Dig Dis Sci* 2019;**64**: 2661-2670.
33. Shamseddeen H, Patidar KR, Ghabril M, et al. Features of blood clotting on thromboelastography in hospitalized patients with cirrhosis. *Am J Med* 2020;**133**:1479-1487.
34. Zanetto A, Campello E, Bulato C, et al. Global hemostatic profiling in patients with decompensated cirrhosis and bacterial infections. *JHEP Rep* 2022;**4**:100493.
35. Zanetto A, Pelizzaro F, Campello E, et al. Severity of systemic inflammation is the main predictor of ACLF and bleeding in individuals with acutely decompensated cirrhosis. *J Hepatol* 2023;**78**:301-311.