Research article

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Global hemostatic profiling in patients with decompensated cirrhosis and bacterial infections

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Background & Aims: Bacterial infections in cirrhosis are associated with increased bleeding risk. To assess the factors responsible for bleeding tendency in patients with bacterial infections, we conducted a prospective study comparing all 3 aspects of hemostasis (platelets, coagulation, and fibrinolysis) in hospitalized patients with decompensated cirrhosis with *vs.* without bacterial infections.

Methods: Primary hemostasis assessment included whole blood platelet aggregation and von Willebrand factor (VWF). Coagulation assessment included procoagulant factors (fibrinogen, factor II, V, VII, VIII, IX, X, XI, XII, XIII), natural anticoagulants (protein C, protein S, antithrombin) and thrombomodulin-modified thrombin generation test. Fibrinolysis assessment included fibrinolytic factors (plasminogen, t-PA, PAI-1, α2-AP, TAFIa/ai) and plasmin-antiplasmin complex (PAP).

Results: Eighty patients with decompensated cirrhosis were included (40 with and 40 without bacterial infections). Severity of cirrhosis and platelet count were comparable between groups. At baseline, patients with cirrhosis and bacterial infections had significantly lower whole blood platelet aggregation, without significant differences in VWF. Regarding coagulation, bacterial infections were associated with reduced procoagulant factors VII and XII, and a significant reduction of all natural anticoagulants. However, thrombomodulin-modified thrombin generation was comparable between the study groups. Finally, although mixed potentially hypo-fibrinolytic (lower plasminogen) and hyper-fibrinolytic (higher t-PA) changes were present in bacterial infections, a comparable level of PAP was detected in both groups. Upon resolution of infection (n = 29/40), platelet aggregation further deteriorated whereas coagulation and fibrinolysis factors returned to levels observed in patients without bacterial infections.

Conclusion: In hospitalized patients with decompensated cirrhosis, bacterial infections are associated with reduced whole blood platelet aggregation and a significant decrease of all natural anticoagulants, which may unbalance hemostasis and potentially increase the risk of both bleeding and thrombosis.

Lay summary: Bacterial infections are a common issue in hospitalized patients with decompensated cirrhosis (*i.e.* patients hospitalized due to severe complications of advanced chronic liver disease). Patients with decompensated cirrhosis who acquire infections may be at increased risk of bleeding complications following invasive procedures (that is a procedure in which the body is penetrated or entered, for instance by a needle or a tube). As bleeding complications in decompensated cirrhosis are associated with a high risk of further decompensation and death, there is an urgent need to understand the factors responsible for such increased bleeding tendency. Herein, we investigated the alterations of hemostasis (that is the physiological process responsible for clot formation and stability) in patients with decompensated cirrhosis and bacterial infections. We found that development of bacterial infections in these patients is associated with alterations of hemostasis (particularly of platelets and clotting cascade) that may increase the risk of both bleeding and thrombotic complications.

Keywords: cirrhosis; infections; platelets; coagulation; fibrinolysis.
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Introduction

Decompensated cirrhosis is associated with multiple alterations of hemostasis that include low platelet count and increased von Willebrand factor, a concomitant decrease of most procoagulant factors and inhibitors, and complex changes in fibrinolysis.[1–4] Current theory posits that these alterations lead to a *rebalanced* hemostatic state in which hemostatic changes promoting bleeding (*i.e.* low platelet count, reduced levels of clotting factors) are counterbalanced by hemostatic changes promoting clotting (*i.e.* increased von Willebrand factor, reduced levels of

anticoagulants).[5–7] However, this rebalanced equilibrium is particularly susceptible to destabilizing factors and easily tilts towards either hypo-coagulability (increased risk of bleeding) or hyper-coagulability (increased risk of thrombosis).[5–7] For instance, recent studies have demonstrated that the development of acute kidney injury (AKI) in hospitalized patients with decompensated cirrhosis is associated with platelet dysfunction[8] and low levels of fibrin-stabilizing factor XIII,[8,9] which may explain the bleeding tendency in patients who undergo paracentesis in the presence of AKI.[10]

Bacterial infections are common complications in decompensated cirrhosis,[11] occurring in up to 47% of hospitalized patients.[12] Previous evidence suggests that bacterial infections in cirrhosis are associated with increased risks of portal hypertensive bleeding[13,14] – in which hemostasis is not implicated[15] – as well as delayed bleeding after endoscopic variceal ligation.[16] On the other hand, the association between bacterial infections and procedure-related bleeding in hospitalized patients with cirrhosis is still under investigation (NCT04076605).

Recent international guidelines recommend screening and treatment of bacterial infections in patients with cirrhosis undergoing procedures.[5–7] Unlike AKI-related bleeding,[8–10] however, the hemostatic factors potentially involved in the purported increased risk of procedure-related bleeding in patients with decompensated cirrhosis and infections have not yet been investigated.[17] Indeed, it is currently unclear whether alterations of hemostasis are truly responsible for the bleeding tendency of these patients. A better understanding of the alterations of hemostasis driven by bacterial infections in decompensated cirrhosis would improve hemostatic and clinical management of these patients.

Herein, we conducted a prospective study to thoroughly assess alterations of hemostasis (platelets, coagulation, and fibrinolysis) in hospitalized patients with decompensated cirrhosis and bacterial infections.

Materials and methods

Patient selection

Adult (>18 years old) patients with acutely decompensated cirrhosis admitted to the Gastroenterology/Multivisceral Transplant Unit and Internal Medicine Unit (5th chair) of Padova University Hospital from October 1st 2020 to September 30th 2021 were prospectively screened to determine eligibility to participate in the study. The diagnosis of cirrhosis was confirmed using available data including histology, radiology, laboratory, and clinical assessment.[18] Acute decompensation of cirrhosis was defined as an acute development of clinically significant ascites, hepatic encephalopathy, portal hypertensive-related gastrointestinal bleeding or bacterial infection or any combination thereof.[18–20] Exclusion criteria were: admission for variceal hemorrhage or variceal hemorrhage and/or any other major bleeding in the 30 days prior to admission[21]; a diagnosis of acute-on-chronic liver failure (ACLF) at the time of screening[22]; transfer from other hospitals; admission to intensive care units.

At screening, patients' medical records, past medical history, and laboratory data were reviewed for the following exclusion criteria: chronic kidney disease; presence and/or history of portal vein thrombosis and/or venous thromboembolism; presence of liver cancer at last available imaging; history of extrahepatic tumors or known hematologic diseases; recent major surgery (within 1 month); HIV infection, history of any organ transplantation; therapeutic anticoagulation and/or anti-platelet therapy and/or anti-fibrinolytic therapy; transfusion of any blood product in the 7 days prior to screening. These exclusion criteria were chosen to mitigate the effects of potential confounders on the assessment of hemostasis.[23–28]

Following admission to the inpatient service and having determined eligibility, patients were categorized into cases (with bacterial infections) and controls (without bacterial infections). Bacterial infections were categorized into spontaneous bacterial peritonitis, pneumonia, urinary tract infection, bloodstream infection, gastro-intestinal infection (including *C. difficile*), and erysipelas/skin/subcutaneous infection, as per standard criteria (see supplementary information for further details regarding definition and screening of infection). Presence of sepsis was defined according to Sepsis-3 criteria.[29]

A third group of hospitalized patients with bacterial infections but without liver disease was included to determine which bacterial infection-driven alterations of hemostasis were specific to cirrhosis. These patients were compared with a group of 40 healthy individuals. Patients' medical records, past medical history, and previous laboratory data were reviewed to apply the aforementioned exclusion criteria used in patients with cirrhosis plus the presence of any signs (clinical, biochemical or imaging) and/or history of liver disease.

Study design

This was a prospective, single-center, cohort study, approved by the Ethics Committee of Padova University Hospital (HIC #0034435). The study was conducted in compliance with the principles of the Declaration of Helsinki and all patients gave written informed consent before enrollment.

Patients with cirrhosis and bacterial infections were recruited within 24 hours of the diagnosis of infection, whether bacterial infections were present at admission or developed during hospitalization. Evaluation of hemostasis was performed twice: at enrollment, and after the resolution of infection. Resolution of infection was defined as complete resolution of clinical signs and/or symptoms related to bacterial infections such that no further antibiotic therapy was required. Microbiological/laboratory documentation of infection resolution was required for patients with urinary tract infection, bloodstream infection, and spontaneous bacterial peritonitis. The re-assessment of hemostasis was not performed in the following cases: initiation of renal replacement therapy, major surgery, transfer of the patient to intensive care units, development of thrombosis, development of major bleeding.[21]

In patients with cirrhosis without bacterial infections the evaluation of hemostasis was performed once, at enrollment. Patients with bacterial infections but without liver disease were recruited within 24 hours of the diagnosis of bacterial infection, and evaluation of hemostasis was performed once, at enrollment.

Sample collection and assessment of hemostasis

Blood sampling

See supplementary information.

Assessment of hemostasis

Assessment of hemostasis included primary hemostasis (platelets), secondary hemostasis (coagulation), and tertiary hemostasis (fibrinolysis).

Primary hemostasis (platelets) was assessed by measuring platelet aggregation by whole blood aggregometry (Multiplate® analyzer, Roche Diagnostics, Switzerland)[30] and von Willebrand factor (platelet adhesive glycoprotein, VWF), both antigen (VWF:Ag) and function (VWF:RCo).

Secondary hemostasis (coagulation) was assessed by measuring fibrinogen, procoagulant factors II (FII), V (FV), VII (FVII), VIII (FVIII), IX (FIX), X (FX), XI (FXI), XII (FXII), fibrin-stabilizing factor XIII (FXIII), natural anticoagulants (protein C [PC] chromogenic and coagulometric, protein S [PS], and anti-thrombin [AT]), as well as thrombin generation with and without thrombomodulin (TM).[27,31] Thrombin-antithrombin complex was determined as a marker of coagulation activation.

Tertiary hemostasis (fibrinolysis) was assessed by measuring plasminogen, tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), α2-antiplasmin (α2-AP), and activated and inactivated thrombin-activatable fibrinolytic inhibitor (TAFIa/ai). TAFIa/ai represents the amount of TAFI that has been activated. Plasmin-antiplasmin complex (PAP) was determined as a marker of fibrinolysis activation.

All tests were performed at the General Internal Medicine and Thrombosis and Hemostasis Unit, Coagulation Laboratories of Padova University Hospital by expert personnel. The tests were performed only for research purposes and results were not shared with the clinical team caring for the patients.

See supplementary information for more details.

Data collection

Data collected from the medical records included reason for admission, patient demographics, presence or absence of AKI at the time of infections,[32,33] and laboratory data. Thrombocytopenia was defined as platelet count <150x10^9/L, and subdivided into mild (100-150x10^9/L), moderate (50-100x10^9/L) or severe (<50x10^9/L).

Data analysis

Study objective

Our primary objective was to compare primary hemostasis, secondary hemostasis, and tertiary hemostasis in patients with decompensated cirrhosis with *vs.* without bacterial infections.

As the measurement of platelet aggregation by whole blood aggregometry depends on platelet count, this comparison was performed at 2 levels: overall and according to the severity of thrombocytopenia. This adjustment was performed to better ascertain the impact of bacterial infections on platelet aggregation.

Sample size determination

See supplementary information for further details.

Statistical analysis

See supplementary information for further details.

Results

Demographics

Eighty patients with decompensated cirrhosis were recruited (40 with and 40 without infection) (Fig. 1). Baseline demographics and severity of cirrhosis by means of Child-Pugh stage were comparable between the 2 groups (Table 1). Abdominal pain or suspected infection and ascites accounted for approximately 80% of admissions. Model for end-stage liver disease score was significantly higher in patients with bacterial infections than in those without bacterial infections (19 *vs.* 16, respectively), due solely to differences in international normalized ratio (INR: 1.6 [1.4-1.9] *vs.* 1.4 [1.2-1.7]). Indeed, bilirubin and creatinine were comparable between the study groups. White blood cell count, C-reactive protein, and procalcitonin were all significantly higher in patients with *vs.* without bacterial infections (Table 1).

Spontaneous bacterial peritonitis was the most frequent infection (32.5%), followed by urinary tract infection (22.5%), and erysipelas/subcutaneous infection (12.5%). Among patients with bacterial infections, 17 (42.5%) had sepsis (Table 1).

At time of enrollment, the prevalence of AKI was comparable between groups (30% *vs.* 20% in patients with *vs.* without bacterial infections, respectively). In both groups, pre-renal azotemia was the most common etiology of AKI — 58% in patients with bacterial infections and 75% in patients without bacterial infections, respectively.

Twenty-nine (72%) patients with cirrhosis and bacterial infections had repeat assessments at resolution of infection (Fig. 1). The median duration of infection was 10 days (IQR: 7-12). At resolution of infection, bilirubin and INR significantly decreased (p <0.01) and became comparable with values in patients with cirrhosis without bacterial infections (Table S1). Indeed, MELD score at resolution was similar to that in patients without infections (17 *vs.* 16, respectively; p = 0.7).

Resolution of infections was also associated with a significant decrease in inflammatory markers (all p <0.0001). White blood cell count and procalcitonin became comparable to baseline values in controls without infections, whereas C-reactive protein remained significantly higher (Table S1).

Ten inpatients with bacterial infections but without liver disease were recruited as controls (M/F 5/5, median age 64 years [IQR: 75-66]). Median platelet count was 202 (182-255) x10^9/L. Pneumonia, urinary tract infection, and subcutaneous infection occurred in 50%, 30%, and 20% of patients, respectively. Sepsis and AKI were present in 30% and 20% of patients, respectively.

Baseline sample collection

In patients with cirrhosis, baseline samples were collected at or near admission (median time 1 day [IQR 1-2] *vs.* 1 day [IQR 1–3] in patients with and without bacterial infections, respectively [p = 0.9]).

Among patients with bacterial infections, 85% (34/40) had infection at admission, whereas the remaining 15% developed bacterial infections during hospitalization with a median time from admission to blood collection of 6 days [range 5-8].

Impact of bacterial infections on primary hemostasis (platelets) in decompensated cirrhosis

As shown in Table 1, platelet count was comparable between patients with and without bacterial infections (86x10^9/L *vs.* 92x10^9/L, respectively). Nearly all patients were thrombocytopenic — mostly moderate thrombocytopenia (Table 1).

At baseline, whole blood platelet aggregation was significantly more altered in patients with cirrhosis and bacterial infections compared to those without bacterial infections (Table 2). Both ADP- and thrombin receptor activating peptide-induced platelet aggregation were significantly reduced in patients with bacterial infections. By contrast, arachidonic acid-induced aggregation was comparable between the 2 groups (Table 2).

Reduced whole blood platelet aggregation in patients with cirrhosis and bacterial infections was in line with the

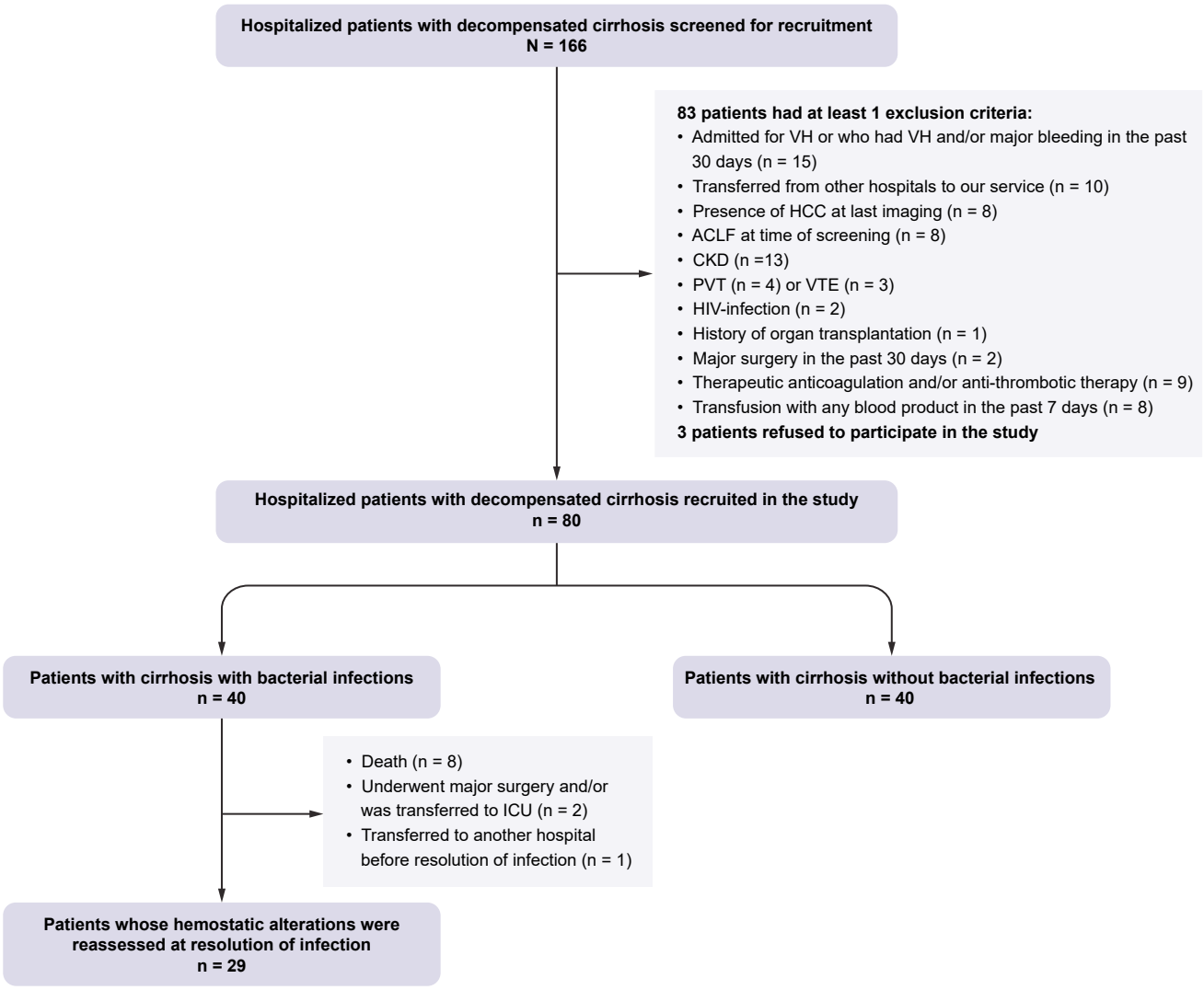

Fig. 1. Flow chart of the study. ACLF, acute-on-chronic liver failure; CKD, chronic kidney disease; HCC, hepatocellular carcinoma; ICU, intensive care unit; PVT, portal vein thrombosis; VH, variceal hemorrhage; VTE, venous thromboembolism.

aforementioned findings in patients with moderate and severe thrombocytopenia but not those with mild thrombocytopenia or normal platelet count (Fig. 2 and Table S2).

Baseline VWF:Ag and VWF:RCo were higher in patients with *vs.* without bacterial infections though the difference was not statistically significant (Table 2). Resolution of bacterial infections was associated with a significant decrease in whole blood platelet aggregation, independently of the severity of thrombocytopenia and agonist used (Fig. 3). In fact, we noted that all agonist-induced platelet aggregation became significantly lower than the baseline values in patients with cirrhosis without bacterial infections, irrespective of the severity of thrombocytopenia (Table S2).

At resolution of bacterial infections, both VWF:Ag and VWF:RCo significantly dropped to near baseline values in patients without bacterial infections (Table S3).

In patients without liver disease and bacterial infections, whole blood platelet aggregation and VWF were significantly lower and higher, respectively, than in healthy individuals (Tables S4 and 5).

Impact of bacterial infections on secondary hemostasis (coagulation) in patients with decompensated cirrhosis

At baseline, patients with cirrhosis and bacterial infections had lower levels of factors VII and XII, as well as natural anticoagulants PC, PS, and AT *vs.* patients with cirrhosis without bacterial infections (Table 2). Fibrinogen was higher in patients with bacterial infections though the difference was not significant (Fig. 4). Levels of FII, FVIII, FIX, FX, FXI, and FXIII were comparable between the 2 groups (Table 2).

Endogenous thrombin potential (ETP) was comparable among patients with cirrhosis with bacterial infections, patients with cirrhosis without bacterial infections, and healthy controls. The addition of TM significantly reduced ETP in healthy individuals but not in patients with cirrhosis (Fig. 5 and Table S6). In fact, ETP with TM was significantly higher in cirrhosis than in healthy individuals, independently of infections (Fig. 5).

Among patients with cirrhosis, ETP (both with and without TM) was comparable between patients with and without bacterial infections, whereas the ETP ratio was significantly higher in those with bacterial infections (Fig. 5). Other parameters of

Table 1. Baseline characteristics in patients with decompensated cirrhosis.

	Infection (n = 40)	No infection (n = 40)	*p* value
Age (years)	61 (53-69)	61 (54-78)	0.7
Male sex (%)	70	68	0.8
Etiology of cirrhosis (%)			0.5
Alcohol	50	60	
Viral	22.5	17.5	
NASH	10	12.5	
Autoimmune	12.5	10	
Other	5	0	
Child class B/C, %	48/52	52/48	0.7
Pugh score^	10 (7-13)	10 (7-12)	0.7
MELD score	19 (15-24)	16 (11-21)	0.04
MELD-Na score	20 (17-25)	18 (12-23)	0.1
Ascites (%)	85	88	0.7
Reason for admission (%)			0.2
Abdominal pain/suspected infection	40	18	
Ascites	43	60	
HE	15	17	
Other	2	5	
AKI (%)	30	20	0.3
VTE prophylaxis (%)	12.5	7.5	0.5
Type of infection (%)*			-
Spontaneous bacterial peritonitis (SBP)	32.5	-	
Urinary tract infection (UTI)	22.5		
Pneumonia	10		
Gastrointestinal (GI)	10		
Erysipelas/subcutaneous	12.5		
Bloodstream infections (BSI)	10		
Primary Biliary Cholangitis (PBS)	2.5		
History of previous HCC (%)	12	12	1
Total bilirubin, mg/dl	4.5 (2.7-8.2)	2.7 (1.5-8.6)	0.1
INR	1.6 (1.4-1.9)	1.4 (1.2-1.7)	0.02
Albumin, g/dl	30 (26-34)	31 (29-34)	0.4
White blood cells, 10^9/L	9 (4-14)	5 (3-8)	0.01
Polymorphonucleate cells, 10^9/L	6 (3-11)	3 (2-6)	<0.0001
C-reactive protein, mg/L	54 (35-79)	6 (<2.9-18)	<0.0001
Procalcitonin, ng/L	1 (0.2-3)	0.2 (0.1-0.4)	0.001
Lactate, mmol/L	2.1 (1.3-2.8)	1.2 (0.8-1.7)	0.005
Presepsin, ng/L	1,253 (450-2,400)	516 (248-1,190)	<0.0001
Hemoglobin, g/dl	9.4 (8.5-12)	9 (8.3-11)	0.3
Platelet count, 10^9/L	86 (57-129)	92 (67-125)	0.6
Thrombocytopenia, (%)			0.3
Present	85	95	
Mild 100-150x10^9/L	27	37	0.6
Moderate 50-100x10^9/L	53	47	
Severe <50x10^9/L	20	16	
Creatinine, mg/dl	0.8 (0.7-1.2)	0.7 (0.6-1)	0.6
Sodium, mmol/L	135 (133-138)	136 (133-138)	0.8
Potassium, mmol/L	3.9 (3.5-4.3)	3.9 (3.7-4.3)	0.5
AST, U/L	50 (30-87)	40 (30-68)	0.6
ALT, U/L	32 (22-57)	27 (17-45)	0.2
GGT, U/L	37 (21-88)	44 (24-76)	0.8
ALP, U/L	126 (101-164)	110 (89-146)	0.3

Median values reported with 25th and 75th percentile values in parenthesis. Mann-Whitney *U* test.
ALT, alanine aminotransferase; AKI, acute kidney injury; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; VTE, venous thromboembolism.
^ Median (range).
* 2/13 patients with PBS (23%), 2/9 patients with UTI (22%), 4/4 (100%) patients with GI infection, 1/4 patients with erysipelas (25%) had also positive blood cultures; 2/4 patients with BSI developed endocarditis; among patients with infection, 42.5% had sepsis.

thrombin generation were comparable between the 2 groups, except for a longer start tail in patients with bacterial infections (Table S6).

After resolution of infection, fibrinogen significantly decreased whereas FVII, FXII, PC, PS, and AT significantly increased (Table S3). In fact, levels of procoagulant factors and inhibitors became comparable to those observed in patients with cirrhosis without bacterial infections. ETP with and without TM remained unchanged and comparable with that observed in patients with cirrhosis without bacterial infections. The ETP ratio slightly decreased and became comparable to that in patients without infection (Table S3).

Baseline thrombin-antithrombin complex was comparable between patients with and without bacterial infections (Table 2), and no significant change was observed upon resolution of infection (Fig. 6).

Contrary to what we observed in patients with cirrhosis, bacterial infections in patients without liver disease were

Table 2. Hemostatic alterations in patients with decompensated cirrhosis with *vs.* without bacterial infections at baseline.

	Infection (n = 40)	No infection (n = 40)	*p* value
Platelets			
Platelet aggregation, AUC			
ADP	35 (24-50)	47 (35-64)	0.003
ASPI	33 (22-48)	38 (27-47)	0.2
TRAP	60 (36-94)	100 (72-122)	<0.001
VWF:Ag, %	303 (244-383)	278 (223-346)	0.06
VWF:RCo, %	369 (264-526)	325 (243-417)	0.07
Coagulation			
Fibrinogen, mg/dl	194 (111-339)	167 (121-242)	0.3
Factor II, %	44 (24-52)	40 (26-59)	0.7
Factor V, %	56 (40-81)	65 (51-91)	0.3
Factor VII, %	29 (17-42)	40 (25-61)	0.01
Factor VIII, %	245 (176-283)	231 (186-265)	0.4
Factor IX, %	60 (44-92)	60 (42-87)	0.8
Factor X, %	53 (44-67)	55 (43-74)	0.7
Factor XI, %	48 (30-68)	53 (39-72)	0.2
Factor XII, %	47 (38-65)	60 (46-85)	0.02
Factor XIII, %	47 (38-71)	50 (46-73)	0.1
Protein C coagulometric, %	21 (12-38)	31 (17-46)	0.03
Protein C chromogenic, %	26 (21-51)	40 (27-59)	0.05
Protein S, %	56 (42-72)	68 (56-85)	0.001
Anti-thrombin, %	32 (21-47)	38 (31-55)	0.001
ETP, nmol/L*min	903 (774-1117)	965 (789-1156)	0.8
ETP + TM, nmol/L*min	853 (709-1054)	865 (698-958)	0.7
ETP ratio	0.95 (0.91-0.99)	0.90 (0.87-0.92)	0.001
TAT, ng/ml	2.6 (2.3-3.7)	3.1 (2.2-3.8)	0.4
Fibrinolysis			
Plasminogen, %	39 (29-53)	47 (37-64)	0.004
t-PA, ng/ml	22 (19-32)	17 (11-22)	0.001
PAI-1, ng/ml	33 (20-54)	29 (19-42)	0.5
α2-AP, %	50 (43-70)	62 (47-80)	0.2
TAFIa/ai, ng/ml	26 (23-33)	24 (20-33)	0.1
PAP, ng/ml	41 (38-46)	42 (39-44)	0.8

Median values reported with 25th and 75th percentile values in parentheses. Mann-Whitney *U* test.
α2-AP, α2-antiplasmin; ASPI, arachidonic acid; ETP, endogenous thrombin potential; PAI-1, plasminogen activator inhibitor; TAFIa/ai, activated inactivated thrombin-activatable; TAT, thrombin-antithrombin complex; TM, thrombomodulin; t-PA, tissue-type plasminogen activator; TRAP, thrombin receptor agonist peptide; VWF:Ag, von Willebrand factor antigen; VWR:RCo, ristocetin cofactor activity.

associated with a significantly increased level of fibrinogen, FV, FVIII, and FIX. Levels of additional procoagulant factors such as FII, FV, FVII, FX, XI, XII as well as natural anticoagulants PC, PS, and AT were within reference range (Table S5). Both ETP with and without TM and ETP ratio were significantly higher than in healthy individuals (Fig. 5).

Impact of bacterial infections on tertiary hemostasis (fibrinolysis) in patients with decompensated cirrhosis

At baseline, patients with cirrhosis and bacterial infections had lower plasminogen and higher t-PA than patients with cirrhosis without bacterial infections. Conversely, PAI-1, α2-AP, and TAFIa/ai were comparable between groups (Table 2).

At resolution of bacterial infections plasminogen increased and t-PA decreased, both to levels comparable with those observed in patients without bacterial infections. TAFIa/ai increased to higher levels than baseline in patients without bacterial infections. PAI-1 and α2-AP remained unchanged (Table S3).

Baseline PAP was comparable between patients with and without bacterial infections (Table 2) and no significant change was observed with resolution of bacterial infections (Fig. 6).

In patients with bacterial infections without liver disease, t-PA and TAFIa/ai were higher than in healthy individuals. No differences in FXIII, plasminogen, PAI-1, and α2-AP were found (Table S5). PAP was significantly reduced *vs.* healthy individuals (35 ng/ml [34–38] *vs.* 48 ng/ml [42-62]; p <0.001]).

Post hoc analyses of hemostatic alterations in patients with cirrhosis excluding those receiving antithrombotic prophylaxis

Similar to the overall analysis, patients with bacterial infections (n = 35) had lower whole blood platelet aggregation than those without infections (n = 37). Resolution of infection was similarly associated with a further reduction in platelet aggregation. Differences in coagulation and fibrinolysis between patients with and without infection were similar to those observed in the overall analysis (Table S7).

Discussion

Although patients with cirrhosis and bacterial infections may be at increased risk of procedure-related bleeding,[5–7] the hemostatic factors eventually responsible for this bleeding tendency have not yet been thoroughly investigated.

While awaiting clarification from multicenter observational studies on the association between bacterial infections and bleeding complications in patients with cirrhosis undergoing procedures (NCT04076605), we conducted a prospective study to extensively investigate alterations of hemostasis (platelets,

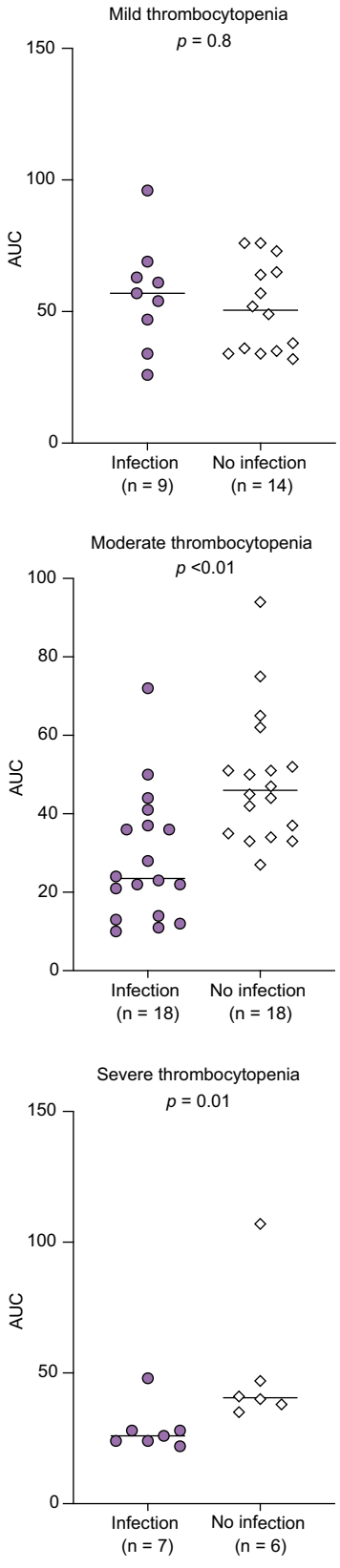

Fig. 2. Whole blood platelet aggregation in patients with cirrhosis. In patients with platelet count <100x10^9/L, ADP-induced platelet aggregation was more altered in patients with *vs.* without bacterial infections. Mann-Whitney *U* test. For numerical values, refer to Table S1.

coagulation factors and inhibitors, and fibrinolysis) in this population.

This study shows, in hospitalized patients with decompensated cirrhosis, that bacterial infections are associated with a prolonged impairment in whole blood platelet aggregation and a significantly reduced level of natural anticoagulants. These alterations may tip the delicate equilibrium of hemostatic balance in hospitalized patients with decompensated cirrhosis towards either hypo-coagulability or hyper-coagulability, thus potentially increasing both bleeding and thrombotic risk.

The primary hemostasis assessment revealed that patients with decompensated cirrhosis and bacterial infections had a significantly reduced whole blood platelet aggregation *vs.* controls with cirrhosis without infections. Interestingly, a deleterious effect of infections on platelet aggregation was also observed in controls with infections without chronic liver disease, thus indicating that such an effect is not specific to cirrhosis. As alterations of platelet aggregation may result in increased bleeding, our results lend support to a possible association between bacterial infections and bleeding complications in hospitalized patients with cirrhosis.[5–7]

In vivo, in case of vessel injury, platelets adhere to subendothelial collagen where they aggregate and form a hemostatic plug.[34] Although whole blood aggregometry mimics *in vivo* conditions of platelet activation and aggregation,[26] it depends on platelet count and does not allow for a direct comparison between thrombocytopenic patients and healthy individuals with a normal platelet count.[35] To obviate this challenge, we used a control group of patients with cirrhosis without bacterial infections and matched cases and controls by severity of thrombocytopenia. The impaired platelet aggregation in bacterial infections was observed only in patients with a platelet count <100x10^9/L, indicating that the deleterious effect of bacterial infections may be dependent on the severity of thrombocytopenia.

Interestingly, resolution of bacterial infections was associated with a further reduction in whole blood platelet aggregation, independently of baseline platelet count and agonist used, indicating that the impaired platelet function may persist or even worsen despite the initial control of infection. Therefore, unlike the reversible platelet dysfunction in patients with decompensated cirrhosis with AKI, which completely resolves with recovery of kidney function,[8] more time seems to be required to achieve a complete resolution of platelet abnormalities driven by infections.

The main function of VWF, a glycoprotein released by endothelial cells, is to facilitate adhesion of platelets to subendothelial collagen. In decompensated cirrhosis, plasmatic VWF is increased due to inflammation and endothelial shear stress.[36] Bacterial infections and sepsis may induce endothelial activation with release of VWF.[37] In fact, we found that patients with infection without liver disease had a significantly higher VWF than healthy individuals.

By contrast, levels of VWF were only slightly elevated in patients with cirrhosis with *vs.* without bacterial infections. A potential explanation for this finding is that chronic release of VWF in decompensated patients is so elevated due to severe portal hypertension that superimposed infections cannot induce a further, significant release of VWF by the endothelium. An alternative explanation is that there is an increased consumption of VWF during infections. To test these hypotheses, further studies should investigate markers of endothelial activation/VWF

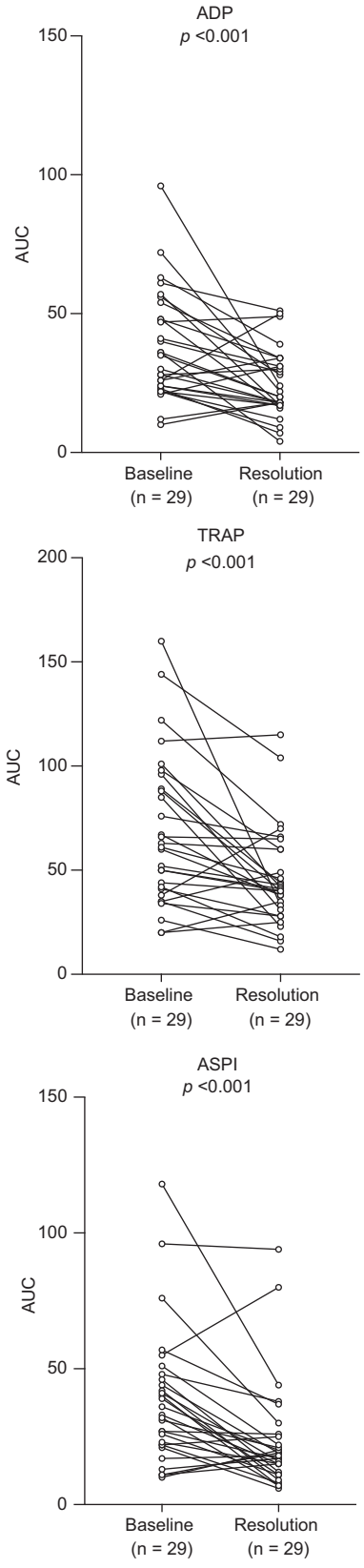

Fig. 3. Evolution of platelet aggregation after resolution of infection. Resolution of infection is associated with a significant reduction of whole blood platelet aggregation, independent of baseline platelet count and agonist used. Wilcoxon matched-pairs signed rank test. ASPI, arachidonic acid; TRAP, thrombin receptor activating peptide.

propeptide in patients with decompensated cirrhosis and infections.

Interestingly, the evolution of whole blood platelet aggregation (impairment at baseline and further deterioration after resolution of infections) indicates that the deleterious effect of bacterial infections on platelet aggregation occurs independently of increased VWF.

Whole blood aggregometry, however, is a static test which cannot specifically explore the contribution of VWF to platelet function. Therefore, studies under experimental conditions of blood flow are needed to strengthen the hypothesis that alterations in whole blood platelet aggregation driven by bacterial infections truly lead to defective primary hemostasis in decompensated cirrhosis.

In opposition to our findings on platelet aggregation, the secondary hemostasis analysis revealed that bacterial infections were mostly associated with prothrombotic changes such as decreased levels of natural anticoagulants PC, PS, and AT. As the severity of cirrhosis was comparable between groups, the reduced levels of natural anticoagulants likely reflect a transient worsening of hepatic synthetic function driven by infections (as also indicated by the lower level of procoagulant factors VII/XII and increased INR). Infection resolution was associated with a significant increase in both pro- and anticoagulant factors, which is further evidence that bacterial infections are truly responsible for the observed alterations in secondary hemostasis.

Despite the significant decrease in anticoagulant factors, the overall clotting capacity – as assessed by TM-modified thrombin generation assay – remained comparable between patients with and without infections.[38] Contrary to our observations in patients without liver disease, in whom infections were associated with significantly increased thrombin generation, this would suggest that infections in decompensated cirrhosis do not lead to a more pronounced hyper-coagulable state.

However, in hospitalized patients with acutely decompensated cirrhosis even small differences in coagulation factors may result in unpredictable changes in the precarious balance between pro and anticoagulant factors, which may not be accurately assessed by TM-modified thrombin generation assays.[39]

Hence, the significant decrease in natural anticoagulants driven by infections may nonetheless destabilize the hemostatic balance towards hyper-coagulability, and thus increase thrombotic risk,[40] as was recently demonstrated in patients with ACLF.[41,42]

In hospitalized patients without liver disease, infections are well-known risk factors for thrombosis, whereas the evidence supporting this association in those with cirrhosis is not as strong.[43] Therefore, further prospective studies are needed to investigate the correlation between bacterial infections/sepsis, decreased anticoagulants, and development of thrombosis in hospitalized patients with decompensated cirrhosis.

Regarding fibrinolysis, our analysis revealed that bacterial infections in decompensated cirrhosis are associated with mixed hypofibrinolytic (low plasminogen) and hyperfibrinolytic (increased t-PA) changes. In patients without liver disease, bacterial infections and sepsis have been associated with a mostly hypo-fibrinolytic status.[44] This is confirmed by our findings in hospitalized patients with bacterial infections without liver disease in whom PAP, a marker of fibrinolysis activation, was significantly reduced. As decompensated cirrhosis is associated with complex changes in fibrinolysis,[1] it may be that the effect of

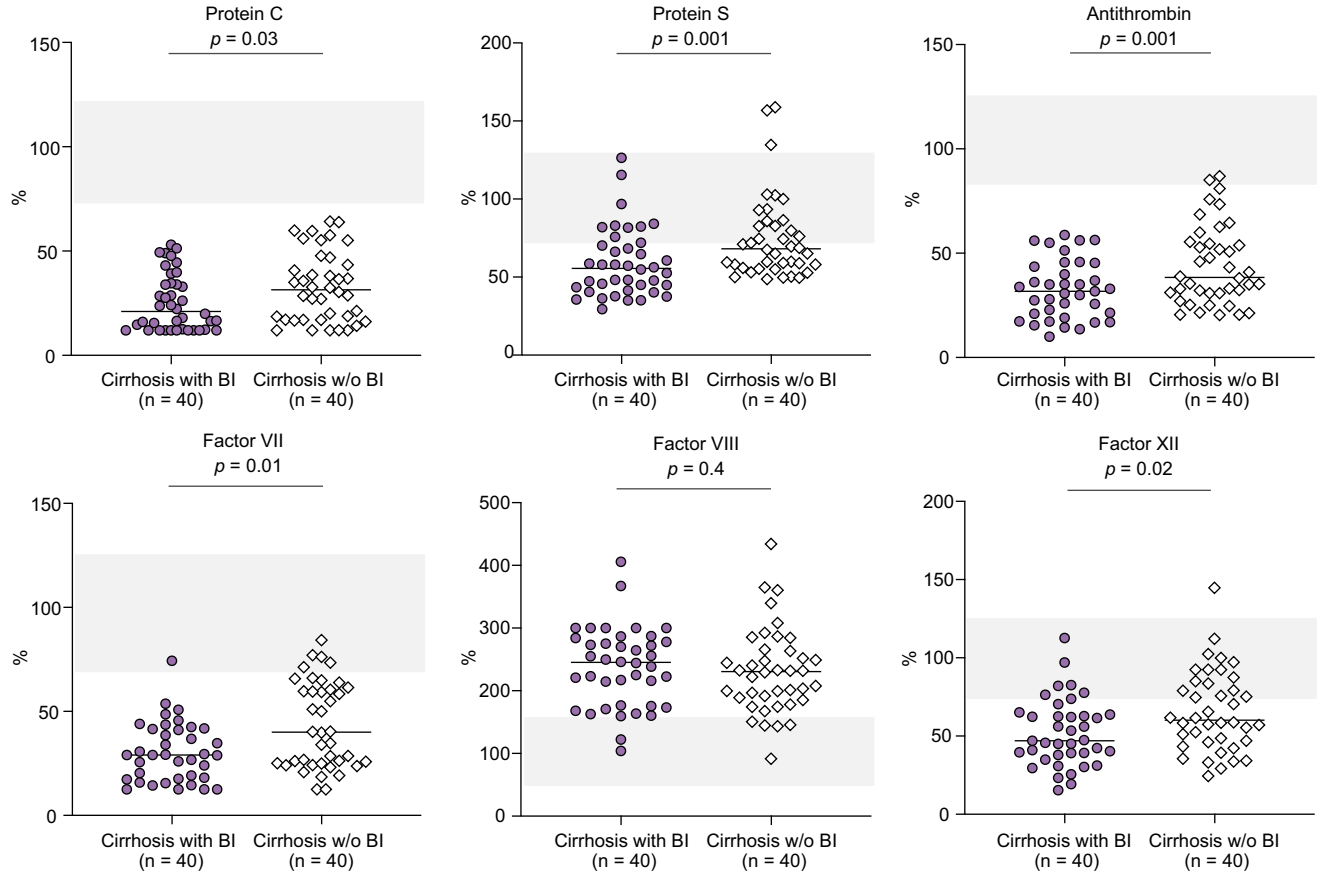

Fig. 4. Levels of procoagulant factors and natural anticoagulants in cirrhosis patients with *vs.* without bacterial infections. The grey area refers to the reference range in healthy individuals. Mann-Whitney *U* test. BI, bacterial infection.

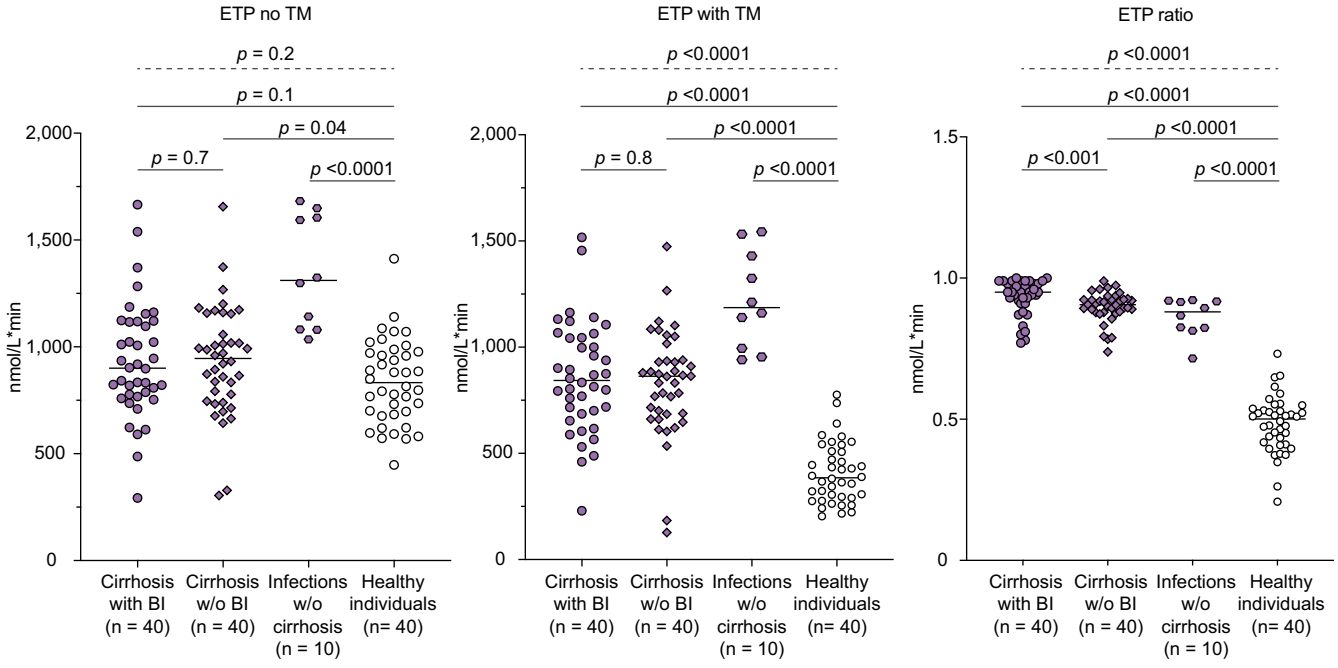

Fig. 5. Thrombin generation results in patients with and without bacterial infections. Mann-Whitney *U* test (solid line); Kruskal-Wallis test (dotted line). BI, bacterial infection; ETP, endogenous thrombin potential; TM, thrombomodulin.

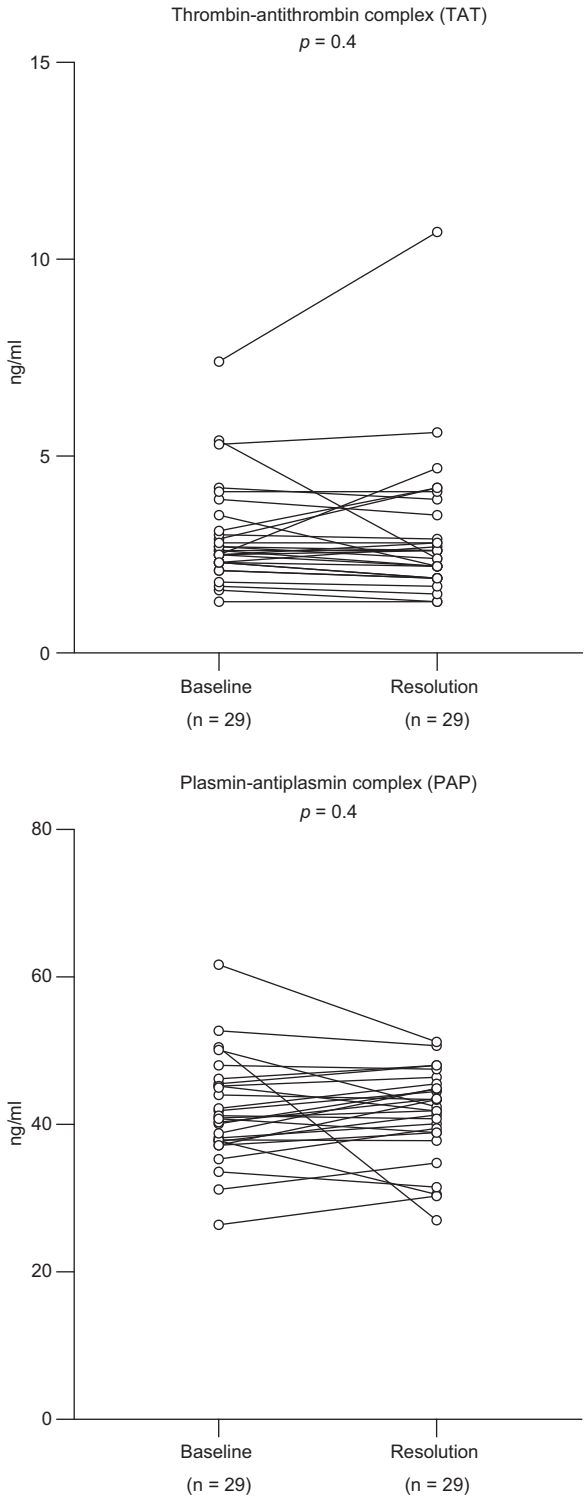

Fig. 6. Evolution of coagulation and fibrinolysis at resolution of infection. Resolution of bacterial infection was not associated with significant changes in activation of coagulation and fibrinolysis, as evidenced by the unchanged levels of thrombin-antithrombin complex (top) and plasmin-antiplasmin complex (bottom). Wilcoxon matched-pairs signed rank test.

bacterial infections in cirrhosis is less uniform than in patients without chronic liver disease, thus explaining the coexistence of hypo-fibrinolytic and hyper-fibrinolytic changes. However, the comparable level of PAP in patients with *vs.* without bacterial infections indicates that there is no major difference in fibrinolysis activation between the 2 groups. As our analysis of a single protein does not encompass cell contribution and regulatory interactions, this hypothesis needs to be confirmed using a more global fibrinolysis assay.

We would be remiss not to mention some of the limitations of our study. Firstly, we did not include an assessment of endothelial alterations that may partially account for the bleeding tendency in patients with decompensated cirrhosis and bacterial infections.[37] Secondly, ongoing medication may have interfered with the assessment of hemostasis though we attempted to mitigate this issue by applying identical inclusion criteria for both groups and excluding additional confounders such as ACLF, recent bleeding/thrombosis, chronic kidney disease, and recent transfusions. Finally, the evaluation of additional markers of primary hemostasis (ADAMTS13, VWF multimers, markers of platelet activation) and coagulation (tissue factor pathway inhibitor), which are not included in our study, may further improve our understanding and management of the complex coagulopathy observed in these patients.

In conclusion, in a prospective cohort of hospitalized patients with decompensated cirrhosis, we demonstrate that bacterial infections are associated with specific alterations of hemostasis such as prolonged impairment of whole blood platelet aggregation and significant reduction of all natural anticoagulants. These bacterial infection-driven alterations of hemostasis may tip the precarious hemostatic balance in hospitalized patients with decompensated cirrhosis either towards hyper- or hypocoagulability, and potentially increase the risk of both bleeding and thrombosis. Further studies are needed to ascertain whether the improvement of hemostasis, particularly platelet function, is associated with reduced incidence and severity of bleeding complications in patients with decompensated cirrhosis and bacterial infections.

Abbreviations

α2-AP, α2-antiplasmin; ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; AT, antithrombin; ETP, endogenous thrombin potential; F, factor; FXIII, fibrin-stabilizing factor XIII; MELD, model for end-stage liver disease; PAI-1, plasminogen activator inhibitor-1; PAP, plasmin-antiplasmin complex; PC, protein C; PS, protein S; TAFIa/ai, activated and inactivated thrombin-activatable fibrinolytic inhibitor; TM, thrombomodulin; t-PA, tissue-type plasminogen activator; VWF, von Willebrand factor.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

AZ: research design, performance of the research (patients' enrollment and laboratory work), interpretation of the data, statistical analysis, writing of the manuscript. EC: laboratory work, interpretation of the data, critical revision of the manuscript. CB: laboratory work. GS: laboratory work. SG: laboratory work. SS: performance of the research (patients' enrollment and laboratory work). PB: acquisition of the data, critical revision of the manuscript. PA: acquisition of the data, critical revision of the manuscript. MS: research design, interpretation of the data, and critical revision of the manuscript. PS: research design, funding of the research, organization of lab facilities and testing, interpretation of the data, critical revision and final approval of the manuscript.

Data availability statement

Data are available from the corresponding authors (Prof. Paolo Simioni and Dr. Marco Senzolo) upon reasonable request.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2022.100493.

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Author names in bold designate shared co-first authorship

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