ORIGINAL PAPER

Endogenous Heparinoids Detected by anti-Xa Activity are Present in Blood during Acute Variceal Bleeding in Cirrhosis. A Prospective Study

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ABSTRACT

Background & Aims: Endogenous heparinoids have been detected by thromboelastography and quantified by clotting based anti-Xa activity assays in patients with cirrhosis, but their presence in variceal bleeding has not been established yet.

Methods: Clotting based anti-Xa activity was measured in A) 30 cirrhotics with variceal bleeding, B) 15 non-cirrhotics with peptic ulcer bleeding, C) 10 cirrhotics without infection or bleeding, and D) 10 cirrhotics with hepatocellular carcinoma (HCC).

Results: Anti-Xa activity was not detected in ulcer bleeders or in cirrhotics without infection or bleeding but was present in seven (23%) variceal bleeders (median levels: 0.03 u/mL (0.01-0.07)) and was quantifiable for 3 days in six of seven patients. Four of seven variceal bleeders with anti-Xa activity present had HCC (p=0.023). Age, creatinine, platelet count and total infections the second day from admission were significantly correlated with the presence of measureable anti-Xa levels (p=0.014, 0.032, 0.004 and 0.019, respectively). In the HCC group, anti-Xa activity was present in three patients (30%) [median levels: 0.05 u/mL (0.01-0.06)].

Conclusions: In this study, variceal bleeders and 30% of the patients with HCC had endogenous heparinoids that were detected by a clotting based anti-Xa activity assay, whereas there was no anti Xa activity present in patients with cirrhosis without infection, or bleeding or HCC, nor in those with ulcer bleeding. Thus, the anti-Xa activity is likely to be a response to bacterial infection and/or presence of HCC in cirrhosis.

Key words: liver cirrhosis – variceal bleeding – anti-Xa activity – endogenous heparinoids – hepatocellular carcinoma.

List of abbreviations: AFP, alpha-fetoprotein; aPTT, activated partial thromboplastin time; CP, Child-Pugh; FXa, activated factor X; GAGS, glycosaminoglycans; Hb, hemoglobin; HCC, hepatocellular carcinoma; HVPG, hepatic venous pressure gradient; INR, International normalized ratio; LMWHs, low molecular weight heparins; MELD, Model for End-stage Liver Disease; PPP, platelet-poor plasma; PRBC, packed red blood cells; PT, prothrombin time; SBP, sponataneous bacterial peritonitis; TEG, thromboelastography; WBC, white blood cells.

INTRODUCTION

Variceal bleeding is the most serious complication of portal hypertension in patients with liver cirrhosis. Despite improvements in prognosis over the past two decades, the 6-week mortality rate remains high, ranging from 15 to 30% [1]. Bacterial infections are a common and severe complication in hospitalized patients with decompensated cirrhosis especially in the setting of upper gastrointestinal bleeding and they are significantly associated with failure to control bleeding and mortality [2]. A possible pathophysiological basis linking infection and variceal bleeding has been proposed [3], suggesting that the release of endotoxin into the systemic circulation during episodes of bacterial infection results in a further increase in portal pressure through the induction of endothelin and possibly vasoconstrictive cyclo-oxygenase products. Furthermore, endotoxin-induced nitric oxide and prostacyclin could inhibit platelet aggregation, thus resulting in variceal bleeding [3].

We have previously reported that in cirrhotic patients, bacterial infections can lead to increased endogenous heparin-like activity, detected by heparinase I-modified thromboelastography (TEG) that is quantifiable by anti Xa (anti-Xa) laboratory assays and therefore, they are thought to inhibit coagulation [4]. The endogenous heparin-like effect seems to be present following infection and then disappears after its resolution (5-10 days after initiation of antibiotics) [5]. The presence of endogenous heparinoids has also been demonstrated in patients with cirrhosis and acute variceal bleeding during the few hours following a bleeding episode, suggesting that the heparin effect could influence failure to control bleeding or early re-bleeding [6]. We conducted this prospective study to assess the presence of endogenous heparinoids that are able to be detected by clotting-based anti-Xa activity assays [7] in plasma samples taken during variceal bleeding in patients with cirrhosis using appropriate control groups.

PATIENTS AND METHODS

This prospective, observational study was devised by CT, VN and AKB and received approval from the Ethics Committee of the University Hospital of Patras, Greece. We evaluated 32 consecutive patients with cirrhosis and acute variceal bleeding. Thirty patients were finally included in the study (Group 1) as two patients with variceal bleeding were excluded because they were unable to give consent due to hemodynamic instability. The control groups were: 15 non-cirrhotic patients with peptic ulcer bleeding (Group 2), 10 cirrhotics without infection or bleeding (Group 3), and 10 cirrhotics with known hepatocelullar carcinoma (HCC) (Group 4). Written informed consent was obtained from each patient. Patients receiving for one week or less before baseline drugs which affect coagulation parameters or platelet aggregation were not included in the study.

The severity of liver disease was assessed by the Child-Pugh (CP) class and Model for End-stage Liver Disease (MELD). Patients with variceal bleeding were treated with packed red blood cells (PRBC) and plasma expanders to maintain hemodynamic stability and hemoglobin levels at approximately 8 g/dl, together with antibiotics, lactulose (when encephalopathy occurred), somatostatin for 5 days and banding ligation performed within 12 hours of admission [8, 9]. No patient had shock at admission and none had received terlipressin.

All patients had routine laboratory tests on admission (full blood count, renal and liver function tests, microscopic urine examination), whereas infection was screened with blood cultures (aerobic and anaerobic), urine culture, ascitic fluid examination/culture and chest radiograph. A clinical and biochemical examination was performed every day of hospitalization. The diagnosis of presence and type of infection was based on internationally accepted criteria [10]. The diagnosis of spontaneous bacterial peritonitis was based on neutrophil count in ascitic fluid of >250/mm^3 as determined by microscopy [11].

Definitions: Diagnosis of cirrhosis was based on histological or compatible clinical, laboratory and imaging data. Variceal bleeding was diagnosed if there was haematemesis or melaena, with either a bleeding varix (active bleeding or clot adherent to varix or variceal ulceration) or presumed to be variceal when there was no other visible lesion at endoscopy. Re-bleeding and bleeding-related mortality was defined according to the Baveno V criteria [8]. Diagnosis of HCC was based on non-invasive criteria or pathology according to the recent clinical practice guidelines [12].

Variceal bleeders: 10 (33.3%) patients with variceal bleeding presented with hematemesis, seven (23.3%) with melena, whereas the rest presented with both. All patients bled from esophageal varices. At initial endoscopy, 10 (33.3%) had visible active bleeding from varices (40% spurting, 60% oozing) and 20 (66.6%) had no active bleeding. Twenty-three patients were transfused with PRBC [median: 4 (range: 1-7)].

Patients with peptic ulcer bleeding (non-cirrhotics): 13 (86.7%) patients presented with melena (6.7% with hematemesis, 6.7% with both). The etiology of gastrointestinal bleeding was gastric erosions (n=1, 6.7%), gastric ulcer (n=4, 26.7%), duodenal ulcer (n=9, 60%) and both gastric and duodenal ulcers (n=1, 6.7%). Three (20%) patients had visible active bleeding (33.3% spurting, 66.7% oozing) at initial endoscopy, whereas the remaining 80% had blood clots on bleeding point. Six patients were transfused with PRBC [median: 3 (2-4)].

Anti-Xa activity: The effect of endogenous glycosaminoglycans (GAGs) was measured using a Xa inhibition clotting assay [13] on the first day of admission and then repeated every day for seven days in patients with variceal bleeding and ulcer bleeding, and at first presentation in the other two groups. Venous whole blood was taken by a single peripheral venepuncture from the antecubital fossa using a 21-gauge butterfly, into two polypropylene tubes containing 0.106M sodium citrate solution (1 part citrate to 9 parts blood dilution). Patient citrated blood samples for anti-Xa assay were centrifuged at 2000g for 15 min. The plasma was removed and further centrifuged 2000g for 15 min to obtain platelet-poor plasma (PPP) which was aliquoted into cryotubes and stored at -70°C. Patients PPP was added to 0.3 u/mL FXa (Diagnostic Reagents Ltd, Thame, United Kingdom), incubated for 90 seconds then sub-sampled into 0.25 M CaCl2. After 120 seconds, a mixture of factor X deficient plasma (Diagnostic Reagents) and platelet substitute (Diagnostic Reagents Ltd) was added and the clotting time recorded. The clotting times for each patient PPP sample were compared to a standard curve constructed using various known concentrations (0.0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 u/mL) of Danaparoid sodium (ORGARAN®, Merck Sharp & Dohme Limited, UK). The clotting times for each standard point were plotted against their respective concentration (units/mL) using a linear scale. To obtain a zero point in the standard curve, the normal plasma used to make up the Danaparoid standard solutions was also measured for Xa inhibition and clotting recorded was used as the 0.0 u/mL value. The lower limit of detection of the assay is 0.01 units/mL and the test was considered positive when the level of anti-Xa was ≥ 0.01units/mL [4].

Statistical analysis

For this exploratory and observational study we recruited consecutive patients with cirrhosis and variceal bleeding in a ratio at least 2:1 (study group/control groups). Numerical data

were expressed as median and range (minimum to maximum) and categorical data as counts and percentages. All variables were tested for normal distribution using the Kolmogorov-Smirnov test. Categorical variables were tested using the chi-square and Fisher's exact test. Continuous variables with and without normal distribution were compared using Student's t-test or the Mann-Whitney U test, respectively. Univariate analysis was used to determine potential independent predictors of 6-week survival in the group of variceal bleeders. All statistical tests were two-sided and significance level was set at <0.05 or less. The SPSS statistical package (version 19.0 for Windows; SPSS Inc, Chicago, Illinois) was used.

RESULTS

All groups were well-matched regarding gender distribution, age and etiology of liver disease. Baseline characteristics of study population are shown in Table I. In patients with HCC (Group 4), median levels of alpha-fetoprotein were 14.1 (range 7.8-2120) ng/ml and median maximum tumor diameter was 5.6 (range 4.8-14.1) cm. Forty per cent had a single node whereas the rest had 3 or more nodes. None of these patients had portal vein thrombosis.

Anti-Xa activity

Anti-Xa activity by the clotting based Xa inhibition assay was not detected in stable cirrhotics (Group 3) or non-cirrhotics with ulcer bleeding (Group 2). It was found in seven (23%) variceal bleeders (median 0.03 u/mL ranging from 0.01-0.07 u/mL) and was present for 3 days in six patients. In the group of variceal bleeders, two patients had a clinically overt infection at admission (both had a chest infection) whereas the second day, infection was clinically present in another six patients (one with SBP, one with aspiration pneumonia, the rest with a chest infection) (total infections, n=8). Blood transfusion (four of seven), infection at admission and severity of liver disease assessed by CP or MELD was not correlated to the raised anti-Xa levels. The factors that positively correlated to the presence of anti-Xa levels were age, HCC, creatinine levels and platelet count at admission (Table II). The median anti-Xa level in patients with HCC was 0.03 u/mL (range 0.0-0.07 u/mL) (Fig. 1). The presence of infection the day after admission (second day) (n=8) was also positively associated

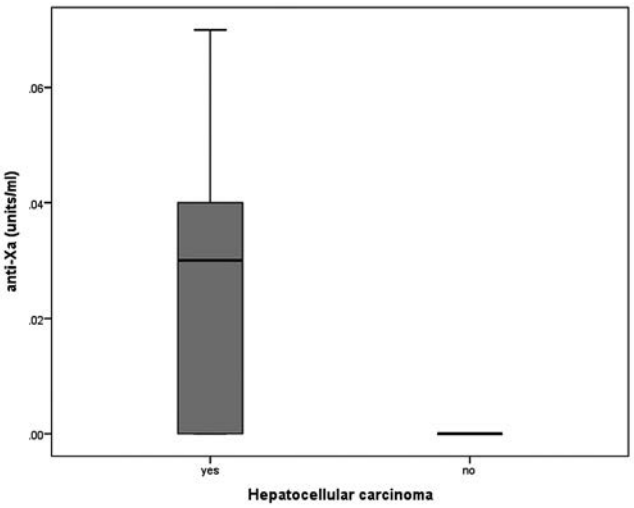

Fig. 1. Box plots of anti-Xa levels in variceal bleeders with hepatocellular carcinoma (HCC) compared to patients without HCC (p=0.009).

Table I. Baseline characteristics of study groups. Group 1: cirrhotics with variceal bleeding, Group 2: non-cirrhotics with peptic ulcer bleeding, Group 3: cirrhotics without infection or bleeding, Group 4: Cirrhotics with hepatocellular carcinoma (HCC)

	Group 1 (n=30)	Group 2 (n=15)	Group 3 (n=10)	Group 4 (n=15)	P value
Age, years (range)	62.5 (40-80)	62 (43-85)	56 (42-72)	63 (53-76)	0.306
Sex, M/F (%)	25/5 (83.3/16.7)	13/2 (86.7/13/3)	7/3 (70/30)	9/1 (90/10)	0.638
Etiology of liver disease, viral/ alcohol/other (%)	9/12/6 (33/44/22)	NA	2/6/2 (20/60/20)	8/2/0 (80/20/0)	0.053
HCC, n (%)	7 (23.3)	NA	0	15 (100)	NA
Child-Pugh score (range)	9 (5-14)	NA	7.5 (5-12)	6 (5-12)	0.016
Child-Pugh A/B/C (%)	4/14/12 (13.3/46.7/40)	NA	4/3/3(40/30/30)	6/3/1 (60/30/10)	0.05
MELD score (range)	14 (8-26)	NA	14 (7-19)	10 (7-33)	0.254
Hemoglobin (g/dl)	8.55 (5.1-12.8)	10.7 (5.1-13)	12.75 (9.9-14)	12.1 (10.3-15.3)	<0.001
WBC count (x109/L)	6.89 (1.5-28.6)	8.2 (5-24.7)	5.09 (2-7.96)	4.81 (3.05-9.5)	0.005
Platelet count (x109/L)	112.5 (30-377)	240 (83-401)	108.5 (38-170)	104.4 (35-290)	0.002
INR	1.355 (1.03-1.95)	1.04 (0.93-1.22	1.575 (1-1.94)	1.15 (0.9-2.71)	<0.001
PT (sec)	16.35 (12.9-20.6)	13.3 (10.9-14.2)	16.9 (13-20.6)	13.95 (11.5-28.5)	<0.001
aPTT (sec)	35.1 (22-48.3)	29 (23-42)	41.8 (30.2-76.6)	32.3 (26.5-50.9)	0.001
Total bilirubin (mg/dl)	2.32 (0.6-10.9)	0.6 (0.43-1.4)	2.34 (0.7-5.5)	1.48 (0.72-24)	<0.001
Albumin (g/dl)	2.8 (2-4)	3.7 (2.8-4.7)	3.35 (2.1-4.3)	3.5 (2.7-4.6)	<0.001
Creatinine (mg/dl)	0.9 (0.5-1.6)	0.9 (0.6-6.5)	0.9 (0.7-1.42)	0.9 (0.6-1.7)	0.848
Serum sodium (mmol/L)	137 (124-145)	139 (133-144)	138 (131-144)	139 (130-143)	0.364

M/F: male/female, WBC: White blood cells, INR: International normalized ratio, PT: prothrombin time, aPTT: Activated partial thromboplastin time, MELD: Model for End-stage Liver Disease, NA: not applicable.

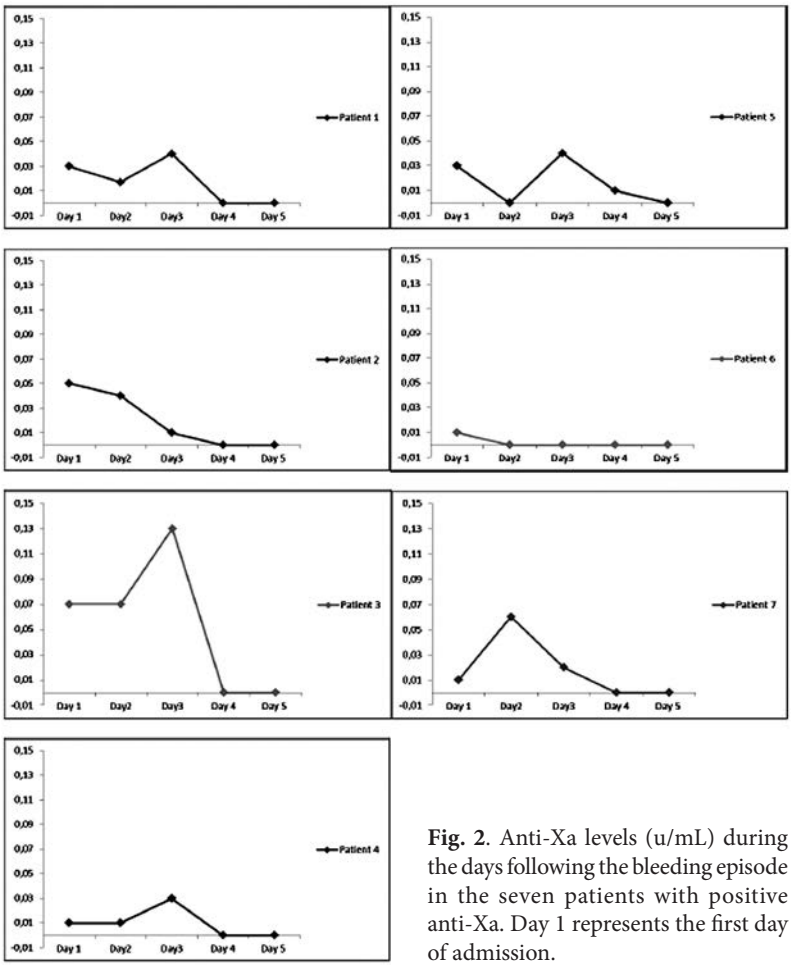

Fig. 2. Anti-Xa levels (u/mL) during the days following the bleeding episode in the seven patients with positive anti-Xa. Day 1 represents the first day of admission.

Table II. Characteristics of patients with variceal bleeding (with and without anti-Xa activity)

	Variceal bleeders with anti-Xa levels present (n=7)	Variceal bleeders without anti-Xa levels (n=23)	P value
Age, years (range)	70 (61-80)	57 (40-76)	0.014
Sex, M/F (%)	6/1 (85.7/14.3)	19/4 (82.6/17.4)	0.776
Child-Pugh score (range)	10 (6-13)	9 (5-14)	0.272
Child-Pugh A/B/C (%)	1/2/4 (14.3/28.6/57.1)	2/12/7 (9.5/57.1/33.3)	0.422
MELD score (range)	14 (8-26)	13 (8-22)	0.67
HCC, n (%)	4 (57.1)	3 (13)	0.023
Hemoglobin (g/dl)	9.9 (6.7-10.7)	8.4 (6.8-12.8)	0.381
WBC count (x10^9/L)	8.7 (3.63-28.6)	5.91 (1.5-12)	0.07
Platelet count (x10^9/L)	242 (87-377)	83 (30-181)	0.004
INR	1.24 (1.04-1.86)	1.36 (1.03-1.95)	0.652
PT (sec)	15.4 (12.9-19.3)	16.5 (13-20.6)	0.577
aPTT (sec)	29.5 (22-46)	35.2 (26.1-48.3)	0.13
Total bilirubin (mg/dl)	2.66 (1.08-10.9)	2.1 (0.6-10.4)	0.411
Albumin (g/dl)	2.7 (2-3.3)	2.8 (2-4)	0.729
Creatinine (mg/dl)	1 (0.6-1.6)	0.9 (0.5-1.3)	0.032
Serum sodium (mmol/L)	134(130-140)	138(124-145)	0.074
Infection (admission) (%)	1 (14.3)	1 (4.35)	0.376
Total Infections (second day) (%)	4 (57)	4 (17.4)	0.019
PRBC	2 (1-3)	2 (1-3)	0.238

M/F: male/female, HCC: hepatocellular carcinoma, WBC: White blood cells, INR: International normalized ratio, PT: prothrombin time, aPTT: activated partial thromboplastin time, MELD: Model for End-stage Liver Disease, PRBC: packed red blood cells

with the presence of anti-Xa levels (p=0.019). Figure 2 presents anti-Xa levels across time (following the bleeding episode). Four out of seven patients with positive anti-Xa levels had peak levels (0.03-0.14 u/mL) the third day after admission, one patient the second day (0.06 u/mL) and the other two patients, the first day (0.01, 0.05 u/mL). One of seven patients with raised anti-Xa levels had an infection the first day of admission. This patient had peak anti-Xa levels of 0.05 u/mL the first day of admission, which declined across time (Fig. 2, patient 2). Three out of seven patients with raised anti-Xa levels presented with infection the second day of admission. The presence of anti-Xa levels in these patients increased the second day of admission (peak levels occurred on the third day of admission and declined across time) (Fig. 2, patients 3, 4 and 5 had anti-Xa levels present of 0.14, 0.03 and 0.04 u/mL, respectively). The patients 1, 6 and 7 did not have a clinically apparent infection.

Anti-Xa activity was detected (median 0.05 units/mL (0.01-0.06) in three (30%) cirrhotic patients with HCC (Group 4). The presence of anti-Xa activity levels in this group of patients was not correlated with any of the other variables tested (Table III).

Re-bleeding

Six patients (20%) with variceal bleeding re-bled in a median of 5 (3-13) days following hospital admission (four of six patients within the first five days). One out of 4 patients (25%) who re-bled within 5 days and 3 of 6 patients (50%) who re-bled during follow-up had raised anti-Xa activity (0.03u/mL and 0.01-0.03 u/mL, respectively). The presence of anti-Xa activity was not associated with the 5-day re-bleeding (p=0.965) or overall re-bleeding (p=0.096).

Bleeding-related mortality

Three patients (10%) with variceal bleeding died in a median of 12 (3-43) days following the variceal bleeding episode. Factors associated with a 6-week mortality were the presence of total infections (second day) (p=0.002) and positive (≥0.01 u/mL) anti-Xa levels (0.01-0.07 u/mL) (p=0.011) (Fig. 3, Table IV).

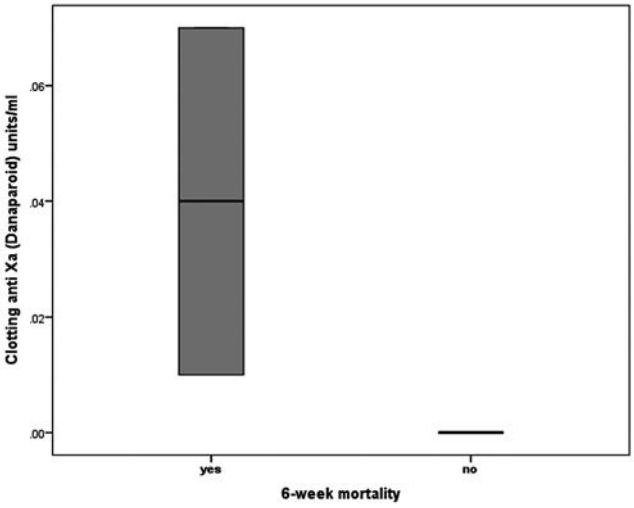

Fig. 3. Box plots of anti-Xa levels comparing variceal bleeders with and without 6-week mortality (p=0.01).

Table III. Characteristics of cirrhotic patients with hepatocellular carcinoma (HCC) (with and without anti-Xa activity)

	HCC patients with anti-Xa levels present (n=3)	HCC patients without anti-Xa activity (n=7)	P value
Age, years (range)	65 (57-76)	62 (53-75)	0.568
Sex, M/F (%)	2/1 (66.7/33.3)	7/0 (100/0)	0.107
Child-Pugh score (range)	7 (5-8)	6 (5-12)	0.639
Child-Pugh A/B/C (%)	1/2/0 (33.3/66.7/0)	5/1/1 (71.4/14.3/14.3)	0.24
MELD score (range)	14 (8-18)	10 (7-33)	0.422
Hemoglobin (g/dl)	11.3 (10.3-15.3)	12.5 (10.5-15)	0.648
WBC count (x10^9/L)	7.79 (3.05-9.5)	4.72 (3.74-6.6)	0.425
Platelet count (x10^9/L)	110 (35-290)	99 (40-215)	0.909
INR	1.03 (0.9-1.21)	1.2 (1.02-2.71)	0.209
PT (sec)	12.3 (11.5-15.1)	14 (13.2-28.5)	0.137
Total bilirubin (mg/dl)	1.26 (1.02-24)	1.7 (0.72-22.2)	0.569
Albumin (g/dl)	3.6 (3.1-4.6)	3.4 (2.7-4.5)	0.425
Creatinine (mg/dl)	1 (0.9-1.7)	0.9 (0.6-1.5)	0.165
Serum sodium (mmol/L)	139(130-140)	139(130-143)	0.565
AFP (ng/ml)	12 (7-173.75)	11.8 (7.8-2120)	0.724
Median maximum tumor diameter (cm)	5.7	5.5	0.77
Number of nodes (%)			
1	33.3	42.8	0.809
2	-	-	
≥3	66.7	57.2	

M/F: male/female, WBC: White blood cells, INR: International normalized ratio, PT: prothrombin time, MELD: Model for End-stage Liver Disease, AFP: alpha-fetoprotein.

Table IV. Univariate models predicting 6-week mortality post variceal bleeding (n=30)

Variables	P value
Age	0.316
Sex	0.414
Etiology of liver disease	0.57
HCC at baseline	0.666
Ascites	0.386
Encephalopathy	0.163
WBC (x10^9/L)	0.284
Hb (g/dl)	0.24
Platelet count (x10^9/L)	0.226
INR	0.917
PT (sec)	0.863
aPTT (sec)	0.213
Total bilirubin (mg/dl)	0.268
Albumin (g/dl)	0.808
Creatinine (mg/dl)	0.328
Sodium (mmol/l)	0.466
CP class	0.559
CP score	0.134
MELD score	0.187
Infection at admission	0.051
Total infections (second day)	0.002
Active bleeding at endoscopy	0.215
Positive antiXa activity	0.011
PRBC	0.311
Systolic blood pressure	0.192
Pulses	0.077
5-day rebleeding	0.474

HCC: hepatocellular carcinoma, CP: Child-Pugh, MELD: Model for End-stage liver disease, PRBC: packed red blood cells, Hb: hemoglobin, WBC: white blood cells, INR: International normalized ratio, PT: prothrombin time, aPTT: Activated partial thromboplastin time.

DISCUSSION

In this pilot study, the presence of endogenous heparinoids (confirmed by measurable anti-Xa levels using a Xa inhibition clotting assay) in patients with cirrhosis and acute variceal bleeding is clearly demonstrated and their association with bleeding-related mortality is documented. Raised anti-Xa levels were detected shortly after the variceal bleeding episode, independently from the presence of cirrhosis, or bleeding, implying a possible response to bacterial infection. Another interesting finding of this study was the presence of raised anti-Xa levels in a high proportion of patients with HCC.

A causal relationship between the presence of infection and acute variceal bleeding as well as early re-bleeding has been proposed [2]. In 84 patients with cirrhosis studied prospectively [14], using routine hemostasis tests and TEG, patients who developed infection (n=30) had a significant deterioration in all routine hemostasis tests and in all TEG parameters, which improved only in the subgroup of patients (n=22) in whom infections were resolved. In a later study [5], the presence of a heparin effect due to endogenous heparinoids was demonstrated in patients with cirrhosis and infection by using heparinase I-modified TEG. This heparin-like effect disappeared following the resolution of infection and was absent in non-infected patients and in infected non-cirrhotics, thus specifically linking this phenomenon with infection in cirrhosis. In a further study, raised anti-Xa levels were detected in 60% of infected patients with cirrhosis, together with a heparinase effect in the heparinase I-modified TEG [4], suggesting that bacterial infections affect hemostasis in cirrhosis by producing endogenous heparin-like substances which inhibit the activated factor X (FXa). In contrast with previous results [5], anti-Xa activity was found in infected patients without cirrhosis [4], suggesting that the mechanisms involved might be independent of cirrhosis. In a prospective study, TEG parameters deteriorated before the day of re-bleeding in all 6 patients with rebleeding of 20 patients with variceal bleeding, compared with those without re-bleeding [15]; patients who re-bled had a higher incidence of uncontrolled infection on the day of re-bleeding. Moreover, a heparin effect detected by raised anti-Xa levels was documented [6] immediately after the bleeding episode in two variceal bleeders, which persisted for 5 days, over a similar time course of antibiotic treatment. Similarly in our study, raised anti-Xa levels were detected in approximately 25% of variceal bleeders shortly after the bleeding episode and persisted for 3 days, but were not detected in patients with stable cirrhosis or those with non variceal bleeding suggesting that neither cirrhosis nor bleeding are specific for its occurrence. Anti-Xa levels were positively associated with the presence of infection the day following admission suggesting that endogenous heparinoids might reflect a response to bacterial infection. Moreover, the association of anti-Xa with bleeding-related mortality might reflect a link with bacterial infections which are a major determinant of outcome in cirrhotics with variceal bleeding. Whether the low levels of anti Xa activity influence haemostasis during variceal bleeding is an open question. With our small sample size we found no association with rebleeding, but this could be a type 2 error.

GAGs are endogenous heparinoids (commonly heparin sulfate, heparin and dermatan sulfate) with anticoagulant properties found on many cell surfaces [16, 17]. Heparan and dermatan sulphates affecting hemostasis are detectable by native TEG and can be completely reversed by heparinase I-modified TEG [18]; these heparinoids are likely to be responsible for the heparin effect seen using TEG in patients with cirrhosis and infection. In another study, an increased heparan sulphate level was found in patients with cirrhosis and variceal bleeding compared to patients without bleeding [19]. Consistent with other studies [4, 5], we found that patients with cirrhosis and raised anti-Xa activity had normal routine coagulation tests (PT, APTT).

In our study, only two variceal bleeders had clinically overt infection at admission and no correlation with anti-Xa level was found. However, the day following admission, infection was present in 8 patients in total and a positive correlation with raised anti-Xa levels was observed. More specifically, 4 of 7 patients with raised anti-Xa levels had infection the second day of admission (1 had infection on the first day). Figure 2

shows that anti-Xa levels in these patients were associated with infection and declined later, probably due to antibiotic therapy. Infection could be either a result of bacterial translocation which was not apparent at the time of admission but became overt the second day, or it was community-acquired due to immune dysfunction as a result of liver cirrhosis, or related to in-hospital infections [20-22]. The other 3 patients with raised anti-Xa levels had no documented infection. However, the possibility that clinically asymptomatic bacterial translocation which is well recognized in cirrhosis [23, 24] could lead to induction of endogenous heparinoids, finally triggering variceal bleeding, cannot be excluded.

There are several mechanisms that could explain how infection induces raised anti-Xa levels [4]. Hepatic injury results in release of heparin-like substances from hepatocytes and endothelial cells. During infection, white blood cells produce extracellular proteases resulting in the unmasking of circulating GAGs, whereas the activation of mast-cells lead to release of heparin and other anticoagulant mediators [4]. Thus the release of endogenous heparinoids might be a common response to infection, which becomes clinically significant in cirrhosis because of decreased liver clearance.

Another interesting finding, not previously reported, was the presence of detectable anti-Xa levels in 7 of the 17 patients (41%) with HCC. Patients with HCC have circulating endogenous GAGs [25], and this is most likely reflected by the detected raised anti-Xa levels. In HCC, there are quantitative and qualitative abnormalities of GAGs in the cancer tissue compared to normal liver [26]. A progressive increase in the content of chondroitin sulfate, low molecular size GAGs, and non-sulfated and di-sulfated chondroitin sulfate disaccharide units, together with a gradual decrease in heparan sulfate, have been found as the differentiation status of HCC becomes worse [26]. The loss of functional activity of GAGs chains in malignant tumors might promote the development of uncontrolled growth and gene expression favoring the neoplastic process [27]. We did not find any correlation between raised anti-Xa levels and tumor size, or number of nodes, but this could be a type 2 error. Experimental studies on hepatoma cells have shown that heparin inhibits cellular invasion [28], whereas clinical studies have described a positive effect of LMWHs on cancer survival [29]. Considering that raised anti Xa levels were predictive for 6-week mortality and that approximately 57% of variceal bleeders with raised anti Xa levels had HCC, this might reflect the confirmed strong association between HCC and bleeding-related mortality in portal hypertensive bleeding [30, 31]. Patients with HCC have higher hepatic venous pressure gradient (HVPG) compared to patients without HCC [32] and HVPG is a known predictive factor of mortality in patients with variceal bleeding [30]. However, this does not explain the higher mortality rate following acute variceal bleeding in patients with HCC; whether endogenous heparinoids in patients with HCC are related to worse short-term survival needs further investigation.

The major limitation of this study is that we did not measure or identify the type of GAG directly. However, the raised anti-Xa levels demonstrate the presence of endogenous heparinoids in variceal bleeding. Secondly, none of the patients had endotracheal intubation during endoscopy and this raises the possibility of aspiration-induced infection, but the absence of anti Xa levels in the patients with cirrhosis and ulcer bleeding makes this highly unlikely.

CONCLUSIONS

The results of the present study add to previous observations supporting the fact that bacterial infections in cirrhosis induce the release of endogenous heparinoids which affect hemostasis by inhibiting the clotting factor Xa, and thus may contribute to rupture of varices, together with platelet dysfunction and increased portal pressure, both induced by bacterial endotoxin. In addition, a novel observation is that endogenous heparinoids were present in patients with cirrhosis and HCC. Our data suggest firstly the need for further investigation of therapeutic agents which inactivate heparin-like substances in the management of patients with cirrhosis and variceal bleeding and secondly to evaluate the role of endogenous heparinoids in the tumour biology of HCC.

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