Association of Heparin-Like Effect, Factor VII/XIII Deficiency and Fibrinolysis with Rebleeding Risk in Cirrhosis with Acute Variceal Bleeding

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Abstract

Background Hyperfibrinolysis and coagulation dysfunction may occur in cirrhotic patients with acute variceal bleed (AVB) despite successful endotherapy.

Aims To prospectively study the association of endogenous heparinoids and coagulation dysfunction with variceal rebleeding and outcome in cirrhosis.

Methods Consecutive patients were assessed with conventional coagulation tests, SONOCLOT™ [(global (gb) and heparinase (h) treated] and factors VII, VIII, XIII, X, tissue plasminogen activator, and plasminogen activator inhibitor ELISA assays in a university hospital. Heparin-like-effect (HLE) was defined as ≥20% difference in paired gb/h-SONOCLOT™ traces for activated clotting time (ACT).

Results Of 143 patients screened, 90 (46.4 ± 11.7 years, males 82.2%, ethanol-related 58.8%) were recruited, who bled from esophageal varices (81, 90.0%), gastric varices (6, 6.6%), or esophageal varices with portal hypertensive gastropathy (3, 3.3%). Twenty (21.7%) had early rebleeding, mainly post-variceal ligation ulcer related (70%). Patients who rebled had low Factor XIII [1.6 (1.2–2.1) vs 2.4 ng/ml (2.0–2.8) P = 0.035] and Factor VII (94.1 ± 46.9 vs. 124.0 ± 50.4, P = 0.023). On receiver operating curve analysis, the gbACT > 252 s (sensitivity 86.8%, specificity 76.9%, P < 0.001), hACT > 215 s (sensitivity 71.1%, specificity 70.3%, P < 0.001), and HLE > 50% (sensitivity 69.5%, specificity 70.3%, P = 0.006) predicted rebleeding. Baseline Factor VIII (HR 1.26; 95% CI 1.17–1.34, P < 0.001), low factor VII (HR 0.89; 95% CI 0.76–0.98, P = 0.035), and lysis (HR 1.25, 95% CI 1.17–1.33, P < 0.001) predicted mortality. Endogenous heparinoids at baseline predicted sepsis (HR 1.8; 95% CI 1.4–6.5; P = 0.022), rebleeding events (HR 1.2; 95% CI 1.1–6.3; P = 0.030), and mortality (HR 1.1; 95% CI 1.0–4.6; P = 0.030).

Conclusions Hyperfibrinolysis, Factor VII/XIII deficiency, and HLE are associated with rebleeding after AVB.

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Heparin-like Effect, Factor VII/XIII deficiency & hyperfibrinolysis are associated with early rebleeding and death in acute variceal bleeding

Consecutive patients with cirrhosis with acute variceal bleeding were assessed with conventional coagulation tests, SONOCLOT™ (globin) and heparinase (h) treated and factors VII, VIII, XIII, X, tissue plasminogen activator and plasminogen activator inhibitor assays.

Of 143 patients screened, 90 patients (46.4± 11.7 years, males 82.2%) were recruited, who bled from esophageal varices (90%), gastric varices (4.4%) or dual sites (3.3%).

Twenty (22.2%) had early rebleeding, mainly post-EVL ulcer related. Repeat Endotherapy Blood components as per VET algorithm.

Baseline Factor VIII (HR 1.25; P<0.001), low factor VII (HR 0.98; P=0.045), and clot lysis (HR 1.25, P<0.001) predicted mortality.

Endogenous heparinoids at baseline predicted sepsis (HR 1.8; CI 1.4-6.5; P=0.022), rebleeding events (HR 1.2; CI 1.1-6.3; P=0.030) and mortality (HR 1.1; CI 1.0-4.6; P=0.030).
Keywords  Endogenous heparinoids · Portal hypertension · Sonoclot · Transfusion algorithms · Acute-on-chronic liver failure

Abbreviations
AST  Aspartate transaminase
ALT  Alanine transaminase
ACT  Activated clotting time
ACLF  Acute-on-chronic liver failure
aPTT  Activated partial thromboplastin time
ATIII  Antithrombin III
ADAMTS13  A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
CI  Confidence interval
BMI  Body mass index
CTP  Child Turcotte Pugh
CR  Clot rate
CRP  High-sensitivity C-reactive protein
EVL  Endoscopic variceal ligation
EST  Endoscopic sclerotherapy
HBV  Hepatitis B virus
HCV  Hepatitis C virus
LDH  Lactate dehydrogenase
HLE  Heparin-like effect
HR  Hazard ratio
INR  International normalized ratio
Lys30 and 60  Lysis at 30 minutes and 60 minutes
MA  Maximum amplitude
MELD  Model for end-stage liver disease
NLR  Neutrophil lymphocyte ratio
PRBC  Packed red blood cells,
FFP  Fresh frozen plasma
PAI-1  Plasminogen activator inhibitor type 1
SDPC  Single donor platelet concentrate
RDPC  Random donor platelet concentrate
SCT  Standard coagulation tests
SIRS  Systemic inflammatory response syndrome
TLC  Total leucocyte count
TEG  Thromboelastography
tPA  Tissue plasminogen activator
vWF  Von Willebrand factor

Introduction

Patients with cirrhosis and acute-on-chronic liver failure (ACLF) have complex dynamic coagulation dysfunction [1]. In patients with cirrhosis who present with acute variceal bleeding (AVB), early appropriate endoscopic intervention, use of portal pressure reducing vasoconstrictor agents, and antibiotic prophylaxis are the main methods to reduce mortality. However, changes in anti- and procoagulant factors, hyperfibrinolysis, and endothelial activation can lead to early rebleeding or even thromboses [2]. Endothelial activation due to systemic inflammation or sepsis in cirrhosis results in an increase in Factor VIII and von Willebrand factor (vWF). This causes glycosaminoglycans (heparin sulfate, heparin, and dermatan sulfate) to be released from the endothelium into the circulation, which cause a ‘heparin-like effect’ (HLE) [3]. This anticoagulant effect cannot be detected by standard coagulation tests (SCTs) like prothrombin time (PT), international normalized ratio (INR), D-dimer, or activated partial thromboplastin time (aPTT) but can be demonstrated on heparinase-treated viscoelastic tests [4]. Bacterial sepsis is a known risk factor for rebleeding due to increased portal pressures, splanchnic arterial vasodilation, and coagulation failure [5].

The rationale for evaluating complex coagulation dysfunction in patients with AVB is predicting the patients at higher risk of rebleeding, sepsis, and decompensation events. It is also unclear if coagulation failure contributes to rebleeding, as portal hypertensive bleeding is directly linked to higher portal pressures and severity of underlying disease. The role of specific coagulation factors like VII, VIII, XIII, and X, vWF, ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), tissue plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1) in the process of clot formation and fibrinolysis in AVB and thromboses needs further evaluation [4, 6–11].

Point-of-care (POC) viscoelastic tests like thromboelastography, Sonoclot, or thromboelastometry can detect hyperfibrinolysis, and help guide use of blood component therapy in patients with diffuse mucosal bleeding, disseminated intravascular coagulation, or bleeding from multiple sites [1, 2, 4, 12]. Assessing the role of specific components like Factor VII concentrate or multifactor concentrate like prothrombin complex concentrate (PCC) has been assessed in a few studies [13–15]. Use of these viscoelastic tests can minimize blood transfusions as an adjunct to the main goal of timely endoscopic interventions and use of drugs to reduce portal pressure like terlipressin and octreotide.

In this prospective study, we evaluated patients with cirrhosis who presented with AVB to our center, assessed them with SCTs and glass bead (gb) and heparinase(h)-treated Sonoclot assays, clot lysis, specific coagulation factor levels (including factors VII, VIII, X, and XIII), vWF, tPA, PAI-1, and ADAMTS13 and determined the role of endogenous heparinoids, viscoelastic tests, coagulation parameters that were associated with fibrinolysis and early rebleeding, sepsis, and outcomes at 30 and 90 days. We aimed to assess the association of endothelial dysfunction and fibrinolysis as a predictor of rebleeding.
Methods

Consecutive patients with cirrhosis with acute variceal bleeding (AVB), aged between 18 and 65 years, either gender who presented to the Department of Hepatology, Postgraduate Institute of Medical Education and Research (PGIMER) Chandigarh were recruited with written informed consent between November 2019 and July 2021. The exclusion criteria were recent blood or blood component transfusion in the last 2 weeks, HIV infection, antiplatelet, anticoagulant or antifibrinolytic therapy, dialysis, pregnancy, portal vein thrombosis or recent deep vein thromboses in the last 1 year, transjugular intrahepatic portosystemic shunt (TIPS) in situ, active malignancy in the last 5 years, chronic heart failure, and/or chronic pulmonary or end-stage renal disease. Patients who tested positive for COVID-19 infection were excluded from analysis as they would also have confounding coagulation abnormalities [16]. The protocol was performed in accordance with the Declaration of Helsinki and adhered to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

Definitions

Cirrhosis was defined based on clinical and imaging criteria with endoscopic presence of varices. Early rebleeding was defined as: (1) recurrent hematemesis, and/or melena, and/or bloody fluid drained by nasogastric tube, occurring 6 to 42 days after the first endoscopy [17]. The presence of infection was assessed by blood, urine, sputum, ascitic fluid, and tracheal aspirate cultures, C-reactive protein, and procalcitonin or new infiltrates on chest radiographs. Major bleeding was defined as patients with fatal bleeding, symptomatic bleeding in a critical area and/or causing a fall in hemoglobin level of ≥ 2 g/dL or leading to transfusion of ≥ 2 units of packed red cells [18]. Sepsis (life-threatening organ dysfunction caused by a dysregulated host response to infection) was defined as per the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [19].

Standard Treatment

Interventions included the use of fluid resuscitation, prophylactic antibiotics, terlipressin, or octreotide as necessary. All patients underwent endoscopic management of AVB as per standard guidelines [17, 20]. Patients with esophageal variceal bleed were managed with endoscopic variceal ligation (EVL). Patients with gastric variceal bleed received endotherapy with N-butyl acrylate glue. Terlipressin or octreotide infusion for 5 days with use of proton pump inhibitors was as per standard guidelines. Standard medical therapy included nutritional intervention, albumin, diuretics, antivirals for hepatitis B and hepatitis C, and vitamin supplements. The standard antibiotic regimen was a cephalosporin, or a beta-lactam, which was changed as per cultures. Administration of blood components was based on our POC viscoelastic test based algorithm which ensures a restrictive transfusion strategy to improve ACT, platelet function or fibrinogen defects following primary endoscopic management where additional coagulation related bleeding was suspected (Supplementary information) [21, 22]. No prophylactic transfusions were given to correct abnormal INR or platelet count, and blood component therapy was as per standard guidelines [20].

COVID-19 Related Changes in Healthcare Delivery During the Study

One of the factors affecting the protocol was that the study was conducted during the COVID-19 pandemic in India. The intensive care, hepatology inpatient wards and routine endoscopy units were diverted to the COVID-19 care for more than a year in our center, and out patient clinics were restricted to admit only a few persons. We were offering emergency services including endoscopy for inpatients, and critically ill patients with AVB, cholangitis etc. Only such patients were admitted in our medical wards. Thus, all patients enrolled in the study were managed as per standard of care during the primary admission, when primary management, coagulation tests and endoscopic interventions were done. After discharge, they were called for outpatient follow ups or teleconsultation. However due to limited slots for surveillance endoscopy at our center, many patients would seek treatment at secondary healthcare centers or accessible endoscopy units. Hence patients with decompensation events were often managed at peripheral medical units and follow up data was completed using the telemedical records.

Outcome Measures

The primary outcomes were to assess the viscoelastic test results, endogenous heparinoids, association of fibrinolysis, and coagulation factor levels with rebleeding and outcomes like sepsis/death at 30 days and 90 days. Predictors of mortality were based on SCTs, Sonoclot tests and coagulation factor assays.

Assessment of Coagulation Parameters

Blood samples were taken at admission to the liver intensive care unit. The complete blood count was performed on a hematology autoanalyzer (Coulter Hmx Hematology...
Coagulation parameters measured included PT, aPTT, INR, D-dimer, and fibrinogen. The individual coagulation factor assays performed were Factor VIII (EL-H3641), vWF (EL-H2168), Factor XIII, PAI (EL-H2104) and tPA (EL-H2106), Factor X (EL-H0761) and ADAMTS13 (EL-H1552) using commercially available ELISA kits from Elabscience; Houston, Texas, USA. Cytokine levels, IL-1β and IL-6 (Assaypro; St. Charles, MO 63301, USA) and serum endotoxin levels were tested using ELISA kits (Pierce Chromogenic Endotoxin Quant kit; A39552) by Thermo Scientific.

Fig. 1 Enrollment flowchart of the study

| Consecutive patients with Cirrhosis with Acute Variceal Bleeding (AVB) screened |
| Inclusion: |
| • Cirrhosis with AVB |
| • Either gender |
| • Age: 18 to 65 yrs |
| 53 were Excluded |
| 16 had recent blood transfusion |
| 8 had HCC |
| 18 had COVID-19 infection |
| 6 had refractory shock at presentation |
| 3 patients were already on dialysis |
| 92 patients meeting criteria were recruited |
| Aged 46.5± 11.6, Male 82.6%; Ethanol related 57.6% |
| Standard medical therapy |
| Vasoconstrictor agent (Terlipressin/Octreotide) |
| Fluid resuscitation |
| Prophylactic antibiotics |
| Blood components as per VET (Sono clot) algorithm |
| Taken up for Endoscopy and Endotherapy within 6 hours of presentation |
| Tests performed were paired gb- and h-SONOCLOT, HLE estimation, Coagulation factor assays (Factors VII/VIII, vWF, XIII, X tPA, PAI-1, ADAMTS-13), markers of inflammation (IL-1 β, IL-6, CRP, ferritin, sepsis (procalcitonin, endotoxin) |
| Standard coagulation and biochemical tests |
| Endoscopic diagnosis |
| Esophageal varices (76%); Gastric varix(4.3%); Erosions(8.7%); Portal hypertensive gastropathy (9.8%); Post EVL ulcer (1.1%) |
| Endotherapy performed |
| EVL (76%); Glue injection (6.5%); Sclerotherapy (3.3%); Hemospray (22.8%) |

Early Rebleeding N=20
Site of Rebleeding
Post EVL Ulcer(14), Gastric Varix rebleed (N=4), spurt from another varix (2) and PHG related (2)
Followed up for incidence of sepsis, bleeding, survival at 30 and 90 days

No Rebleeding N=72
Table 1 Baseline characteristics, endoscopic management, investigations, and blood transfusion details of patients with cirrhosis with acute variceal bleeding

| Parameter                              | Total patients | Bleeding | No rebleeding | P value |
|----------------------------------------|----------------|----------|---------------|---------|
| **Parameter**                          | **N = 90**     | **N = 20** | **N = 70**    | **P value** |
| Age (years)                            | 46.4 ± 11.7    | 46.4 ± 12.1 | 46.5 ± 11.7 | 0.994  |
| Sex (male)                             | 74 (82.2%)     | 17 (85.0%) | 57 (81.4%)   | 0.502  |
| BMI (kg/m²)                            | 24.2 ± 6.3     | 21.8 ± 4.1 | 24.9 ± 6.7   | 0.079  |
| Height (cm)                            | 164.4 ± 21.1   | 167.0 ± 8.9 | 163.6 ± 23.7 | 0.568  |
| Weight (kg)                            | 68.8 ± 18.6    | 64.1 ± 15.8 | 70.4 ± 19.2 | 0.218  |
| DM-2                                   | 20 (22.2%)     | 7 (35.0%)  | 13 (18.5%)   | 0.370  |
| HTN                                    | 21 (23.3%)     | 5 (25.0%)  | 16 (22.8%)   | 0.848  |
| **Etiology of liver disease**          |               |          |              |         |
| • NASH                                 | 21 (23.3%)     | 4 (20.0%) | 17 (24.2%)   | 0.988  |
| • Ethanol                              | 53 (58.8%)     | 14 (70.0%) | 39 (55.7%)   | 0.967  |
| • AIH                                  | 2 (2.2%)       | 0         | 2 (2.8%)     | 0.841  |
| • Viral HBV/HCV                        | 16 (17.7%)     | 2 (10%)   | 14 (20.0%)   | 0.724  |
| CTP*                                   | 9 (8–12)       | 9 (8–14)  | 9 (8–11)     | 0.887  |
| MELDNa*                                | 18.5 (14.8–21.1) | 20.1 (15.6–24.4) | 17.4 (13.8–22.2) | 0.074  |
| Hemoglobin (g/dl)                      | 8.1 ± 2.3      | 7.7 ± 1.6 | 8.1 ± 2.4    | 0.498  |
| TLC (x 10^9/l)*                         | 11.2 (9.2–13.2) | 9.7 (6.0–13.3) | 11.8 (9.3–14.2) | 0.376  |
| Platelet Count (x 10^9/l)*             | 94.8 (80.2–109.5) | 103.7 (57.5–150) | 92.0 (78.5–105.5) | 0.497  |
| Urea (mg/dl)*                          | 69.6 (59.3–79.9) | 75.0 (55.9–94.0) | 67.8 (55.4–80.3) | 0.558  |
| Creatinine (mg/dl)*                    | 1.9 (0.9–2.6)  | 1.8 (1.2–2.4) | 2.0 (1.6–2.4) | 0.627  |
| AST (U/L)*                             | 133.9 (88.5–179.4) | 189.3 (186.3–360.5) | 116.2 (85–147.4) | 0.170  |
| ALT (U/L)*                             | 68.9 (41.6–96.1) | 68.8 (23.8–113.8) | 68.9 (35.1–102.7) | 0.998  |
| LDH (U/L)*                             | 357.5 ± 55.1   | 303.6 ± 62.5 | 365 ± 76.1   | 0.538  |
| Total bilirubin (mg/dl)                | 2.8 ± 0.9      | 3.2 ± 0.5 | 2.1 ± 0.8    | 0.289  |
| Total protein (g/dl)                   | 5.9 ± 1.0      | 5.8 ± 1.1 | 6.0 ± 0.9    | 0.584  |
| Albumin (g/dl)                         | 2.7 ± 0.5      | 2.6 ± 0.5 | 2.7 ± 0.5    | 0.543  |
| Lactate (mmol/l)                       | 3.0 (2.5–3.1)  | 3.1 (2.2–3.9) | 3.0 (2.4–3.6) | 0.809  |
| IL-1β (ng/ml)                          | 14.4 ± 5.4     | 17.2 ± 6.8 | 13.6 ± 4.7   | 0.010  |
| IL-6 (ng/ml)                           | 13.2 (10.6–15.7) | 15.2 (10.3–19.9) | 12.7 (9.8–15.6) | 0.414  |
| CRP (mg/l)*                            | 46.8 (21.3–58.6) | 50.1 (18.6–70.3) | 45.6 (18.2–60.1) | 0.075  |
| Prolactin (ng/ml)*                     | 1.1 (0.04–46.2) | 1.9 (0.12–61.9) | 0.9 (0.04–29.4) | 0.129  |
| Ferritin (ng/ml)                       | 186.5 ± 43.5   | 165.7 ± 67.3 | 194.2 ± 32.5 | 0.083  |
| NLR*                                   | 10.4 (8.7–12.1) | 13.4 (9.1–17.6) | 9.5 (7.7–11.3) | 0.057  |
| Serum endotoxin level (EU/mL)          | 17.8 ± 5.2     | 19.7 ± 6.2 | 16.8 ± 4.7   | 0.065  |

**Endoscopic diagnosis (active bleeding from one or more sites)**

| Diagnosis                             | Total patients | Bleeding | No rebleeding | P value |
|---------------------------------------|----------------|----------|---------------|---------|
| Esophageal varices                     | 81 (90.0%)     | 16 (75.0%) | 65 (92.8%)   | 0.069  |
| Gastric varices                        | 4 (4.4%)       | 1 (5.0%)  | 3 (4.2%)     | 0.784  |
| Esophageal varices with severe portal hypertensive gastropathy | 3 (3.3%) | 2 (10.0%) | 1 (1.4%) | 0.782 |
| Post-EVL ulcer                         | 2 (2.2%)       | 1 (5.0%)  | 1 (1.4%)     |         |

**Endotherapy (monotherapy or combination of interventions used)**

| Intervention                        | Total patients | Bleeding | No rebleeding | P value |
|-------------------------------------|----------------|----------|---------------|---------|
| Endoscopic sclerotherapy            | 3 (3.3%)       | 1 (5.0%)  | 2 (2.8%)     | 0.065  |
| Endoscopic varice ligation (EVL)    | 75 (83.3%)     | 15 (75.0%) | 50 (71.4%)   | 0.422  |
| EVL + glue injection                | 6 (6.5%)       | 0         | 6 (8.3%)     | 0.632  |
| Glue injection                      | 6 (6.6%)       | 1 (5.0%)  | 5 (6.9%)     | 0.280  |
| Hemospray                           | 21 (23.3%)     | 5 (25.0%) | 16 (22.2%)   | 0.089  |
| New thrombosis at any site          | 8 (8.8%)       | 3 (15.0%) | 5 (7.1%)     | 0.056  |
| Blood/component received (N, %)     | 55 (61.1%)     | 11 (55.0%) | 44 (62.8%)   | 0.043  |
| PRBC*                               | 2.1 (1.5–2.7)  | 2.3 (1.1–3.7) | 2.1 (1.4–2.8) | 0.864  |
| FFP*                                | 2.2 (2.1–4.2)  | 2.3 (1.9–3.9) | 1.5 (1.3–2.4) | 0.746  |
**Table 1** (continued)

| Parameter         | Total patients | Bleeding N=20 | No rebleeding N=70 | P value |
|-------------------|----------------|--------------|--------------------|---------|
| SDPC*             | 0.7 (0.3–1.2)  | 1.1 (0.8–2.4) | 0.5 (0.3–1.1)     | 0.056   |
| Cryoprecipitate*  | 3.4 (1.3–3.5)  | 4 (1.4–5.1)   | 3.1 (1.2–3.9)     | 0.032   |
| RDPC*             | 2.2 (1.2–4.1)  | 2.5 (1.4–4.8) | 2.9 (1.1–4.2)     | 0.055   |

*Data are presented as mean with 95% confidence intervals

**Table 2** Conventional coagulation parameters and Sonoclot results of the rebleeding study participants

| Parameter | Total patients N=90 | Early rebleeding N=20 | No rebleeding N=70 | P value |
|-----------|----------------------|------------------------|---------------------|---------|
| INR       | 2.0 (1.5–2.2)        | 2.2 (1.4–2.9)          | 1.9 (1.5–2.1)       | 0.190   |
| aPTT (s)  | 49.5 ± 22.8          | 58.9 ± 31.1            | 46.4 ± 18.6         | 0.037   |
| D-Dimer (ng/ml) | 523.8 (213.4–632.2)  | 586.4 (275.4–674.5)   | 475.4 (259.4–765.4) | 0.015   |
| Fibrinogen (g/L) | 2.2 (1.6–2.5)        | 2.3 (1.7–2.4)          | 2.2 (1.7–2.5)       | 0.945   |
| Sonoclot variables |                   |                        |                     |         |
| gbACT (s)  | 256.9 ± 72.5         | 343.1 ± 60.3           | 230.8 ± 53.3        | 0.000   |
| gbCR (units/min) | 24.7 ± 11.2          | 15.9 ± 9.5             | 27.3 ± 11.6         | 0.000   |
| gbPF (units) | 2.3 (2.0–2.6)        | 1.8 (1.1–2.6)          | 2.5 (2.1–2.8)       | 0.102   |
| gbPeak Amplitude (units) | 78.8 ± 25.2          | 75.1 ± 28.5            | 79.8 ± 24.4         | 0.494   |
| gbTime to peak (min) | 12.7 ± 5.2           | 12.8 ± 4.9             | 12.7 ± 5.3          | 0.931   |
| hACT (s)   | 208.3 ± 54.5         | 241.3 ± 55.7           | 198.5 ± 50.6        | 0.002   |
| hCR (units/min) | 23.6 (20.1–27.8)    | 16.7 (9.8–23.7)        | 25.6 (21.7–29.6)    | 0.029   |
| hPF (units) | 2.9 (2.5–3.2)        | 2.2 (1.7–2.8)          | 3.1 (2.6–3.5)       | 0.045   |
| hPeak-Amplitude (units) | 89.4 ± 22.9          | 76.7 ± 26.3            | 92.9 ± 20.7         | 0.006   |
| hTime to peak (min) | 11.5 (4.8–42.5)      | 14.3 (4.8–46.1)        | 12.3 (5.3–38.8)     | 0.408   |
| Clot lysis % | 8.9 (7.7–10.1)       | 10.4 (7.5–13.2)        | 7.2 (6.0–9.7)       | 0.041   |
| Heparin-like effect (HLE) |             |                        |                     |         |
| No HLE     | 44 (48.9%)           | 6 (30%)                | 38 (54.3%)          | 0.060   |
| Mild HLE   | 33 (36.7%)           | 10 (30.0%)             | 23 (32.9%)          | 0.044   |
| Severe HLE | 13 (14.4%)           | 4 (20%)                | 9 (12.9%)           | 0.030   |
| Coagulation factor assays |         |                        |                     |         |
| Factor XIII (ng/ml) | 2.4 (1.9–2.5)       | 1.6 (1.2–2.1)          | 2.4 (2.0–2.8)       | 0.035   |
| Factor VII (ng/ml) | 117.3 ± 51.0        | 94.1 ± 46.9            | 124.0 ± 50.4        | 0.023   |
| Factor X (μg/ml) | 6.0 ± 2.4           | 5.2 ± 2.4              | 6.2 ± 2.4           | 0.141   |
| VWF:Ag (ng/ml) | 24.8 ± 14.5         | 27.8 ± 15.4            | 23.9 ± 14.2         | 0.316   |
| Factor VIII:Ag (IU/ml) | 5.6 (4.6–6.9)     | 7.6 (5.4–9.9)          | 5.0 (3.9–6.1)       | 0.026   |
| TPA (ng/ml) | 22.1 ± 11.0         | 22.2 ± 10.0            | 22.0 ± 10.8         | 0.954   |
| PAI-1 (ng/ml) | 6.9 (6.1–7.8)       | 7.9 (6.6–9.3)          | 6.8 (5.6–7.7)       | 0.211   |
| ADAMTS13 (ng/ml) | 670.5 ± 108.5      | 512.3 ± 138.7          | 688.5 ± 120.5       | 0.040   |

*Data are presented as Mean with 95% confidence intervals

**ACT** activated coagulation time, **aPTT** activated partial thromboplastin time, **CR** clot rate on glass bead (gbCR) and heparinase-treated (hCR) curve, **HLE** heparin-like effect, **ADAMTS13** a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, **INR** international normalized ratio, **NLR** neutrophil lymphocyte ratio, **MELDNa** model for end-stage liver disease, **PF** platelet function on glass bead (gbPF) and heparinase-treated (hPF) curve, **vWF:Ag** von Willebrand Factor Antigen, **tPA** tissue plasminogen activator, **PAI-1** plasminogen activator inhibitor

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**AIH** autoimmune hepatitis, **AST** aspartate transaminase, **ALT** alanine transaminase, **BMI** body mass index, **CTP** Child Turcotte Pugh, **CRP** high-sensitivity C-reactive protein, **DM-2** Diabetes mellitus, **EVL** endoscopic variceal ligation, **EST** endoscopic sclerotherapy, **TN** hypertension, **HBV** hepatitis B virus, **HCV** hepatitis C virus, **LDH** lactate dehydrogenase, **MELD** model for end-stage liver disease, **NLR** neutrophil lymphocyte ratio, **PRPC** packed red blood cells, **FFP** fresh frozen plasma, **SDPC** single donor platelet concentrate, **RDPC** random donor platelet concentrate, **TLC** total leucocyte count

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Glass bead (gb) and heparinase-treated (h) Sonoclot tests in patients with AVB with calculation of heparin-like effect (HLE) as a predictor of rebleeding and mortality

**SONOCLOT™ Analysis**

Global and heparinase-treated coagulation analysis was done on the analyser (SONOCLOT Coagulation and Platelet Function Analyzer, Sienco Inc., Arvada, CO, USA). HLE was calculated with the percentage correction of the ACT time on SONOCLOT with the addition of heparinase (Supplementary Fig. 1).

\[
HLE (%) = \frac{ACT \text{ (global)} - ACT \text{ (heparinase - treated)}}{ACT \text{ (heparinase - treated)}} \times 100
\]

HLE was diagnosed when the correction of the ACT time is greater than 20%, and severe HLE was defined as a value greater than 50% [1]. In addition to the AVB cohort, we also performed coagulation factor assays in chronic hepatitis C related cirrhosis (N=20) and healthy controls (N=20) to get population reference ranges for these tests (Supplementary Table 1).

**Statistical Analysis**

Descriptive statistics are expressed as mean with standard deviation (SD) for parametric data and median with inter-quartile range (IQR) for non-parametric continuous data. Categorical data are expressed as numbers (n, %). We used Student’s t-test to compare continuous data and Pearson’s Chi-square test or Fisher's exact test (as appropriate) to compare categorical data among the two groups. The main outcomes are reported as estimated effect sizes along with precision (95% confidence intervals [CIs]). Cox proportional hazard analysis was performed for the predictors of 90-day mortality or rebleeding events using models incorporating coagulation factor assays or Sonoclot components for hyperfibrinolysis or HLE. Kaplan Meier survival analysis was also carried out to assess the incidence of rebleeding and its effect on mortality at days 30 and 90. Statistical significance was set at \( P < 0.05 \). All statistical tests were performed using SPSS ver. 25.0 (IBM Corp., Armonk, NY, USA). Sample size was estimated assuming the incidence of HLE in cirrhosis with AVB to be 10–30% as per prior published data [4, 11, 12]. It was estimated that a total sample size of 75 patients with cirrhosis would be required, with an effect size of 0.5, alpha 0.05, and power 0.85. Due to the onset of the COVID-19 pandemic, we recruited an additional 20% cases to account for attrition.

**Results**

A total of 143 patients with cirrhosis with AVB were assessed between December 1, 2019, and July 31, 2021, of whom 90 were enrolled in the study. Fifty-three patients were excluded due to recent blood transfusions (16, 30.1%), presence of hepatocellular carcinoma (8, 15.0%), COVID-19 infection (18, 33.9%), refractory septic shock (6, 11.3%) or were on dialysis (3, 5.6%). Two (3.7%) patients were excluded from analysis as they only had portal hypertensive gastropathy (PHG) related mortality was 39 (43.3%), with 90-day mortality of 15 (75%) patients. The baseline characteristics of the patients are shown in Table 1. The site of bleeding was due to esophageal varices (81, 90%), gastric varices (6, 6.6%), or from both esophageal varices and portal hypertensive gastropathy (PHG; 3, 3.3%). The primary modalities of management were EVL (75, 83.3%), glue injection (6, 6.6%), EVL & glue injection (6, 6.6%) EST (3, 3.3%), and hemospray (21, 22.8%) as monotherapy or in combination. Overall, rebleeding was noted in 20 (21.7%). The main causes of rebleeding were post-EVL ulcers (14, 70%), rebleed from gastric varix (4, 20%), spurt from another varix (1, 5%), diffuse PHG related bleed (1.5%). They were managed with one or more endotherapy techniques, including banding below the ulcers (4, 20%), hemospray (8, 40%), repeat glue injection (5, 25%), SX-Ella Danis stent (2, 10%), and 1 (5%) patient underwent rescue TIPS successfully. No significant differences were noted in baseline hemoglobin, platelet count, INR, albumin, MELDNa (Model for End-stage Liver Disease) or Child score between those who did or did not rebleed. However, IL1β \( (P = 0.010) \), aPTT \( (P = 0.045) \), D-dimer \( (P = 0.013) \) were significantly different between the two groups. Overall mortality was 39 (43.3%), with 90-day mortality of 15 (75%) and 24 (34.2%) in patients with and without rebleeding, respectively.

Table 2 lists the results of SCTs and paired gb and heparinase-treated Sonoclot assays. There was significantly higher gb-ACT (343.1 ± 60.3 vs 230.8 ± 53.8, \( P < 0.001 \)), hACT (241.3 ± 55.4 vs 198.7 ± 50.6, \( P = 0.001 \)) and lower gb-clot rate (15.9 ± 9.5 vs 27.3 ± 11.6, \( P < 0.001 \)) in rebleeders vs non-rebleeders, respectively. Also, the hSonoclot assay showed that those without rebleeding had a higher peak amplitude \( (P = 0.006) \), and lower clot Lysis \( (P = 0.041) \). Figure 2 shows the presence of the HLE in paired Sonoclot tests showing partial correction of the prolonged gbACT and gbCR on the corresponding hACT and hCR curves.
Endogenous Heparinoids and Risk of Rebleeding

Endogenous heparinoids were identified in 46 (51.1%) patients at enrollment. HLE was present in 14 (70%) and 32 (44%) of patients with and without rebleeding, respectively (P = 0.038). The presence of HLE indicates the presence of an unstable clot with lysis of 12.9% (5–20%) as compared to 4.7% (1.7–8%) in rebleeders vs. non-rebleeders, respectively (P < 0.001). The presence of HLE was also associated with increased cryoprecipitate requirements (P = 0.027), lower fibrinogen level (P = 0.021), and high NLR (P < 0.001). However, IL-6, procalcitonin, endotoxin, lactate, or ferritin levels did not differentiate the risk of rebleeding. Patients with rebleeding showed reduced Factor XIII [1.6 (1.2–2.1) vs. 2.4 (2.0–2.8) ng/ml, P = 0.035], Factor VII (94.1 ± 46.9 vs. 124.0 ± 50.4 ng/ml, P = 0.023), and ADAMTS13 (512.3 ± 138.7 vs 688.5 ± 120.5 ng/ml, P = 0.040). Supplementary Table 1 provides the reference ranges for the factor assays in cirrhotics and healthy controls.

Supplementary Figure 2 shows the results of individual coagulation factor assays in patients with and without rebleeding stratified by outcome. There were reductions in Factors VII, X, & XIII, ADAMTS13, and PAI-1 in patients with rebleeding, while there was an increased

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**Table 3** Clinical and coagulation parameters in patients who developed sepsis (N = 28) and those who did not (N = 62)

| Parameter                                      | Total patients N=90 | Sepsis N=28 | No Sepsis N=62 | P value |
|------------------------------------------------|---------------------|-------------|----------------|---------|
| Age (years)                                    | 46.4 ± 11.7         | 46.0 ± 10.9 | 48.6 ± 12.1    | 0.816   |
| Incidence of rebleeding                        | 20 (22.2%)          | 10 (35.7%) | 10 (16.1%)     | 0.039   |
| Mortality                                      | 39 (43.3%)          | 28 (100%)  | 11 (17.7%)     | 0.000   |
| MELDNa                                         | 16.6 (14.5–22.3)    | 17.5 (15.6–23.4) | 16.5 (13–21.5) | 0.377   |
| INR                                            | 1.9 (1.4–2.1)       | 2.0 (1.4–2.4) | 1.7 (1.6–1.9)  | 0.584   |
| Platelet count × 10⁹/l                         | 94.8 (80.2–107.5)   | 89.7 (75.3–104.5) | 105.2 (70.0–131.1) | 0.310 |
| aPTT (s)                                       | 49.5 ± 22.8         | 55.5 ± 23.3 | 46.8 ± 19.9    | 0.119   |
| D-Dimer (ng/ml)                                | 481.4 (213.4–632.2) | 555.6 (435.6–1256.7) | 468.0 (355.6–894.6) | 0.166   |
| Fibrinogen (g/L)                               | 2.2 (1.6–2.8)       | 1.8 (1.2–2.7) | 2.4 (1.6–3.3)  | 0.341   |
| Procalcitonin (ng/ml)                          | 4.3 (0.7–7.8)       | 6.9 (3.7–17.7) | 3.0 (0.7–5.3)  | 0.302   |
| IL-6 (ng/ml)                                   | 14.4 ± 5.4          | 18.7 ± 4.1  | 12.3 ± 4.6     | 0.000   |
| IL-1β (ng/ml)                                  | 13.2 (10.8–15.7)    | 15.8 (11.1–20.6) | 12.0 (9.2–14.8) | 0.146   |
| Serum endotoxin level (EU/mL)                 | 10.3 ± 3.4          | 11.6 ± 2.3  | 9.8 ± 3.4      | 0.291   |
| Presence of HLE                                | 44 (48.9%)          | 13 (46.4%)  | 31 (50.0%)     | 0.938   |
| No HLE                                         | 33 (36.7%)          | 11 (39.3%)  | 22 (35.5%)     |         |
| Mild HLE                                       | 13 (14.4%)          | 4 (14.3%)   | 9 (14.3%)      |         |
| Sonoclot parameters                            |                     |             |                |         |
| gbACT (s)                                      | 255.2 ± 72.9        | 290.6 ± 10.8 | 239.4 ± 75.6   | 0.002   |
| hACT (s)                                       | 207.3 ± 54.7        | 242.7 ± 41.0 | 191.9 ± 53     | 0.000   |
| Lysis (%)                                      | 8.8 (4.5–12.8)      | 10.8 (4.5–14.5) | 6.9 (3.2–9.8) | 0.043   |
| Coagulation factor assays                      |                     |             |                |         |
| Factor VIII (IU/ml)                            | 5.5 (2.5–8.7)       | 9.1 (3.7–11.2) | 3.8 (2.1–6.4) | 0.000   |
| vWF (ng/ml)                                    | 25.1 (18.4–35.8)    | 27.1 (18.5–35.6) | 23.4 (15.6–28.5) | 0.227   |
| Factor VII (ng/ml)                             | 116.5 ± 51.2        | 91.5 ± 42.4  | 128.5 ± 51.1   | 0.001   |
| tPA (ng/ml)                                    | 22.0 ± 9.1          | 25.8 ± 11.1  | 20.2 ± 9.8     | 0.027   |
| PAI-1 (ng/ml)                                  | 6.8 (5.1–11.8)      | 7.3 (3.1–9.8) | 6.8 (3.9–10.1) | 0.580   |
| Factor X (μg/ml)                               | 6.0 ± 2.4           | 5.1 ± 1.5   | 6.4 ± 2.6      | 0.014   |
| Factor XIII (ng/ml)                            | 2.3 (1.9–2.6)       | 2.5 (1.9–3.1) | 2.1 (1.7–2.8) | 0.276   |
| ADAMTS13 (ng/ml)                               | 648.5 ± 108.5       | 485.8 ± 88.7 | 523.5 ± 10.3   | 0.065   |

ACT activated coagulation time, aPTT activated partial thromboplastin time, CR clot rate, HLE heparin-like effect, ADAMTS13 a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, INR international normalized ratio, NLR neutrophil lymphocyte ratio, MELDNa model for end-stage liver disease, vWF:Ag von Willebrand Factor Antigen, tPA tissue plasminogen activator, PAI-1 plasminogen activator inhibitor
Factor VIII Ag and tPA level (Table 2). The presence of HLE > 50% at day 0 predicted new episode of sepsis (HR 1.8; CI 1.4–6.5, \( P = 0.022 \)), rebleeding events (HR 1.2; CI 1.1–6.3, \( P = 0.030 \)), and 90-mortality risk (HR 1.1; CI 1.0–4.6, \( P = 0.030 \)).

### Incidence of Sepsis

At 30 days, 31.1% (28/90) of patients from the entire cohort developed evidence of sepsis, including 10 (50%) of those with rebleeding. The most common sites of sepsis were spontaneous bacterial peritonitis (16.57.1%), pneumonia (4.14.2%), systemic sepsis (6.21.4%), and urinary tract infection (2.7.1%). Cultures were positive in 50% (14/28) of the patients, with multidrug-resistant *Klebsiella pneumonia*, *Escherichia coli*, and *Acinetobacter baumannii* being the common isolates. Table 3 shows that patients who developed sepsis were more likely to have rebleeding (\( P = 0.033 \)) and had increased mortality (\( P = 0.000 \)). Patients with sepsis showed significant difference in ACT on both gb and h-SONOCLOT traces, with increased clot lysis and endotoxin levels, but both groups did not differ on severity of HLE. Patients with sepsis had higher Factor XIII deficiency with viscoelastic measurements of clot lysis and endotoxin levels, but both groups did not differ on severity of HLE. Patients with sepsis had higher Factor VIII Ag, high tPA, and lower Factor VII and Factor X. Plasma ADAMTS13 levels (547.8 ± 187.6 vs. 653.5 ± 105.6, ng/ml; \( P = 0.037 \)) were significantly lower in patients with endotoxin levels ≥ 18 EU/mL, as were Factor VII levels (95.6 ± 35.6 vs. 120.5 ± 57.2 ng/ml; \( P = 0.043 \)). All patients who developed sepsis in the post-rebleeding did not survive as compared with only 17.7% mortality in those who did not develop sepsis (\( P < 0.001 \)).

### Predictors of Rebleeding and Thrombotic Events

On Kaplan–Meier analysis, the overall incidence of rebleeding at day 30 was found to be 17.8% (5/28, 95% CI 8.7–30.9, \( P = 0.061 \)) in patients without HLE, 13.6.0% (3/22, 95% CI 7.8–28.3, \( P = 0.075 \)) in those with mild HLE, and 50% (3/6, 95% CI 24.5–46.7, \( P = 0.012 \)) in those with severe HLE (Fig. 3). On ROC curve analysis, the following parameters were predictive of rebleeding: gbACT > 252 s (sensitivity 86.8%, specificity 76.9%, \( P < 0.001 \)), hACT > 215 s (sensitivity 71.1%, specificity 70.3%, \( P < 0.001 \)), and HLE > 50% (sensitivity 69.5%, specificity 70.3%, \( P = 0.006 \)) (Fig. 4). Eight (8.8%) patients also demonstrated new thrombosis, with the sites being clotting of central lines (5.625%), partial portal vein thrombosis (1.125%), and superior mesenteric vein thrombosis (1.125%).

### Predictors of Mortality

The overall mortality in the cohort of patients was 39 (43.3%) with 15 (75%) and 24 (34.2%) mortality in those with and without rebleeding, respectively (\( P = 0.001 \)). Most of the patients were managed successfully for rebleeding (14/20, 70%) but later succumbed to sepsis or progressive liver failure as causes of death, or following another decompensation event. Age, gender, etiology of liver disease, conventional tests like, INR, PT, aPTT, creatinine, bilirubin, and D-dimer were not found to be predictive of mortality on logistic regression. MELDNa (OR 1.2, 95% CI 1.1–2.1, \( P = 0.046 \)) showed a trend to increased mortality on univariable analysis, but not on multivariable analysis. We tested the use of blood component therapy, packed red cell transfusion, or use of terlipressin or octreotide, but it had no bearing on mortality in this cohort. On Cox proportional hazards analysis, predictors of mortality at day 0 were Factor VIII (HR 1.26; 95% CI 1.17–1.34, \( P < 0.001 \)), low factor VII (HR 0.98; 95% CI 0.88–0.94, \( P < 0.001 \)), and lysis (HR 1.25, 95% CI 1.17–1.33, \( P < 0.001 \)). In multivariate analysis on Sonoclot tests (Model 1), gbCR (HR 0.93; 0.89–0.98, \( P = 0.008 \)), lysis (HR 1.21, 95% CI 1.17–1.31, \( P = 0.005 \)), and presence of HLE (HR 1.1, 95% CI 1.0–4.6, \( P = 0.030 \)) were significant predictors of outcome. In model 2 based on multivariate analysis of coagulation factors, Factor VIII Ag (HR 1.21; CI 1.14–1.31, \( P < 0.001 \)) & Factor VII (HR 0.98, 95% CI 0.98–0.99, \( P = 0.006 \)) remained significant for predicting mortality (Table 4). When adjusted for baseline MELD, prolonged gbACT HR 1.009 (\( P = 0.003 \)), reduced gbCR (HR 0.839, \( P = 0.004 \)), increased lysis (HR 1.129, \( P = 0.015 \)), and FVIII levels (HR 1.120, \( P = 0.040 \)) remained predictive of mortality. (Supplementary Table 2).

### Discussion

Our study demonstrates the association of factor XIII and factor VII deficiency with viscoelastic measurements of clot lysis and endogenous haptinoids in cirrhosis with AVB, which are associated with early rebleeding and mortality. The primary variceal bleeding event is due to high portal pressure, and early appropriate endoscopic intervention and use of portal pressure reducing vasoconstrictor agents are the main therapeutic strategies. Rebleeding from post-EVL ulcers, erosions, PHG, or post-glue ulcers should be addressed by reducing portal pressure, endoscopic interventions, use of TIPS, and appropriate measures to assess coagulation dysfunction. We have shown that the ‘HLE’ was associated with altered tPA–PAI-1 interaction, increased vWF/ADAMTS13 ratio, elevated plasma endotoxin, increased cytokines (IL-1β, IL-6), and hyperfibrinolysis. Patients with rebleeding showed elevated D-dimer, reduced factor XIII (fibrin stabilizing factor), and factor VII, which is not uncommon in cirrhosis [4, 11, 15].
In normal hemostasis, tPA and PAI-1 act reciprocally to control fibrinolysis. tPA is a natural fibrinolytic agent released from the endothelium and works specifically at the fibrin clot surface. An increase in tPA, results in increased plasmin, which lyses the clot. PAI-1 inhibits tPA, and increase in PAI-1 is associated with delayed bleeding [6]. Ferguson et al. had shown that high tPA, with reduced PAI-1 was the hallmark of hyperfibrinolysis in alcohol-associated cirrhosis due to failure to clear tPA from the circulation [23]. However, we found that both tPA and PAI are increased in cirrhosis with AVB (Supplementary Fig. 4). Elevation of D-dimer observed in our study could be due to increased fibrinolysis or after rebleeding. There is poor correlation between hyperfibrinolysis and D-Dimer level with Sonoclot signature [24]. High D-Dimer observed in patients with cirrhosis may be due to reduced hepatic clearance and not solely due to increased fibrinolysis [25]. The study by Saxena et al. [24] points out that although D-Dimer was very high in 8 patients, hyperfibrinolysis on Sonoclot was seen in only one patient.

ADAMTS13, also called vWF cleaving protease, is synthesized in the hepatic stellate cells and cleaves multimeric vWF. Deficiency of ADAMTS13 predisposes to further liver injury due to increased large multimeric vWF, causing microthrombosis and changes in capillary circulation in the setting of sepsis [7, 8]. Takaya et al. reported similar increased vWF:Ag and reduced ADAMTS13 with increasing cytokine concentrations (IL-6 and IL-8) in patients with acute liver failure with endotoxemia [26]. Reduced activity of ADAMTS13 in the setting of SIRS in cirrhosis increases the prothrombotic function of vWF [27].

In this cohort, patients with post-bleeding sepsis had higher IL1β and serum endotoxin levels, increased ACT and clot lysis, increased factor VIII:Ag and tPA, and reduced factor VII levels. Increased Factor VIII/vWF and reduced ADAMTS13 can explain the thromboses that occurred in 8 (8.8%) patients in this cohort due to complex coagulation dysfunction [28].

Supplementary Fig. 5 provides a schematic diagram explaining the coagulation dysfunction associated with sepsis, HLE, and rebleeding. In our study, multiple mechanisms could have contributed to the increased endogenous heparinoids, including (i) failure of the liver to clear the circulating GAGs following AVB, (ii) sepsis or bacterial translocation with endotoxemia causing neutrophil-mediated injury of the hepatocytes that can release heparan sulfate, and (iii) the direct release of GAGs from the endothelial surface following AVB, which was associated with the characteristic Sonoclot signature. On Kaplan–Meier analysis, the overall incidence of rebleeding at day 30 was found to be 17.8% (P = 0.061) in patients without HLE, and 50% (P = 0.012) in those with severe HLE.

The typical coagulation defect was seen in the Sonoclot signature as prolonged ACT, reduced CR, and increased clot lysis. Therefore, these POC tests can be used as a quick bedside tool to assess the hemostatic defect and hyperfibrinolysis, which can be an indicator of rebleeding risk [29].

We followed a restrictive transfusion strategy based on POC tests, which is in accordance with the new BAVENO VII consensus guidelines [30]. We reiterate that AVB is due to increased portal pressures, and therapy should be focused on the management of portal hypertension, timely endotherapy, and preventing rebleeding. There is no role of prophylactic correction of coagulation abnormalities in portal hypertension related AVB, as it may cause more harm with limited benefit as portal pressure remains the primary cause of bleeding [31].

Use of tranexamic acid for hyperfibrinolysis or substitution of factor VII or 4 factor prothrombin complex concentrate (PCC) containing Factors II, VII, IX, and X has been described. However, more data from randomized trials are required to support use of such drugs as PCC has also been associated with increased risk of thromboembolism [13–15].

A major limitation of our study is that recruitment commenced just before the COVID-19 wave started in India. The overall mortality in the patients was 39 (43.3%) in patients who presented with AVB, which is quite high compared to our center’s data in the pre-COVID-19 era [21]. These patients were successfully managed for the AVB episode but failed to report for follow-up after their discharge. This is due to late presentation of patients due to COVID-19 restrictions, limited beds in non-COVID intensive care units, delays in COVID-19 testing, and impaired endoscopic surveillance of stable patients with cirrhosis during the pandemic. Patients were unable to report to tertiary care units following acute decompensation or report for follow-up endoscopy due to conversion of inpatient wards, endoscopy units, and intensive care units for COVID-19 care. Access to speciality procedures like TIPS or liver transplantation was also limited. Many cases were managed using telemedicine.

Fig. 3 Kaplan–Meier (KM) survival analysis for overall transplant-free survival in patients with and without rebleeding (panel A), risk of mortality in presence of heparin-like effect (HLE) (panel B), and outcomes based on severity of HLE (panel C)
at peripheral sites or were not referred to our center due to significant restriction of access to specialist care [32]. Thus, our data highlight the collateral damage due to COVID-19 in specialist care units such as ours, with increased mortality due to changes in healthcare delivery and diversion of health resources for COVID-19 care. Nonetheless, the principles of usage of POC tests, and interaction between sepsis, endothelial activation, endogenous heparinoids, and hyperfibrinolysis leading to rebleeding and mortality in cirrhosis and AVB remain pertinent. Another limitation of our study was that we measured only platelet count and Sonoclot as a marker of platelet function. We did not measure tests of platelet aggregation and secretion to assess platelet function. Patients with decompensated cirrhosis and AKI have reduced platelet aggregation and secretion measured by whole blood lumiaggregometry which improved to baseline after resolution of AKI [33]. Similarly, another study demonstrated reduced platelet aggregation in patients with decompensated cirrhosis, which reduced further in those with bacterial infection. However, this reduction in platelet aggregation persisted even after resolution of infection [34]. Further studies assessing role of platelet aggregation in early rebleeding after AVB may help in bridging the knowledge gap.

Although viscoelastic tests are considered better than standard coagulation tests, they are not free of limitations. These are in vitro and do not assess contribution of injured endothelium to clot formation, and hence cannot be used to predict risk of bleeding. However, these tests may provide a guide to specific hemostatic abnormality in a clinically bleeding patient. Also, contribution of platelet and fibrinogen defect to abnormal clot rate is difficult to assess by means of Sonoclot alone and functional fibrinogen assays should be performed.

**Conclusion**

Our results demonstrate the presence of a HLE and a possible association of Factor VII & XIII deficiency, with increased fibrinolysis with early rebleeding in patients with AVB. The main focus in such patients should be on the management of portal hypertension with drugs like terlipressin, timely endoscopy, referral for TIPS or transplantation, and follow-up to

| Test                          | Cut-off (%) | Sensitivity (%) | Specificity (%) | Area under curve (%) | 95% Confidence Interval (%) | Significance |
|-------------------------------|-------------|----------------|----------------|----------------------|----------------------------|--------------|
| gbACT (s)                     | ≥ 252.5     | 86.8           | 76.9           | 0.836                | 0.748-0.925                | 0.000        |
| hACT (s)                      | ≥ 215.0     | 71.1           | 70.3           | 0.768                | 0.652-0.861                | 0.000        |
| IL-1β (ng/ml)                 | ≥ 11.8      | 92             | 87.4           | 0.897                | 0.701-0.988                | 0.007        |
| Lactate mmol/l                | ≥ 2.8       | 66.7           | 86.7           | 0.804                | 0.619-0.944                | 0.000        |
| Factor X (IU/ml)              | ≤ 6.4       | 79.5           | 64.8           | 0.726                | 0.616-0.837                | 0.000        |
| Factor VII (ng/ml)            | ≤ 5.5       | 78.0           | 67.7           | 0.802                | 0.707-0.897                | 0.000        |
| Tissue plasminogen activator (ng/ml) | ≥ 20.3   | 70.7           | 65.7           | 0.704                | 0.591-0.817                | 0.000        |
| HLE (%)                       | >50%        | 69.5           | 70.3           | 0.855                | 0.768-0.942                | 0.006        |

Fig. 4 Receiver operating Characteristics (ROC) curves for predictors of mortality in patients with cirrhosis and acute variceal bleeding

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Table 4  Results of cox proportional hazards analysis for mortality and rebleeding using relevant parameters, and two predictive models based on Sonoclot results and coagulation factor assays

| Predictors of mortality | Parameter | HR  | 95% CI      | P value | Parameter | HR  | 95% CI      | P value | Parameter | HR  | 95% CI      | P value |
|-------------------------|-----------|-----|-------------|---------|-----------|-----|-------------|---------|-----------|-----|-------------|---------|
| Univariable analysis    | gbACT (s) | 1.010 | 1.009–1.018 | 0.000   | gbACT    | 1.009 | 1.003–1.015 | 0.003   | Factor VIII | 1.217 | 1.142–1.319 | 0.000   |
|                         | gbCR (units/min) | 0.915 | 0.882–0.949 | 0.000   | gbCR    | 0.939 | 0.891–0.983 | 0.008   | Factor VII | 0.989 | 0.982–0.997 | 0.006   |
|                         | hACT (s)  | 1.020 | 1.010–1.025 | 0.000   | Lysis   | 1.215 | 1.174–1.312 | 0.005   | Presence of HLE   | 1.1 | 1.0–4.6 | 0.030   |
|                         | hCR (units/min) | 0.969 | 0.943–0.996 | 0.024   | Presence of HLE   | 1.1 | 1.0–4.6 | 0.030   |
|                         | Lysis (%) | 1.250 | 1.173–1.332 | 0.000   | Presence of HLE   | 1.3 | 1.1–6.8 | 0.015   |
|                         | Factor VIII (ng/ml) | 1.260 | 1.175–1.349 | 0.000   | Presence of HLE   | 1.3 | 1.1–6.8 | 0.015   |
|                         | Factor VII (ng/ml) | 0.980 | 0.979–0.992 | 0.000   | Presence of HLE   | 1.3 | 1.1–6.8 | 0.015   |
|                         | Factor X (ng/ml) | 0.790 | 0.693–0.908 | 0.001   | Presence of HLE   | 1.3 | 1.1–6.8 | 0.015   |
|                         | Presence of HLE > 50% | 1.300 | 1.1–6.8 | 0.015   | Presence of HLE   | 1.3 | 1.1–6.8 | 0.015   |

| Predictors of rebleeding | Parameter | HR  | 95% CI      | P value | Parameter | HR  | 95% CI      | P value | Parameter | HR  | 95% CI      | P value |
|--------------------------|-----------|-----|-------------|---------|-----------|-----|-------------|---------|-----------|-----|-------------|---------|
|                         | Factor XIII | 0.810 | 0.78–0.95 | 0.008   | Factor XIII | 0.90 | 0.87–0.96 | 0.030   |
|                         | Factor VII | 0.890 | 0.76–0.98 | 0.035   | Factor VII | 0.92 | 0.84–0.98 | 0.043   |
|                         | Presence of HLE > 50% | 1.500 | 1.1–5.6 | 0.044   | Presence of HLE | 1.2 | 1.1–6.3 | 0.030   |

| Predictors of sepsis event | Parameter | HR  | 95% CI      | P value | Parameter | HR  | 95% CI      | P value | Parameter | HR  | 95% CI      | P value |
|-----------------------------|-----------|-----|-------------|---------|-----------|-----|-------------|---------|-----------|-----|-------------|---------|
|                            | Presence of HLE > 50% | 1.900 | 1.3–7.3 | 0.018   | Presence of HLE > 50% | 1.8 | 1.4–6.5 | 0.022   |
|                            | IL1β      | 1.200 | 1.1–4.5 | 0.045   | Presence of HLE > 50% | 1.2 | 1.1–4.5 | 0.045   |

CI confidence interval, HLE heparin-like effect, HR hazard ratio, ACT activated clotting time, gbACT glass bead-activated ACT, h ACT heparinase-modified ACT, CR clot rate, gb GR glass bead-activated CR, hCR heparinase-treated CR
prevent further acute decompensation or sepsis. A restrictive transfusion strategy is preferred, and use of specific blood component therapies as supportive care requires further research. Patients with sepsis and SIRS have worsening coagulation function, with increased Factor VIII/vWF, endogenous heparinoids, and endotoxin levels. Our data argue for the need for careful evaluation of sepsis and secondary coagulation anomalies in cirrhosis following an episode of AVB.

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Author’s contribution MP, AVK, AR, and RM were all involved in the manuscript preparation. The study design and execution were done by MP and KK. NV, AD, VS, ST, and AD were the attending consultants and were involved in data curation and clinical management of cases. JA, NK, and SD were involved in coagulation assessment and interpretation of tests. AG, KK, HK, and HK performed the point-of-care tests. All the authors have read and approved the manuscript.

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Declarations

Conflict of interest None of the authors have potential conflicts (financial, professional, or personal) which are relevant to this manuscript.

Ethical approval Ethical clearance was obtained from the Institutional Review Board (INT/IEC/2019/002239 dated 15/10/2019) and the study was registered at NCT04111120 available from https://clinicaltrials.gov/ct2/show/NCT04111120.

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