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Coagulopathy is not predictive of bleeding in patients with acute decompensation of cirrhosis and acute-on-chronic liver failure

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Abstract

Background & Aims: Understanding factors responsible for the increased bleeding tendency in acute-on-chronic liver failure (ACLF) would improve the management of these complications. We investigated coagulation alterations in ACLF and assessed whether they were predictive of bleeding.

Methods: Cirrhosis patients with ACLF (cases) and acute decompensation (AD, controls) were prospectively recruited and underwent an extensive haemostatic assessment including standard tests, pro and anticoagulant factors, thrombomodulin-modified thrombin generation (TG) and thromboelastometry (ROTEM®). In study part 1 (case-control), we compared coagulation in ACLF vs AD. In study part 2 (prospective), all patients were followed for bleeding, and predictors of outcome were assessed.

Results: Ninety-one patients were included (51 with ACLF, 40 with AD). Infections and ascites/renal dysfunction were the most common precipitating and decompensating events. Platelet count was lower while INR and activated partial thrombin time were longer in ACLF cohort vs AD. Regarding clotting factors, fibrinogen and factor VIII were comparable between groups while protein C and antithrombin were significantly reduced in ACLF. Endogenous thrombin potential by TG was comparable between groups. Clotting formation time and clot stability by ROTEM® were significantly lower in ACLF, indicative of a more hypocoagulable state. No haemostasis alteration could discriminate between patients who had bleeding complications during hospitalization and those who did not.

Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; aPTT, activated partial thromboplastin time; AT, antithrombin; CFT, clotting formation time; CT, clotting time; ETP, endogenous thrombin potential; FVIII, procoagulant factor VIII; MCF, maximum clot firmness; MELD, Model for End-Stage Liver disease; PC, anticoagulant protein C; PH, portal hypertension; TG, thrombin generation; TM, thrombomodulin.

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Conclusion: We found coagulation changes in ACLF to largely overlap with that of AD and evidence of preserved coagulation capacity in both groups. ROTEM alterations were indicative of a more pronounced hypocoagulable state in ACLF; however, no correlation was found between such alterations and bleeding.

KEYWORDS

acquired coagulopathy, coagulation, haemorrhage, thrombin generation, thromboelastometry

1 | INTRODUCTION

Cirrhosis is associated with profound alterations of haemostasis that include thrombocytopenia and increased levels of Von Willebrand factor, reduced levels of most procoagulant factors and inhibitors and complex changes in fibrinolysis.[1-5]

The extent of such alterations is proportional to the severity of cirrhosis (compensated vs decompensated); however, their net effect appears to be relatively independent of disease severity.[6,7] In fact, both compensated and decompensated patients are in a *rebalanced* haemostatic state that is maintained by a parallel decline in pro- and anti-haemostatic pathways.[8-10]

It seems plausible, however, that this *rebalanced* equilibrium becomes progressively more unstable with increasing severity of liver dysfunction,[11-15] which may explain the increased risk of bleeding in patients with acute-on-chronic liver failure (ACLF) (ie the sickest patients in whom acutely decompensated cirrhosis is complicated by systemic inflammation and extra-hepatic multiorgan failure).[16,17]

As such, complications may be life-threatening or lead to further decompensation, there is an urgent need for trials to assess best practices for prophylaxis and treatment of bleeding in patients with ACLF,[6,18] and observational studies on their haemostatic status may help in the design of such trials.

Here, we investigated alterations of coagulation in hospitalized patients with acutely decompensated cirrhosis and ACLF and evaluated whether such alterations were predictive of bleeding complications.

Lay summary

- Understanding factors responsible for the increased bleeding tendency in patients with cirrhosis and acute decompensation would improve the management of these complications.
- Patients with acute-on-chronic liver failure have a *rebalanced* coagulation system that is largely comparable with that of acutely decompensated cirrhosis.
- Thromboelastometry, despite multiple alterations indicative of a more marked hypocoagulable state, was not useful to identify patients at higher risk of bleeding.

2 | MATERIAL AND METHODS

2.1 | Patient selection

Adult (>18 years old) patients with acutely decompensated cirrhosis admitted to Gastroenterology/Multivisceral Transplant Unit and 5th Unit of Internal Medicine of Padova University Hospital from 1 November 2016 to 1 May 2019 were prospectively screened to determine eligibility to participate in the study.

Diagnosis of cirrhosis was confirmed with available data including histology, radiology, laboratory and clinical assessment. Acute decompensation (AD) and ACLF were defined according to the criteria of the CANONIC study.[19]

At screening, patient's medical records, past medical history and laboratory data were reviewed for the following exclusion criteria: presence and/or history of portal vein thrombosis and/or venous thromboembolism; hepatocellular carcinoma outside Milan criteria; presence of extra-hepatic tumours; known hematologic or coagulation diseases; recent surgery (within 30 days); HIV-infection, history of any organ transplantation, including liver.

Patients on therapeutic anticoagulation and/or antithrombotic and/or anti-fibrinolytic therapy, and those who received transfusion of platelets, cryoprecipitate or fresh-frozen plasma in the 7 days prior to screening were also excluded. Patients on antithrombotic prophylaxis with low molecular weight heparin were eligible for recruitment.

Upon admission to the inpatient ward and having determined eligibility, patients were categorized into cases (with ACLF) and controls (with AD) and underwent coagulation assessment.

2.2 | Study design

This was a two-part study, and all patients were included in both parts 1 and 2. In study part 1 (case-control), we compared alterations of coagulation in patients with ACLF (cases) vs AD (controls). In study part 2 (prospective), we investigated whether alterations of coagulation at recruitment could discriminate between patients who then had in-hospital, bleeding complications and those who did not. With this goal, we prospectively followed all patients with ACLF and AD throughout hospitalization until one of the following outcomes,

whichever came first: occurrence of bleeding complications, liver transplantation, death or discharge. Duration of follow-up was calculated as the time (days) between patient recruitment (ie: when baseline tests of coagulation were performed) and development of outcome. Only bleedings unrelated to portal hypertension (PH) were included in secondary outcome analysis (see below).

The study was approved by the Padova University Hospital Ethical Committee (3103/A0/14), it was conducted in compliance with the Declaration of Helsinki and all patients gave written consent before enrolment.

2.3 | Sample collection and coagulation assessment

2.3.1 | Blood sampling

Peripheral blood was collected via venipuncture in citrate-containing vacutainer tubes using 21 g needles and tourniquet. Platelet-poor plasma was prepared within 1 hour by double centrifugation (2 × 10 minutes at 1500 g) at room temperature. Aliquots (1 mL) were immediately frozen and then stored at −80°C until use.

2.3.2 | Coagulation assessment

Coagulation assessment included conventional coagulation tests, plasmatic coagulation proteins, thrombin generation (TG) with and without thrombomodulin and whole blood rotational thromboelastometry (ROTEM®). All tests were performed at the Lab of Thrombotic and Hemorrhagic Disease Unit of Padova University Hospital.

Conventional tests and plasmatic coagulation proteins

Conventional tests included platelet count, INR and activated partial thrombin time (aPTT). Plasmatic coagulation proteins included fibrinogen (n.v. 150-450 mg/dL), procoagulant factor VIII (FVIII, n.v. 60%-160%), protein C coagulometric (PC coag, n.v. 80%-120%), protein C chromogenic (PC chromo, n.v. 70%-130%) and antithrombin (AT, n.v. 80%-120%) as previously described.[20-23]

Thrombin generation with and without thrombomodulin

Thrombin generation (TG) was determined in platelet poor plasma with the calibrated automated thrombogram method (Thrombinoscope BV), as previously described.[4]

Briefly, 80 μL of plasma was dispensed into the wells of a 96-well microtiter plate, and coagulation was triggered with 20 μL of platelet poor plasma-Reagent Low (Thrombinoscope BV), a mixture of tissue factor (1 pmol/L final concentration) and synthetic phospholipids (4 μmol/L final concentration). The reaction was initiated by adding 20 μL of a mixture composed of a thrombin fluorogenic substrate and $CaCl_2$ (FluCa-Kit, Thrombinoscope BV). Thrombin calibrator (Thrombinoscope BV) was used to correct each curve for inner filter effects and substrate consumption. Fluorescence was read in a Fluoroskan Ascent® reader (Thermo Labsystems), and TG curves were calculated using the Thrombinoscope Software version 5.0.0.742 (Thrombinoscope BV).

TG curves were described in terms of lag-time, peak height, time to peak and endogenous thrombin potential (ETP).

TG was run both with and without thrombomodulin (TM). TM is the main cofactor in the thrombin-induced activation of protein C (natural anticoagulant). In normal plasma, the addition of TM significantly reduced the generation of thrombin, thus leading to a reduction of ETP. In this study, the concentration of TM (1.5 nmol/L) was chosen to reduce the ETP by 50% in normal pool plasma (resulting in an ETP ratio of 0.52). Plasma from 53 normal healthy subjects was also tested to evaluate the effect of TM on ETP and acted as control group for TG. This group consisted of 23 males and 30 females without history of cardiovascular, autoimmune and acute diseases and not taking antithrombotic, antibiotic and hormonal therapy. All tests were performed in duplicate. The ETP ratio was calculated as follows: ETP with TM/ETP without TM, and it reflects the 'resistance' to the anticoagulant effect of PC. The lower the ETP ratio, the better preserved the level and the function of PC. Conversely, higher ETP ratio means more severe PC deficiency/PC resistance and a potentially greater susceptibility for thrombosis.

Rotational thromboelastometry (ROTEM®)

ROTEM® (TEM International GmbH) is a whole blood viscoelastic test evaluating clot formation and stability as the result of the interplay between plasma coagulation factors and blood cells, especially platelets.[24] ROTEM® was performed according to the standard protocols supplied by the manufacturer, as previously reported.[25-27] The following tests were performed: INTEM (to assess intrinsic coagulation pathway), EXTEM (to assess extrinsic coagulation pathway) and FIBTEM (to assess fibrinogen contribution to clot formation and stability). For EXTEM and INTEM tests, the following ROTEM® parameters were collected: clotting time (CT), clotting formation time (CFT), maximum clot firmness (MCF), α-angle and maximum lysis for EXTEM and INTEM tests. Only MCF was collected for FIBTEM test. A 'hypocoagulable' trace at ROTEM® was defined if all the 4 parameters assessing clot formation and stability (CT, CFT, MCF and α-angle) were below normal range. A 'likely hypocoagulable' trace was defined if 2 or 3 of these 4 parameters were below normal range.

2.4 | Data collection

Data collected from the medical records included reasons for decompensation and decompensating events, patient demographics, presence or absence of infections at inclusion, laboratory data and in-hospital bleeding and thrombotic complications.

Model for End-Stage liver disease (MELD) score, Child-Pugh score and ACLF grade were calculated on the day of enrolment.

Bleeding complications were categorized in PH-related and PH-unrelated. PH-unrelated bleedings were further subclassified in procedure-related and spontaneous bleedings. According to the

International Society of Thrombosis and Hemostasis guidelines for non-surgical patients,[28] 'major' bleeding was defined as fatal bleeding and/or symptomatic bleeding in a critical area or organ and/or bleeding causing a fall in haemoglobin level of 2 g/dL or more or leading to transfusion of two or more units of whole blood or red cells; 'clinically relevant non major bleeding' was defined as any sign or symptom of bleeding that fit at least one of the following: required medical intervention, lead to increased level of care, prompted a face to face evaluation and 'minor' bleeding was defined as every bleeding that did not fit the previous criteria.

2.5 | Data analysis

The primary objective of this study was to compare alterations of coagulation in patients with cirrhosis and ACLF (cases) vs patients with cirrhosis and AD (controls).

The secondary objective was to evaluate whether baseline alterations of coagulation were able to discriminate between patients who then experienced in-hospital bleeding complications and those who did not. For secondary objective, only procedure-related and spontaneous, PH-unrelated bleedings were considered. Bleedings related to PH (ie oesophageal variceal haemorrhage) were not included because alterations of haemostasis are not responsible for this type of bleeding.

Sample size was calculated for primary objective. In a previous seminal study evaluating the difference of the haemostatic status in patients with ACLF vs AD, those with ACLF had a significantly lower level of antithrombin compared with those with AD (27% vs 48% respectively).[13] We based our sample-size calculation on that evidence. Sample size was calculated assuming a continuous endpoint compared between two independent groups, two-sided type I error of 0.05 and statistical power of 0.90. The required per-group sample size was 40. Qualitative data are described using frequency and percentage. Quantitative data are described using median with 25% and 75% quartile ranges. Comparisons between independent groups were performed using the Mann-Whitney U test and t-test for continuous variables and Chi-square test of Fisher's exact test (when the cell value was small, ≤5) for categorical variables. Statistical significance was set at $P \leq .05$. All analyses were completed using SPSS version 26.

3 | RESULTS

3.1 | Demographics

Ninety-one patients with cirrhosis were recruited (51 with ACLF and 40 with AD) (Figure S1). Baseline demographics and aetiology of cirrhosis were comparable between the groups (Table 1). Alcohol and alcohol plus HCV were the most common aetiology in both groups, accounting for 65% of the patients. Infection was the most common precipitating events in both patients with ACLF and AD (54% and

TABLE 1 Baseline characteristics in patients with acutely decompensated cirrhosis

	ACLF (n = 51)	AD (n = 40)	*P* values
Age (years)	60 (51-66)	60 (54-66)	.9
Male gender (%)	58	42	.5
Etiology of cirrhosis (%)			.3
Alcohol	51	48	
HCV ± alcohol	12	23	
NASH	24	8	
Autoimmune	6	8	
HBV or HBV/HDV	8	15	
Child-Pugh score	11 (7-14)	10 (7-12)	.2
MELD score	27 (23-33)	17 (14-23)	<.0001
ACLF grade (%) *1/2/3*	51/26/23	—	—
Precipitant (%)			.5
(Suspected) Infection	54	57	
Bleeding/ dehydration	14	18	
Procedure	14	5	
Alcoholic hepatitis	8	15	
Other[b]	10	5	
Decompensating event (%)			.4
Ascites	27	35	
AKI	37	23	
Jaundice[d]	31	15	
AMS/HE	14	27	
Bacterial infection (%)[a]	55	60	.6
Hepatocellular carcinoma, %	16	10	.4
Thromboprophylaxis with LMWH[c], %	18	22	.6
Total bilirubin, mmol/L	160 (52-367)	63 (33-150)	.01
Albumin, g/dL	29 (25-32)	27 (23-30)	.3
Hemoglobin, g/dL	9.4 (8.5-11)	9.5 (8.9-11.1)	.1
Platelet count, 10^9/L	60 (39-81)	73 (49-115)	.04
Thrombocytopenia, (%)			.1
Present	96	88	
Mild 100-150 × 10^9/L	6	18	
Moderate 50-100 × 10^9/L	49	56	
Severe <50 × 10^9/L	45	26	
WBC, 10^9/L	8 (5-12)	6 (4-10)	.5
Creatinine, mmol/L	146 (94-219)	78 (51-119)	<.0001
Sodium, mmol/L	133 (128-137)	135 (132-138)	.1

(Continues)

TABLE 1 (Continued)

	ACLF (n = 51)	AD (n = 40)	*P* values
AST, U/L	68 (42-116)	45 (30-73)	.1
ALT, U/L	32 (18-49)	30 (14-51)	.9
PCR, mg/dL	29 (18-51)	32 (21-66)	.5
PCT, ng/L	1.2 (0.5-3)	0.5 (0.3-0.9)	.01

Note: Median values reported with 25th and 75th percentile values in parenthesis.

Abbreviations: ALT, alanine aminotransferase; AMS, altered mental status; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HE, hepatic encephalopathy; LMWH, low molecular weight heparin; MELD, Model for End-Stage Liver Disease; NASH, non-alcoholic steatohepatitis; PCR, C reactive protein; PCT, procalcitonin; WBC, white blood cells.

[a]Bacterial infections were: spontaneous bacterial peritonitis (30%), urinary tract infection (15%), pneumonia (11%), sepsis/septicaemia (30%), cutaneous or subcutaneous (7%) and others (7%) in patients with ACLF and spontaneous bacterial peritonitis (21%), urinary tract infection (4%), pneumonia (25%), sepsis/septicaemia (21%), cutaneous or subcutaneous (21%) and others (8%) in patients with AD.

[b]Other reasons for decompensation were: heart failure (1×), vasculitis (1×), portal vein thrombosis (1×), bowel occlusion (1×) and atrial fibrillation (1×) in patients with ACLF and accidental trauma (2×) in patients with A.

[c]LMWH was administered once a day at 6 PM Per study protocol, all samples were collected at 6-7 AM, thus allowing approximately 12 hours between administration of LMWH and blood samples collection.

[d]Jaundice was defined as clinical evidence of yellow skin and sclera together with increased level of serum bilirubin (>2 mg/dL).

57% respectively), followed by bleeding and procedure in ACLF and bleeding and alcoholic hepatitis in AD. Regarding decompensating events, acute kidney injury and jaundice were the most frequent in patients with ACLF while patients with AD presented mostly with ascites and altered mental status (Table 1). In patients with ACLF, grade was 1, 2, and 3 in 51%, 26%, and 23% of the cases respectively.

MELD score was significantly higher in patients with ACLF than in patients with AD (27 vs 17), due to significant differences in serum bilirubin (160 mmol/L vs 63 mmol/L), INR (1.9 vs 1.6) and serum creatinine (146 mmol/L vs 78 μmol/L). Frequency of bacterial infections was comparable between patients with ACLF and patients with AD (55% vs 60%), with spontaneous bacterial peritonitis and septicaemia/sepsis being the most common types of infections in both groups. White blood count and plasmatic levels of C reactive protein were comparable between patients with ACLF and AD, whereas procalcitonin was significantly higher in those with ACLF (1.2 ng/L vs 0.5 ng/L).

Conventional tests and coagulation proteins in patients with ACLF vs patients with AD: ACLF is associated with lower platelet count, longer INR and aPTT and lower levels of anticoagulants protein C and antithrombin.

Patients with ACLF had a lower platelet count compared with those with AD (60×10^9/L vs 73×10^9/L respectively). Nearly all patients were thrombocytopenic (96% among those with ACLF and 88% among those with AD); however, the frequency of severe thrombocytopenia (platelet count $<50 \times 10^9$/L) was relatively higher in patients with ACLF than in those with AD (Table 1).

Regarding standard tests assessing coagulation, patients with ACLF had longer INR (1.9 [1.5-2.5] vs 1.6 [1.3-1.9]; $P = .007$) and aPTT (44 seconds [35-54] vs 35 seconds [30-42]; $P < .001$) compared with patients with AD.

Figure 1 shows levels of procoagulant factors and inhibitors in patients with ACLF and AD. Levels of fibrinogen and procoagulant FVIII were comparable between groups (141 mg/dL [107-203] vs 190 mg/dL [100-245] and 143% [103-181] vs 177% [98-240] respectively). In contrast, levels of anticoagulant PC and AT were both significantly lower in patients with ACLF than in patients with AD (PC chromogenic: 22 [16-29] vs 31 [21-50]; PC coagulometric: 20% [13-25] vs 24% [18-38]; and antithrombin 26% [16-37] vs 36% [25-48]) (Figure 1).

Among patients with ACLF, INR and aPTT were longer while platelet count, PC and antithrombin were lower, respectively, in patients with more vs less severe ACLF (INR: 2.5 [2.1-2.6] vs 1.7 [1.4-2.1], $P < .0001$; aPTT: 61 seconds [54-73] vs 38 seconds [32-49], $P < .0001$; platelet count: 42×10^9/L [35-67] vs 62×10^9/L [41-82], $P = .04$; PC chromogenic: 16% [15-22] vs 24% [17-35], $P = .02$; PC coagulometric: 13% [12-20] vs 21% [14-36], $P = .1$; antithrombin; 19% [15-21] vs 26% [16-39], $P = .04$; in grade 3 vs. grade 1 + 2 ACLF). Conversely, FVIII (145% [78-218] vs 162% [114-231]; $P = .4$) and fibrinogen (145 mg/dL [81-215] vs 200 mg/dL [119-214]; $P = .2$) were comparable across groups.

In both patients with ACLF and AD, no significant difference was found in standard coagulation tests and levels of pro and anticoagulant factors between infected and non-infected patients (data not shown).

Global tests of haemostasis in patients with ACLF vs patients with AD: ACLF is associated with comparable TG but lower clot formation and stability

ETP with and without TM was significantly lower in patients with AD and ACLF than in healthy subjects (Table S1). As expected, the addition of TM significantly reduced the ETP in healthy subjects but not in patients with cirrhosis. In fact, the ETP ratio was significantly higher in patients with ACLF and AD vs healthy subjects (Figure 2 and Table S1).

No large difference was found in profiles of TG between patients with ACLF and patients with AD (Table 2). Lag-time, time to peak, ETP with and without TM and ETP ratio were all comparable between the two groups (Figure 2). The only difference between groups was a significantly lower peak-height in those with ACLF (Table 2).

In contrast, ROTEM® showed profound differences in patients with ACLF vs patients with AD (Table 2). In particular, patients with ACLF demonstrated longer CT and CFT (INTEM and EXTEM), a lower maximum clot firmness (INTEM, EXTEM, FIBTEM) and a smaller α-angle (INTEM and EXTEM) (Figure 3). A hypocoagulable trace at

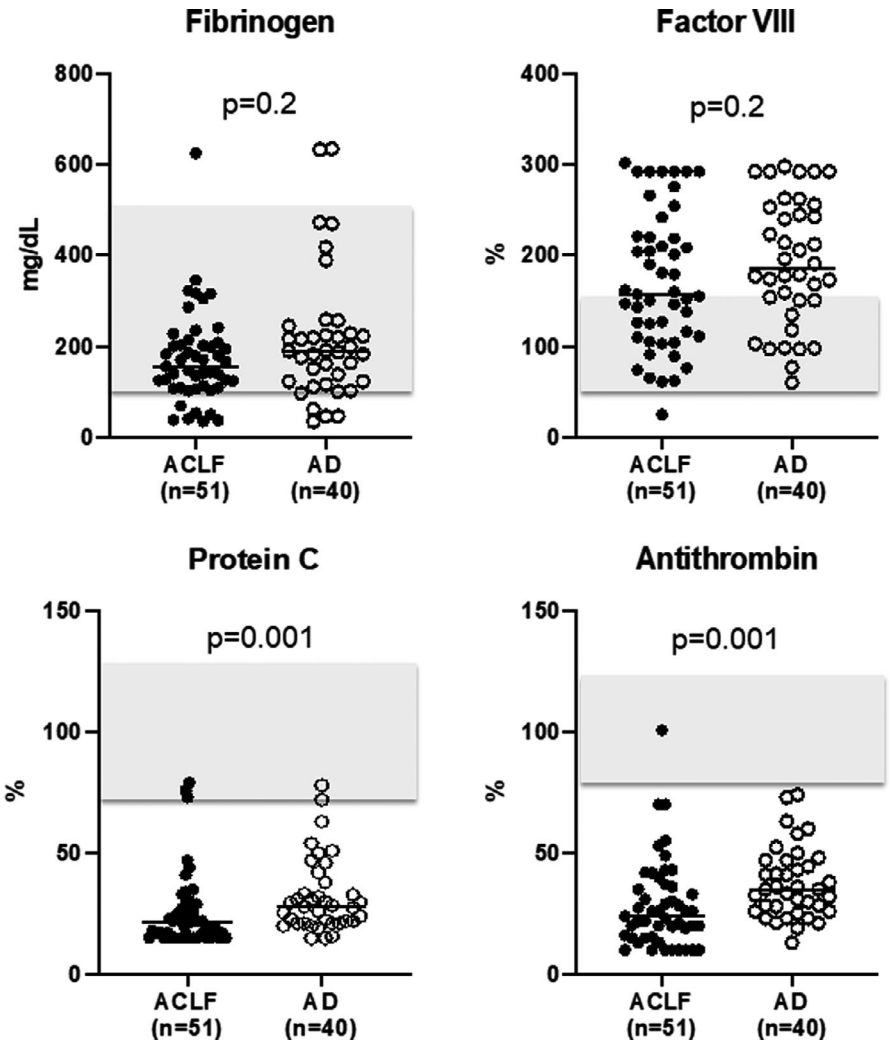

FIGURE 1 Levels of procoagulant factors and inhibitors in patients with ACLF and AD. Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation. Grey areas refer to normal reference ranges: fibrinogen: 150-450 mg/dL; FVIII: n.v. 60%-160%; PC chromogenic: 70%-130%; antithrombin: AT 80%-120%

ROTEM® was significantly more frequent in patients with ACLF than in patients with AD (78% vs 42% of patients respectively). No difference was found regarding maximum lysis at both INTEM and EXTEM tests between groups.

In the subgroup of patients with ACLF, those with more vs less severe ACLF demonstrated a significantly more deranged TG, specifically a lower ETP and a lower peak height (Supplementary Table 2). Similarly, ROTEM® markers of clot formation and stability were more markedly deranged in patients with more vs less severe ACLF (Table S2).

In both patients with ACLF and AD, no significant difference was found in TG and ROTEM® parameters between infected and non-infected patients (data not shown).

3.2 | Bleeding complications and their correlation with alterations of haemostasis

Median duration of follow-up was 15 days (IQR 9-24). Of 91 patients (51 with ACLD and 40 with AD) followed prospectively thorough hospitalization, 5 developed PH-related bleeding (oesophageal variceal haemorrhage), 7 developed spontaneous, PH-unrelated bleeding (1 haematuria, 1 intraocular, 2 chest wall haematoma requiring embolization, 1 ileo-psoas, 1 abdominal wall bleeding requiring embolization, and 1 without unidentified source), 1 experienced a procedure-related bleeding (post-TIPS haemoperitoneum), 5 patients underwent liver transplantation, 12 died due to multiorgan failure or sepsis and 61 were discharged. Median time from patient's recruitment and occurrence of bleeding was 11 days (range: 6-16). Bleeding was major in 5 patients (63%), clinically relevant non major in 2 patients (25%) and minor in 1 patient (12%) (Table 3).

None of the patients developed portal vein thrombosis or any other thrombotic complication during hospitalization.

Table 4 shows the differences between patients who had in-hospital bleeding complications (spontaneous, PH-unrelated and procedure-related) and those who did not. No significant difference in standard tests assessing haemostasis, presence or absence of infection and renal dysfunction, levels of pro and anticoagulant factors and markers of TG and ROTEM® was found between the two groups (Table 4).

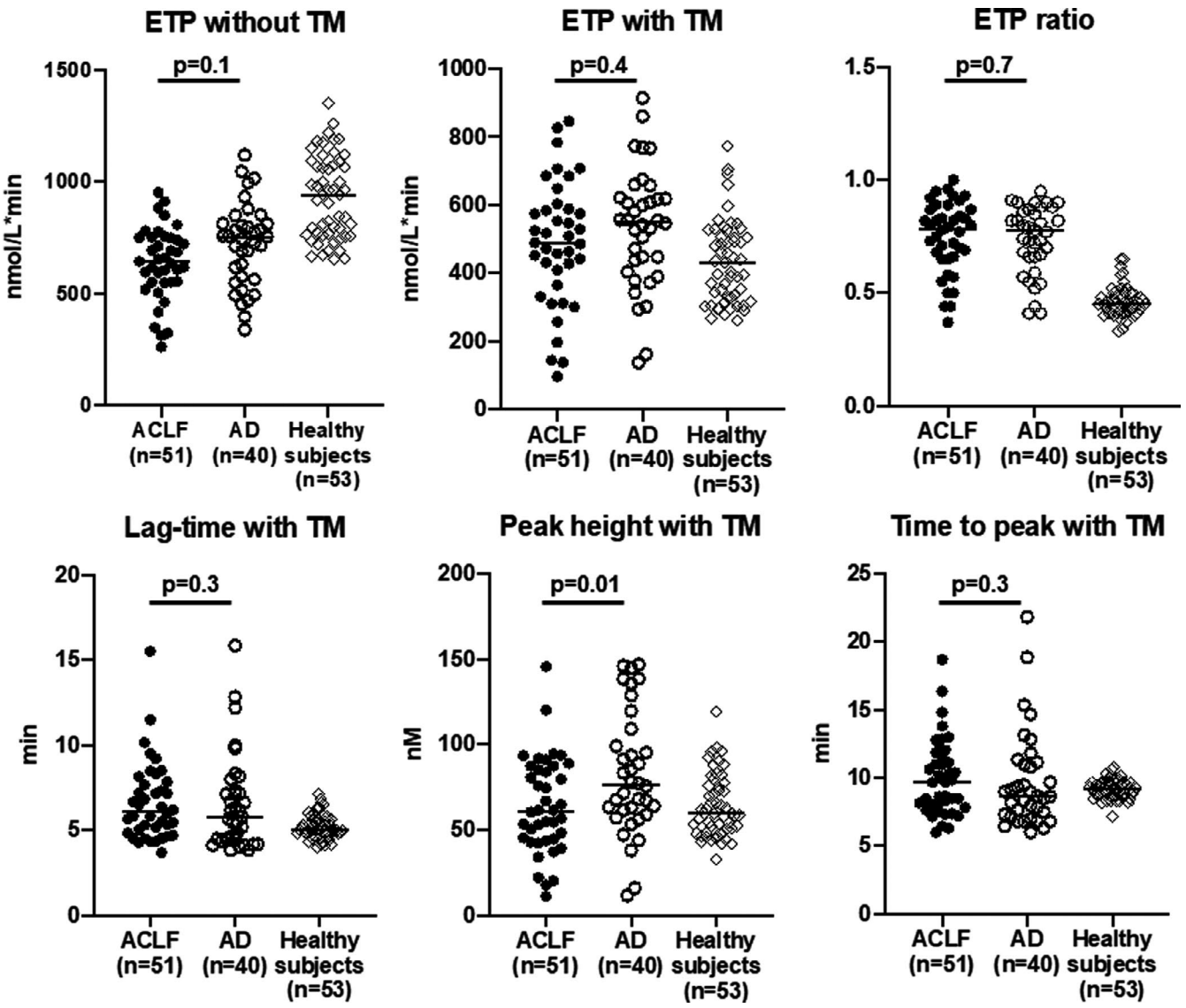

FIGURE 2 Thrombin generation profile was largely comparable between patients with ACLF and those with AD. Both patients with AD and ALCF had higher thrombin generation compared with healthy controls. For numerical values, see Table 2 and Table S1. Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; ETP, endogenous thrombin potential; TM, thrombomodulin

Only 2/21 (10%) patients with ACLF who had a clear hypocoagulable trace at ROTEM® experienced bleeding. As indicated in Figure 4, MCF could not discriminate between patients who had bleeding complications and those who did not.

4 | DISCUSSION

Patients with AD of cirrhosis, including those with ACLF, are in a *rebalanced* haemostatic state that is maintained by a simultaneous decline in both pro and anti-haemostatic factors.[1,2,6] Yet, compared with patients with AD but no extra-hepatic inflammation and organ failures, the *rebalanced* haemostatic system in those with ACLF appears more unstable and susceptible to external perturbations (ie acute kidney injury and infections),[12-14] which likely explains their purported increased risk of bleeding.[16,17]

Understanding the factors responsible for this bleeding tendency, and investigating whether there may also be an increased clotting tendency, have implications for prevention and treatment of these potentially catastrophic complications.[18]

Here, we used TM-modified TG and rotational thromboelastometry (ROTEM®) to investigate alterations of haemostasis in hospitalized patients with ACLF.

We found that the ETP with TM, the parameter of TG that better reflects the coagulation system in cirrhosis,[29] was similar between patients with ACLF and controls with AD. In agreement with recent findings by other groups,[13,14] this confirms that the overall coagulation capacity in ACLF is comparable with that of AD but no extra-hepatic inflammation and multi-organ failure.

However, we also found that patients with ACLF had a significantly lower level of TG peak height.[13] This parameter of TG reflects the maximum amount of thrombin generated and, in patients without

TABLE 2 Global coagulation tests in patients with ACLF vs. patients with AD

	ACLF (n = 51)	AD (n = 40)	*P* value
Thrombin generation			
Lag time, min	5.5 (4.4-6.9)	5.2 (4-6.7)	.3
Peak-height, nM	69.5 (54.6-95.8)	88.5 (72.7-112.6)	.008
Time to peak, min	8.9 (7.7-11.4)	8.6 (6.9-10.9)	.3
ETP, nmol/L*min	650 (551-751)	750 (567-813)	.1
Lag time +TM, min	6.1 (4.9-7.8)	5.7 (4.5-7.3)	.3
Peak-height +TM, nM	61 (43.9-86.9)	76.5 (59.6-106.7)	.01
Time to peak +TM, min	9.7 (7.9-11.7)	8.7 (7.2-11.1)	.3
ETP +TM, nmol/L*min	500 (377-600)	551 (413-621)	.4
ETP ratio	0.78 (0.65-0.87)	0.77 (0.66-0.86)	.7
ROTEM			
INTEM CT, sec	242 (221-280)	201 (175-235)	<.0001
INTEM CFT, sec	204 (120-397)	134 (85-271)	.02
INTEM MCF, mm	41 (33-47)	48 (35-57)	.03
INTEM α-angle, °	59 (49-68)	68 (54-74)	.01
INTEM ML, %	2 (0-6)	4 (0-8)	.3
EXTEM CT, sec	85 (71-109)	67 (60-84)	.001
EXTEM CFT, sec	229 (132-497)	139 (84-258)	.01
EXTEM MCF, mm	41 (32-49)	48 (36-58)	.03
EXTEM α-angle, °	55 (45-66)	68 (52-73)	.008
EXTEM ML, %	3 (0-8)	5 (1-12)	.2
FIBTEM MCF, mm	7 (4-11)	12 (7-17)	.001
Hypocoagulable profile at ROTEM (yes/likely/no), %	41/37/22	32/10/58	.001

Note: Median values reported with 25th and 75th percentile values in parenthesis.

Abbreviations: CFT, clotting formation time; CT, clotting time; ETP, endogenous thrombin potential; MCF, maximum clot firmness; ML, maximum lysis; TM, thrombomodulin.

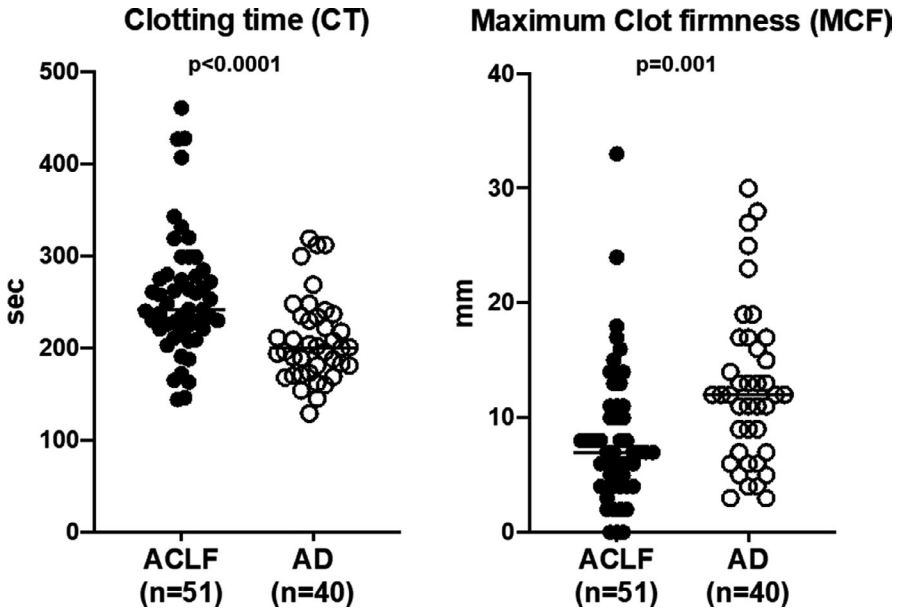

FIGURE 3 Markers of clotting formation and clot stability by thromboelastometry ROTEM were significantly more altered (more hypocoagulable) in patients with ACLF compared with those with AD. Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; CF, clotting time at INTEM test; MCF, maximum clot firmness at FIBTEM test

TABLE 3 Characteristics of patients with ACLF and AD who had bleeding (procedure-related and spontaneous, PH-unrelated) bleeding

Gender	Years	Etiology	Group	MELD	Infection	Creatinine (mmol/L)	INR	Platelet count ($\times 10^9$/L)	Type of bleeding	Time from recruitment to bleeding (days)	Severity of bleeding
M	67	Alcohol	AD	10	Yes	102	1.2	133	Post-TIPS haemoperitoneum	2	Major
M	40	Alcohol	ACLF I	31	Yes	470	1.3	224	Haematuria	6	Minor
M	73	Alcohol	ACLF II	38	Yes	160	4.8	40	Ocular bleeding	14	CRNM
M	52	PBC/AI	ACLF I	22	No	64	1.7	24	Chest wall haematoma	16	Major
M	63	Alcohol	ACLF III	30	No	120	2.5	36	Chest wall haematoma	20	Major
M	57	HBV	ACLF II	41	No	390	1.8	23	Ileo-psoas bleeding	14	Major
M	49	HBV/HDV	AD	28	No	53	3.1	19	Abdominal wall haematoma	8	CRNM
M	58	Alcohol	ACLF I	29	Yes	331	1.4	37	Unidentified source	6	Major

Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; CRNM, clinically relevant non major bleeding; HBV, Hepatitis B virus; HCV, Hepatitis C virus; MELD, Model for End-Stage Liver Disease; PBC/AI, primary biliary cholangitis/autoimmune; TIPS, trans-jugular intrahepatic shunt.

TABLE 4 Characteristics in patients who had bleeding (spontaneous, PH-unrelated and procedure-related) vs patients who did not

	Bleeding (n = 8)	Non-bleeding (n = 83)	*P* value
Clinical and laboratory data			
Age, years	63 (58-68)	63 (53-68)	.7
MELD	30 (29-34)	22 (15-26)	.1
Infection, %	50	42	.7
Creatinine, mmol/L	161 (140-246)	108 (74-178)	.2
Albumin, g/dL	26 (25-31)	28 (25-32)	.5
Bilirubin, mg/dL	328 (219-450)	85 (33-159)	.04
Platelet count, $\times 10^9$/L	37 (36-39)	54 (37-81)	.1
Hemoglobin, g/dL	8.8 (8.4-8.8)	9.4 (8.7-10.3)	.3
INR	2.5 (1.9-3.6)	1.8 (1.4-2.2)	.9
aPTT	70 (55-75)	48 (32-58)	.1
Pro and anticoagulant factors			
Factor VIII, %	162 (153-182)	150 (98-191)	.9
Fibrinogen, mg/dL	142 (100-208)	202 (164-208)	.6
Protein C chromogenic, %	17 (16-26)	24 (20-33)	.5
Protein C coagulometric, %	12 (10-19)	20 (15-28)	.8
Antithrombin, %	19 (16-25)	26 (21-42)	.7
ROTEM parameters			
INTEM CT, sec	280 (255-343)	258 (230-290)	.1
INTEM CFT, sec	479 (330-680)	277 (167-422)	.1
INTEM MCF, mm	27 (25-36)	42 (34-52)	.2
EXTEM CT, sec	100 (81-109)	79 (64-109)	.3
EXTEM CFT, sec	535 (365-563)	225 (118-470)	.2
EXTEM MCF, mm	27 (26-36)	42 (33-52)	.2
FIBTEM MCF, mm	3 (2-6)	8 (5-12)	.3
Thrombin generation parameters			
ETP without TM	763 (589-868)	692 (551-784)	.4
Peak-height without TM	94 (77-105)	82 (62-101)	.4
ETP with TM	603 (506-724)	472 (342-585)	.5
Peak-height with TM	92 (71-93)	64 (45-85)	.5
ETP ratio	0.89 (0.85-0.91)	0.72 (0.65-0.83)	.2

Note: Median values reported with 25th and 75th percentile values in parenthesis.

Abbreviations: CFT, clot formation time; CT, clot time; ETP, endogenous thrombin potential; MCF, maximum clot firmness; MELD, Model for End Stage Liver Disease; TM, thrombomodulin.

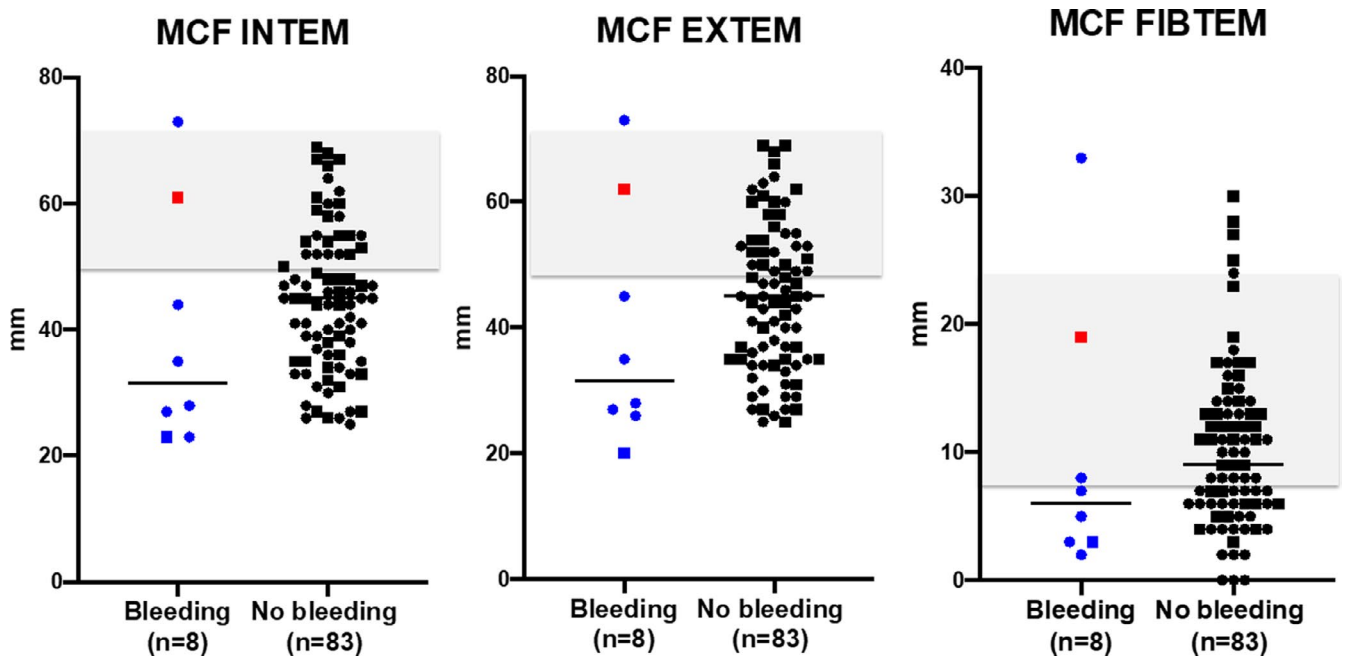

FIGURE 4 Maximum clot firmness (MCF), a marker of clot stability, could not discriminate between patients with ACLF (circles) and AD (squares) who had bleeding (procedure-related in red and spontaneous, PH-unrelated in blue) and those who did not. Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation. Grey areas represent ROTEM reference ranges in healthy subjects: MCF INTEM: 50-72 sec.; MCF EXTEM: 50-72 mm; MCF FIBTEM: 9-25 mm

chronic liver disease, has been proposed as a more sensitive predictor of bleeding than the ETP.[30] Therefore, although the overall clotting capacity remains comparable between patients with ACLF and AD, those with ACLF may still represent a patient group with an increased bleeding tendency.[13] To test this hypothesis, larger studies are required to evaluate the correlation between ETP, peak height and risk of bleeding in these patients.

Interestingly, we also demonstrated that, in the vast majority of patients with ACLF, the thrombin-generating capacity was similar or even increased than that of healthy subjects, which has two main implications for the management of ACLF. First, a restrictive use of procoagulants in these patients would be warranted to prevent further activation of coagulation and potential thrombotic complications.[31] Second, use of anticoagulant therapy should not be contraindicated a priori and instead may be beneficial, including in patients who appear 'naturally anti-coagulated' due to significant prolongation of INR or low platelet count.[32,33]

Contrary to our findings in TG, ROTEM® indicated that patients with ACLF had slower clot formation and lower clot stability both indicative of a more markedly hypocoagulable state (more pro-haemorrhagic).[34] However, ROTEM® is relatively insensitive to increased levels of platelet adhesive glycoprotein Von Willebrand factor and reduced anticoagulant protein C.[35] As these factors compensate for the low platelet count and low levels of procoagulant factor in decompensated cirrhosis,[36-38] it is possible that ROTEM® underestimates the true haemostatic potential in patients with ACLF.[39]

This explains why we could not find any association between degree of ROTEM® alterations and occurrence of bleeding. By contrast, 90% of patients who had a clear hypocoagulable trace at ROTEM® (all 4 parameters reflecting clot formation and stability below normal range) did not experience bleeding, which suggests that ROTEM® cannot be used to identify patients with ACLF at higher risk of bleeding. This is in line with recent findings from a multicentre, prospective cohort study including 200 patients with acute liver injury and acute liver failure.[40] In fact, despite multiple and significant alterations of ROTEM® all indicative of a severe hypocoagulable state, Stravitz et al [40] found no correlation between alterations of ROTEM®, performed at admission and occurrence of bleeding complications during hospitalization.

In the only previous study that investigated the role of ROTEM® in patients with ACLF, Seessle et al [41] found that CFT and MCF at INTEM test were significantly more altered in patients who bled (n = 9) than in those who did not (n = 13) and concluded that ROTEM® could be helpful to estimate bleeding risk in ACLF. However, 60% of events were oesophageal variceal bleedings for which portal pressure, but not haemostatic failure, is responsible.[42] Therefore, it is likely that the association between a more profound derangement of ROTEM® and bleeding merely reflected a more advanced liver dysfunction with higher portal hypertension in patients who bled.[41] By contrast, we carefully excluded all the bleeding events directly and primarily correlated with portal hypertension. In fact, in our series, only the haemoperitoneum post-TIPS might have been somehow related, yet indirectly, to portal hypertension. This allowed us to evaluate more clearly the potential correlations between alterations of ROTEM® and occurrence of bleeding in acutely decompensated cirrhosis.

However, as our primary objective was the assessment of haemostatic alterations, it is likely that our study was not adequately powered to detect a clinically meaningful association between ROTEM® and development of bleeding. Therefore, our finding of no significant correlation between alterations of ROTEM® and occurrence of bleeding in hospitalized patients with AD and ACLF needs confirmation in larger cohorts.

Our ROTEM® data further show that, while markers of clot formation and stability were significantly different in ACLF vs AD, maximum lysis time (marker of fibrinolysis) was similar between groups. This appears at odds with recent data that demonstrated a hyper-fibrinolytic status in patients with ACLF[12]; however, it could be explained by the relatively low sensitivity to hyperfibrinolysis of viscoelastic tests.[43]

Our study has some limitations. First, as we aimed to investigate alterations of coagulation, no marker of platelet function and fibrinolysis was included. Second, potential cofounders such as renal dysfunction, infections and use of prophylactic anticoagulant therapy with low molecular weight heparin might have interfered with our ability to assess haemostasis. Yet, we intentionally did not exclude such factors to evaluate coagulation in a real-life cohort of patients with ACLF. Third, while we studied coagulation in sample taken at hospital admission or at time of ACLF diagnosis, it is well known that AD and particularly ACLF are dynamic conditions in which a longitudinal assessment may perhaps be more informative, especially regarding prediction of bleeding.[14,15,40] Finally, although we carefully excluded bleeding events unrelated to haemostatic failure, the correlation between alterations of coagulation and bleeding was limited by the relatively low number of outcomes. Therefore, further studies with larger sample size and a longitudinal design are required to truly assess whether ROTEM® is not useful to predict occurrence of bleeding in hospitalized patients with AD and ACLF.

In conclusion, we demonstrate that patients with ACLF have a *rebalanced* coagulation system that is largely comparable with that of acutely decompensated cirrhosis. We also demonstrate that, in this cohort and with this study design, ROTEM®, despite multiple alterations indicative of a more marked hypocoagulable state, was not useful to identify patients at higher risk of bleeding. Whether and how haemostasis is implicated in the pathogenesis of bleeding complications in hospitalized patients with ACLF requires further investigation.

ETHICS APPROVAL STATEMENT AND PATIENT CONSENT STATEMENT

This study was approved by the Padova Hospital Ethical Committee (3103/A0/14). The study was conducted in compliance with the Declaration of Helsinki and all patients gave written informed consent before enrolment.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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