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Laboratory evidence for hypercoagulability in cirrhotic patients with history of variceal bleeding

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ABSTRACT

Aim: We aimed to assess the relationship between procoagulant imbalance and the occurrence of variceal bleeding in patients with liver cirrhosis.

Methods: We compared the results of chromogenic assay for the functional evaluation of the Protein C anticoagulant pathway (ThromboPath®), thromboelastometry and the levels of factor VII, VIII, and antithrombin in two groups of cirrhotic patients: Group 1 (n = 25) — patients with moderate or large esophageal or gastric varices, who had never experienced acute gastrointestinal bleeding and Group 2 (n = 24) — patients with a history of variceal bleeding.

Results: Despite the differences in MELD score and the results of basic laboratory tests indicating more severe cirrhosis and suggesting a greater risk of bleeding in Group 2, the results of thromboelastometry did not differ significantly between groups. The ThromboPath® test results [ThP B: 67.8 ± 13.4 versus 59.09 ± 12.4%, $p = 0.023$] and factor VII level [69.04 ± 24.16 vs 53.54 ± 25.06, $p = 0.032$] confirmed greater plasma procoagulant activity in Group 1 compared to Group 2. However, there were no statistically significant differences in thrombin generation after activation of the protein C. Plasma of patients in Group 2 was more resistant to anticoagulation with protein C compared to Group 1 (PICI%: 65.58 ± 7.24 versus 55.64 ± 13.07%, $p = 0.001$).

Conclusion: The results of our study confirm the lack of influence of coagulation disorders on the occurrence of variceal bleeding. Moreover, the results of ThromboPath® assay indicate hypercoagulability in patients with a history of variceal bleeding and more severe liver cirrhosis, compared to patients who have never bled.

1. Introduction

Liver cirrhosis has been considered as a pro-bleeding state for many years. This belief was resulted from the relatively high incidence of variceal bleeding and the abnormalities noted in routine clotting tests in cirrhotic patients. However, studies of recent years have shown that despite deranged results of standard coagulation tests, the efficacy of hemostatic system in patients with liver cirrhosis is not significantly impaired [1]. The main reason for this is the simultaneous reduction of coagulation factors and natural anticoagulants synthesized in the liver. In addition, the majority of patients with cirrhosis have elevated levels of factor VIII and von Willebrand factor [2,3]. Therefore, the new concept of coagulopathy of chronic liver disease assumes that in patients with cirrhosis the main issue is not the deficiency of clotting factors, but the imbalance between coagulation factors and natural anticoagulants [4]. Theoretically, disturbances of procoagulants and anticoagulants may be the cause of an increased thrombotic tendency or an increased tendency to bleed. Nevertheless, the clinical implications of these disorders are not well studied.

Disturbances in the coagulation system in patients with cirrhosis can

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be evaluated by thrombin generation assay (TGA) [5]. A simplified alternative to TGA is the ThromboPath® test [6]. The usefulness of this test to detect procoagulant imbalance in patients with cirrhosis has been confirmed in previous study [7]. However the influence of the imbalance between clotting factors and natural anticoagulants on the occurrence of variceal bleeding has not been studied before.

Currently, therapeutic correction of coagulopathy in patients with cirrhosis, based on the results of standard clotting tests is not recommended [8]. Nevertheless, the results of a recent meta-analysis indicate that some patients with variceal bleeding may benefit from recombinant activated factor VII (rVIIa) administration [9]. However, the administration of rVIIa was also associated with an increased risk of thrombotic events. A better understanding of the mechanisms of coagulation disorders in patients with cirrhosis may allow the selection of patients in whom correction of coagulopathy may be appropriate.

In our study we have performed comprehensive assessment of the coagulation system in two groups of cirrhotic patients: Group 1 — patients with at least medium-sized varices without history of GI bleeding, Group 2 — patients after variceal bleeding. We used: (1) ThromboPath® test — to evaluate the procoagulant imbalance, (2) thromboelastometry — to assess the global efficiency of the coagulation system and additionally (3) the measurement of traditional coagulation tests and factor VII, factor VIII and antithrombin levels to assess their association with TEM and thrombin generation assay.

2. Aim of the study

The aim of our study is to assess the relationship between procoagulant imbalance and the occurrence of variceal bleeding in patients with liver cirrhosis.

3. Patients and methods

A total of 49 patients with cirrhosis [31 (61.22%) males, and 19 (38.78%) females, mean age 55.4 ± 10.94 years] were enrolled in this cross-sectional study after approval of Ethics Committee of the Medical University of Bialystok, Poland (Resolution No. R-I-002/118/2012). Written informed consent was obtained from each patient. The diagnosis of cirrhosis was based on clinical, laboratory and imaging evidence. Data was collected for patient demographics, etiology of liver cirrhosis, severity of liver failure (Model of End-stage Liver Disease (MELD), Child-Turcotte-Pugh score) [10,11]. Routine laboratory tests including: peripheral blood count, liver enzymes, creatinine, bilirubin, C-reactive protein (CRP), sodium, potassium, albumin, fibrinogen and D-dimer concentrations as well as prothrombin time (PT) and activated partial thromboplastin time (APTT) were obtained.

Both in-patients and ambulatory ones were included into the study. The most frequent reasons for hospitalization were: decompensation of liver cirrhosis (ascites or encephalopathy), hepatic venous pressure gradient (HVPG) measurement and variceal band ligation. Endoscopic evaluation of varices was performed most often during the same hospitalization in Group 1. The time interval between variceal bleeding and coagulation studies in the Group 2 was at least 3 weeks, usually several months.

Inclusion criteria in Group 1 were as follows: (1) diagnosis of cirrhosis based on clinical, laboratory, and imaging data, and (2) the presence of medium or large esophageal, gastro-esophageal or gastric varices, confirmed in gastroscopy. The major inclusion criteria in Group 2 were the same as in Group 1, but the additional criterion in Group 2 was documented variceal bleeding from the upper gastrointestinal tract at least 21 days before inclusion. This period was determined arbitrarily based on the half-life of clotting factors and platelets lifetime — in order to limit the effect of treatment with blood products on the results of performed tests.

Pregnant women, and those with prothrombotic disorders or congenital coagulation disorders, were excluded. Additional criteria for exclusion were the use of drugs known to interfere with blood coagulation (anticoagulants, anti-platelet agents, freshly frozen plasma, cryoprecipitate, concentrates of coagulation factors, fibrinolysis inhibitors and fibrinolytic agents), ongoing bacterial infections, recent trauma or surgical treatment requiring hospitalization, end stage renal insufficiency due to chronic kidney disease, advanced chronic heart failure, hepatocellular carcinoma and extrahepatic malignancy. Any previous GI bleeding was the exclusion criterion in Group 1.

3.1. Definitions

The acute gastrointestinal variceal bleeding was defined as: (1) endoscopically confirmed active variceal bleeding, or (2) clinical symptoms of acute upper gastrointestinal bleeding (hematemesis or bloody stools with simultaneous hemodynamic instability — systolic blood pressure below 100 mm Hg, heart rate above 100/min.) and endoscopic features of recent variceal hemorrhage (e.g. white nipple on the varix), or (3) medium or large esophageal or gastric varices with red markings and clinical symptoms of acute upper gastrointestinal bleeding in patient no other potential source of bleeding within upper gastrointestinal tract.

The size of the esophageal varices was evaluated according to the Minimal Standard Terminology 3.0 (MST 3.0), proposed by the World Endoscopy Organization (WEO). The size of gastric varices was assessed in a three-point scale: grade 1 — small (< 5 mm), grade 2 — medium (5 to 10 mm), and grade 3 — large (> 10 mm) [12].

3.2. Laboratory analyses

3.2.1. Thrombopath®

Measurement of tissue factor–induced endogenous thrombin generation was performed with the ACL Elite Pro analyzer (Instrumentation Laboratory Instrumentation Laboratory Company, Lexington, USA) using the ThromboPath® chromogenic assay (Hemosil™ Instrumentation Laboratory Company, Lexington, USA) designed to evaluate the functionality of the protein C system [13]. Lyophilized reagents were reconstituted with distilled water before use according to the manufacturer's specification. The plasma samples were divided into two parts (10 μL each), which were diluted with a 40 μL of ThP Diluent. The first part was incubated with ThP Activator A containing the snake venom fraction *Agkistrodon contortix contortix* (Protac®) — the activator of protein C, the other part of the plasma was incubated with ThP Activator B, which did not contain snake venom. ThP Thromboplastin (containing tissue factor and phospholipids) and ThP Substrate containing the chromogenic substrate (S-2796 *Z*-D-Arg-Sar-Arg-pNA2HCl 1.46 g/L) were then added to the test samples. After incubation thrombin activity was assessed by recording changes in optical density at a wavelength of 405 nm for 45 s in the presence (ThP A) or absence (ThP B) of Protac®. The measurement results are expressed as a percentage of inhibition of thrombin generation induced by snake venom — Protac-induced coagulation inhibition percentage (PICI %), calculated by the following equation: PICI% = [(ThP B − ThP A) / (ThP B)] × 100%, where: ThP A — optical density for plasma in the presence ThP A activator, ThP B — optical density for plasma in the presence of ThP B activator.

3.2.2. Rotational thromboelastometry (ROTEM®)

Rotational thromboelastometry (ROTEM ®, TEM International GmbH, Munich, Germany) is a modification of classic thromboelastography (TEG) [14]. The methodology of ROTEM® has previously been described in detail [15]. Blood for ROTEM® was drawn by sterile venipuncture from a peripheral vein and collected in vacuum tubes (S-Monovette® 3 mL 9NC SARSTEDT AG & Co. Nümbrecht, Germany) containing 0.3 mL sodium citrate at a concentration of 0.106 mol/L as anticoagulant in the proportion of 1/9 parts of anticoagulant/blood. After collection, the blood was gently mixed with an anticoagulant.

Thromboelastometry was performed within 0.5–4 h of blood collection. Prior to analysis citrated blood was stored at room temperature. Before the test, blood and cuvettes (cup and pin cells, Pentapharm GmbH, Munich Germany) were incubated to a temperature of 37 °C. Thromboelastometry was performed, according to the manufacturer's recommendations, using ROTEM Gamma® Analyzer (TEM International GmbH, Munich, Germany). All pipetting steps and the mixing is performed in a standardized manner by following an automated programme. All patient's whole blood samples were analysed by INTEM ROTEM® assay. A star-TEM® (20 μL) reagent (Pentapharm GmbH, Munich, Germany) containing 0.2 mol/L $CaCl_2$ was used to neutralize citrate. Twenty μL of in-TEM® reagent (containing ellagic acid and phospholipids) and 300 μL of citrated blood were added to the cup. The mixture of reagents and blood was then withdrawn and pipeted back into the cup using an electronic pipette. The cups were set immediately onto the pins in selected channels of the thromboelastometer. Tests were started automatically and run for 60 min. Following parameters were recorded: (1) clotting time (CT) — the period in seconds from analysis start until the trace to reach an amplitude of 2 mm, (2) clot formation time (CFT) — the period from initial fibrin formation (CT) until an amplitude of 20 mm is reached, (3) alpha angle (in degrees) — the angle between the centre line and a tangent to the TEMogram at the point where the amplitude reach 2 mm (CT), (4) maximum clot firmness (MCF) — the peak amplitude of the TEMogram in millimetres, (5) amplitude (A10, A20) — amplitude after 10 and 20 min respectively from the beginning of clot formation (CT) and (6) maximum lysis (ML) — the ratio of the lowest amplitude observed after reaching MCF to MCF expressed as a percentage. Interpretation of ROTEM data is based on normal ranges provided by the manufacturer: CT_{INTEM} (s) 100–240, CFT_{INTEM} (s) 30–110, alfa $angle_{INTEM}$ (degree) 70–83, MCF_{INTEM} (mm) 50–72, and ML_{INTEM} (%) < 15.

3.2.3. Factor VII, factor VIII and antithrombin (AT) activity

The factor VII activity in citrated plasma was measured by PT-based chromogenic assay using STA®-Deficient VII (Diagnostica Stago S.A.S. France), STA®-Neoplastine CI Plus, (Diagnostica Stago S.A.S. France) and STA®-Neoplastine CI Plus (Diagnostica Stago S.A.S. France). We analysed also factor VIII activity by APTT based assay on the STA Compact Max® analyzer (Diagnostica Stago, Inc.) using STA®-Deficient VIII, Diagnostica Stago S.A. S. France, STA®-PTT A, Diagnostica Stago S.A.S. France. STA® CaCl2 0.025 M, Diagnostica Stago S.A.S. France. Antithrombin activity was analysed by chromogenic assay STA®-Stachrom® AT III (Diagnostica Stago S.A.S. France). The FVII, FVIII and AT measurements were performed on STA Compact Max® analyzer (Diagnostica Stago, Inc.) according to the manufacturer's instructions. Results were expressed as percentage of a normal pooled plasma arbitrarily set at 100% of normal. The reference values of FVII, FVIII and AT are 55–170%, 60–150% and 80–120% respectively [16].

3.3. Statistical analyses

Continuous variables were expressed as means (± SD) or median (interquartile range [IQR]) as appropriate. Categorical variables were reported as counts (percentage). All groups of results were tested for normality by the Shapiro-Wilk test. Continuous variables were tested for statistical significance with the nonparametric Mann-Whitney *U* test or Student *t*-test as appropriate. Categorical variables were analysed by Chi Square test. Correlation of continuous data were analysed by Spearman rank correlation or Pearson coefficient test. All data were analysed using Statistica 13 software package (StatSoft Inc. USA). Only two-tailed probabilities were used for testing statistical significance. *p* values of < 0.05 was considered statistically significant. Additionally, to analyse the relationship between variceal bleeding history and coagulation disorders, we ran a multiple regression analysis, considering MELD score, prothrombin index, hemoglobin level, serum bilirubin, AST, ThP B, PICI% and level of factor VII and AT as possible determinants of the occurrence of variceal bleeding. Odds ratios (95% confidence intervals) were calculated as a measure of the relative risk of having the history of variceal bleeding with a procoagulant imbalance defined as PICI% lower than the median value of the distribution of results for patients who have never bleed.

4. Results

The study included 49 patients (Group 1: 25 patients, Group 2: 24 patients) with liver cirrhosis, presenting to our gastroenterology and hepatology units between September 2012 and June 2014. The etiology of liver cirrhosis was as follows: 26 (53.06%) alcoholic, 8 (16.33%) viral hepatitis, 8 (16.33%) PBC (primary biliary cholangitis) and PSC (primary sclerosing cholangitis), 5 (10.2%) cryptogenic, 1 (2.04%) AIH (autoimmune hepatitis), and 1 (2.04%) mixed. The presence of varices was confirmed endoscopically in all patients [39 (79.59%) esophageal varices, 9 (18.37%) gastroesophageal varices type 2 (GOV2), 1 (2.04%) gastroesophageal varices type 1 (GOV1)]. The patients were classified according to Child-Turcotte-Pugh classification: 11 (22.45%) were of A class, 24 (48.98%) were of class B, 14 (28.57%) were of class C. Among groups age, sex, etiology and severity of cirrhosis according to Child-Turcotte-Pugh and distribution of varices location were similar.

There were no significant differences in the results of laboratory tests between study groups except lower hemoglobin level and higher serum bilirubin, activity of aspartate transaminase (AST) as well as Model of End Stage Liver Disease (MELD) score in patients with history of variceal bleeding (Table 1).

4.1. Traditional coagulation tests

There was a slight rise in INR (1.19 ± 0.18 vs 1.31 ± 0.21, $p = 0.027$) with a decrease of prothrombin index (79.76 ± 14.43 vs 69.38 ± 12.21%, $p = 0.009$) in patients with history of variceal bleeding (Group 2). These standard clotting tests indicated more

Table 1
Laboratory and clinical characteristics of patients according to variceal bleeding history.

	Group 1 (n = 25)	Group 2 (n = 24)	*p*
WBC (IQR) $10^3/\mu L$	4.74 (4.0–6.0)	5.98 (3,47-8,05)	0.54
Hemoglobin ± SD g/dL	**12.83 ± 1.76**	**11.39 ± 2.64**	**0.029**
RBC ± SD $10^6/\mu L$	3.98 ± 0.76	3.80 ± 0.76	0.411
Albumin ± SD g/dL	3.34 ± 0.58	3.20 ± 0.68	0.452
Bilirubin (IQR) mg/dL	**1.48 (0.99–2.01)**	**2.06 (1.63–2.73)**	**0.014**
AST (IQR) IU/L	**42.0 (28.0–61.0)**	**62.00 (39.0–78.0)**	**0.010**
ALT (IQR) IU/L	33.0 (22.0–42.0)	33.0 (26.5–44.0)	0.494
Urea (IQR) mg/dL	30.5 (27.0–35.0)	31 (22.0–32.0)	0.590
Creatinine (IQR) mg/dL	0.76 (0.63–0.83)	0.72 (0.65–0.87)	0.881
CRP (IQR) mg/L	4.8 (1.65–13.05)	6.3 (2.6–19.0)	0.317
MELD score ± SD	**6.91 ± 2.95**	**9.36 ± 3.47**	**0.010**
Child-Turcotte-Pugh score (IQR)	8.0 (6.0–9.0)	8.5 (7.5–10.0)	0.143
Class A [n (%)]	8 (32%)	3 (12.5%)	0.258
Class B [n (%)]	11 (44%)	13 (54.17%)	
Class C [n (%)]	6 (24%)	8 (33.3%)	
Etiology [n (%)]			
Alcoholic	11 (44%)	15 (62.5%)	0.380
Viral	5 (20%)	3 (12.5%)	
AIH	1 (4%)	0	
PBC/PSC	6 (24%)	2 (8.3%)	
Cryptogenic	2 (8%)	3 (12.5%)	
Mixed	–	1 (4.17%)	

Autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), white blood cells (WBC), red blood cells (RBC), aspartate transaminase (AST), alanine transaminase (ALT), Model of End Stage Liver Disease (MELD), C-reactive protein (CRP), standard deviation (SD), interquartile range (IQR).
Statistically significant values (p < .05) were highlighted in bold.

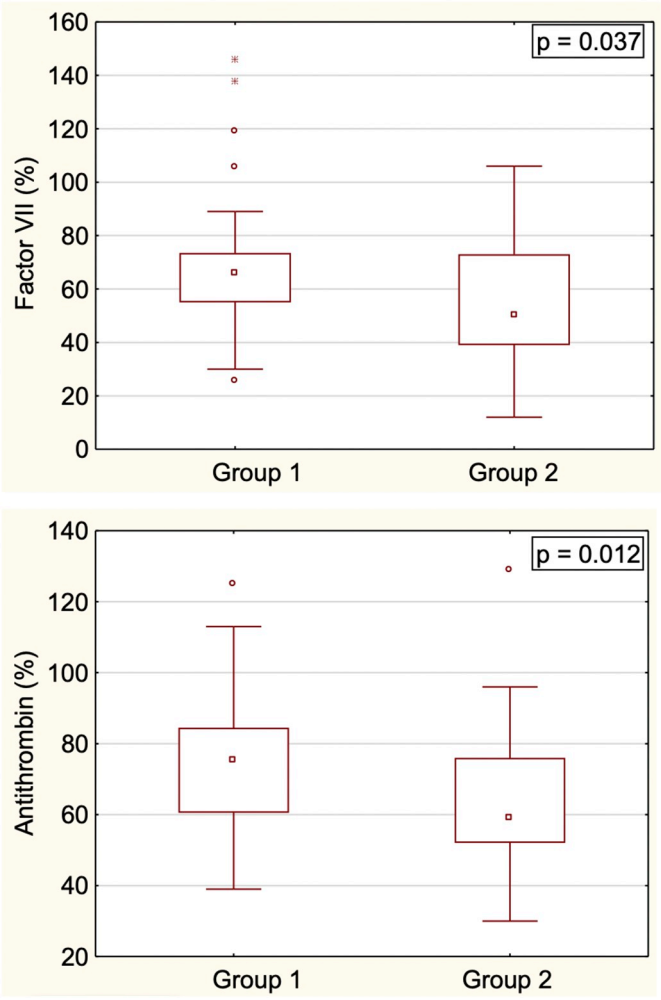

Fig. 1. Factor VII and antithrombin level for patients without history of gastrointestinal bleeding (Group 1) and patients with history of variceal bleeding (Group 2). Box plots of the distribution of values (median, lower and upper quartile; outliers identified as °, extreme values identified as *).

hypocoagulable profile in Group 2 compared to Group 1. Platelet count [109.0 (69.0–160.0) vs 73.50 (54.5–115.5) × 10^3/μL, $p = 0.053$)], and fibrinogen level [(279.13 (± 87.12) vs 247.12 (± 82.61) mg/dL, $p = 0.237$)] were similar in both groups.

4.2. Activity of factor VII, factor VIII and AT

Activity of factor VII in Group 2 was significantly lower than in Group 1 [53.54 ± 25.06 vs 69.04 ± 24.16, $p = 0.032$] (Fig. 1). However, decreased factor VII activity in Group 2 was accompanied by decreased antithrombin activity [76.48 ± 17.51 vs 63.33 ± 21.57 $p = 0.023$; Group 1 vs Group 2 respectively] (Fig. 1).

We have found also an increased factor VIII activity - in both groups the mean activity of factor VIII significantly exceeded the upper limits of the norm [Group 1 vs Group 2: 268.0 (222.0–346.0) % vs 251.5 (205.5–270.5) % respectively, $p = 0.122$]. However, the level of factor VIII did not differ between groups.

4.3. Tissue factor-induced thrombin generation and the effectiveness of anticoagulant activity of protein C system (ThromboPath®)

The ThromboPath® test confirmed greater plasma procoagulant activity in Group 1 compared to Group 2 [ThP B: 67.78 ± 13.41% vs 59.09 ± 12.42%, $p = 0.023$]. However, there were no statistically significant differences regarding the efficacy of thrombin generation after activation of the protein C [ThP A: 23.2 (20.4–26.1) % vs 24.85 (21.35–29.30) %, $p = 0.121$]. Moreover, the plasma of patients in Group 2 was more resistant to anticoagulation with protein C compared to Group 1 — PICI%: 65.58 ± 7.24 vs 55.64 ± 13.08%, $p = 0.0018$ in Group 1 versus Group 2 respectively.

Table 2
The results of thromboelastometry — INTEM test.

	Group 1 (n = 25)	Group 2 (n = 24)	p
CT (IQR) (s)	211.0 (187.0–282.0)	204.0 (194.50–256.50)	0.641
CFT (IQR) (mm)	111.0 (94.0–157.0)	129.0 (100.0–166.0)	0.532
MCF (mm)	50.64 ± 8.70	47.54 ± 9.54	0.240
Alpha (IQR) (°)	71.0 (61.0–75.0)	68.5 (62.5–73.5)	0.558
ML (%)	9 ± 8.0–11.0	10.0 ± (6.0–13.0)	0.775

Clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), maximum lysis (ML).

4.4. Thromboelastometry (INTEM®)

We did not show statistically significant differences between groups regarding the parameters obtained in INTEM® test in thromboelastometry. This indicates similar efficiency of clot formation in both groups (Table 2). Twelve patients (24%) had completely normal studies; 9 patients (18%) had 1, 8 (16%) had 2, 13 patients (26%) had 3, and 7 patients (14%) had 4 TEM parameters in a hypocoagulable range. Only one (2%) patient had 1 parameter in hypercoagulable range.

Of ThromboPath® parameters ThP B value was significantly directly correlated with AT and factor VII levels and significantly indirectly correlated with PT (Fig. 2). ThP A and PICI% value did not correlate with any TEM parameter or basic coagulation test result.

A multivariate analysis including hemoglobin concentration, prothrombin index, bilirubin level, AST activity, MELD score, level of factor VII and AT, ThP B, and PICI% values was performed to evaluate the impedance of correlations. PICI% was the only factor that independently determined the presence of variceal bleeding history — OR = 0.909 (95% C.I. 0.850–0.972; $p = 0.005$).

5. Discussion

Previous studies showed that the risk of variceal bleeding is significantly higher in patients who have had previous episode of variceal bleeding, which indicates a chronically increased bleeding tendency in these patients [8]. This increased bleeding tendency in patients with a history of variceal hemorrhage, most often goes hand in hand with other known bleeding risk factors, including the size and location of varices, the severity of cirrhosis. On the other hand, 65–75% of patients with liver cirrhosis will never bleed [17]. This may indicate the presence of a protective mechanism that prevents bleeding in cirrhotic patients without history of variceal hemorrhage or the contribution of additional variceal bleeding risk factor in patients with recurrent hemorrhages. Thus, we hypothesized that the risk of variceal bleeding in cirrhotic patients may be related to procoagulant imbalance.

Reliable assessment of the relationship between coagulation disorder of chronic liver disease and acute gastrointestinal bleeding in cirrhotic patients is difficult for many reasons. First, the results of standard coagulation tests do not correlate with the occurrence of variceal bleeding. Routine tests do not reflect imbalance between coagulation factors and natural anticoagulants. The assessment of these complex disorders is possible by thrombin generation assay (TGA). Nevertheless, this method is quite complicated and is not widely available. Second, due to unpredictable nature of acute gastrointestinal hemorrhage, a prospective study, which would detect transient disturbances of hemostasis preceding bleeding episode is difficult to perform. On the other hand, the massive gastrointestinal bleeding

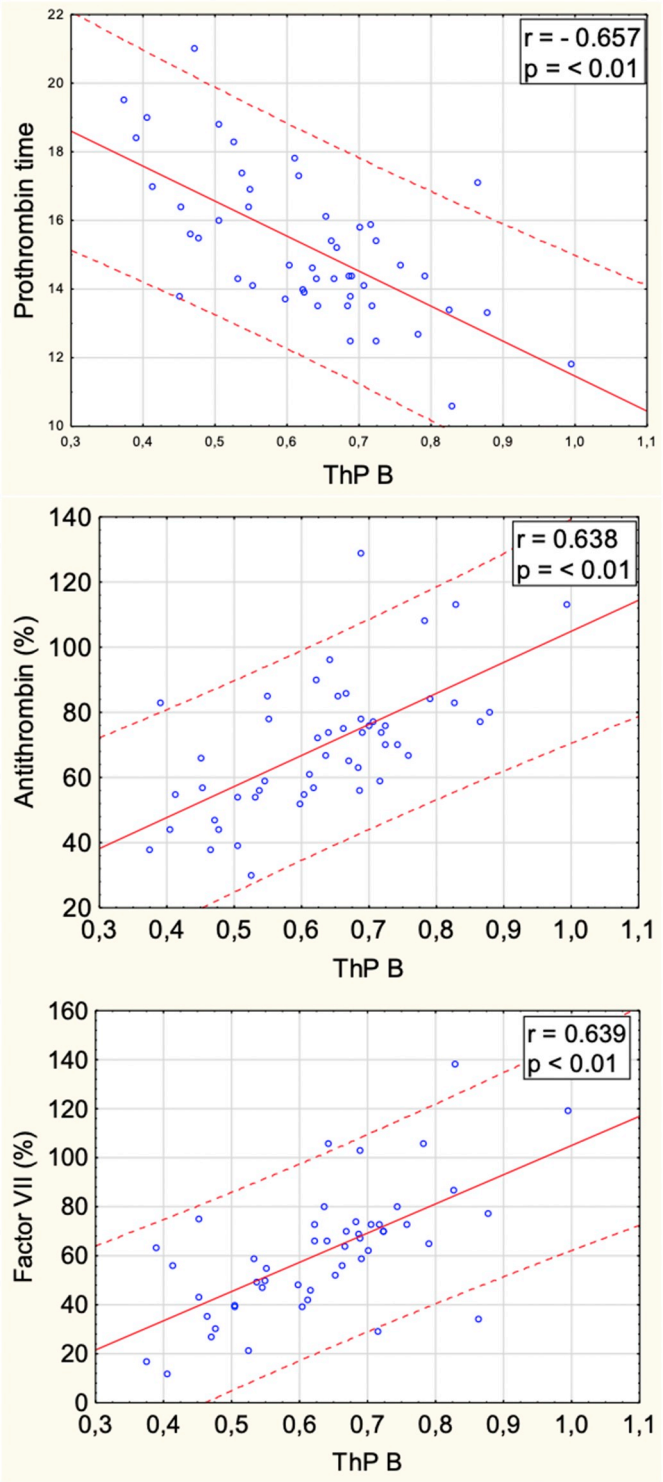

Fig. 2. Correlations of factor prothrombin time, antithrombin and factor VII vs ThP B.

significantly affects the hemostatic system through the loss of the clotting factors and blood platelets. In addition, the variceal hemorrhage usually leads to significant liver decompensation which directly affects the hemostatic system. Thus, the assessment of coagulation disorders in the first days following the bleeding episode does not reflect hemostatic system condition preceding the onset of acute hemorrhage. And finally, several risk factors for bleeding are currently known including the size and location of varices, severity of cirrhosis and infections. These factors should be considered in the assessment of the relationship between the occurrence of variceal bleeding and coagulation disorders.

In our study, we compared the results of the ThromboPath test, in two groups of cirrhotic patients: Group 1 — patients with no history of GI bleeding, Group 2 — patients after variceal bleeding. To better understood the potential relationship between coagulation system imbalance and the occurrence of variceal bleeding we measured factor VII, factor VIII and antithrombin levels. Additionally, in both groups we performed thromboelastometry to assess the global efficiency of the coagulation system. These tests were performed at least three weeks following the episode of GI bleeding. This period seems long enough to "restore" the hemostatic system to the state before bleeding. To limit the influence of other variceal-related bleeding risk factors, we included only patients with at least medium varices with a comparable distribution of varices location. The severity of cirrhosis according to Child-Turcotte-Pugh score was comparable in both groups. Patients with infections or HCC were not included in the study. Standard clotting tests and the results of the factor VII level assessment suggested an increased bleeding tendency in the group with history of variceal bleeding. In addition, the results of the ThromboPath® test without protein C activator (ThP B) confirmed increased procoagulant activity in patients without history of variceal bleeding. The ThP B parameter, being a measure of tissue factor-induced endogenous thrombin generation, significantly directly correlated with the activity of natural procoagulant — factor VII and significantly indirectly correlated with PT. In practice, the ThP B parameter, as well as factor VII and PT reflected the more severe liver cirrhosis in Group 2. Nevertheless, the reduced activity of procoagulants in cirrhosis is accompanied by a reduced activity of anticoagulants. Moreover, natural anticoagulants are suitable for assessing the severity of liver disease. Thus, we also demonstrated a correlation of the ThP B parameter with antithrombin activity. Nevertheless, it is well known that the results of basic coagulation tests do not correlate with the risk of bleeding in patients with liver cirrhosis. We ran multiple regression analysis which confirmed that basic coagulation tests and procoagulant activity were not independent determinants of variceal bleeding. Thus, decreased activity of procoagulants in group with history of bleeding was most likely due to the higher proportion of patients with more severe cirrhosis in comparison with patients who have never bleed.

Previous reports indicated the usefulness of thromboelastometry in the assessment of coagulation disorders in patients with chronic liver disease [18]. The results of parameters assessing the quality and the dynamics of clot formation, obtained in thromboelastometry (INTEM® test) showed similar effectiveness of blood clotting in both groups. Thus, we were not able to identify patients at increased risk of variceal bleeding using thromboelastometry. The results of thromboelastometry suggested a lack of relationship between the occurrence of variceal bleeding and coagulation disorders in patients with cirrhosis.

To assess the relationship between the occurrence of bleeding and the imbalance of procoagulant vs anticoagulant activity, we have performed the ThromboPath® test. It is a simplified thrombin generation assay, performed in the presence and absence of Protac® — a snake venom, which activates protein C in a manner similar to thrombomodulin. The assay results are expressed as Protac induced coagulation inhibition (PICI%). Generally low PICI% translates into hypercoagulability. In contrast to the classic thrombin generation assay (TGA), in which the global generation of thrombin is evaluated and expressed as endogenous thrombin potential (ETP), ThromboPath® evaluates only the tissue factor induced thrombin generation. However, ETP-ratio obtained by classic TGA, reflecting the ratio of ETP in the presence of thrombomodulin to ETP in its absence and PICI% obtained by means of ThromboPath® are significantly inversely correlated as previously shown [6]. To our surprise, the median PICI% was lower in patients with history of variceal bleeding than patients who have never bled. This means that in patients with variceal bleeding history, the assay for the functional evaluation of the Protein C anticoagulant pathway

paradoxically indicated hypercoagulability. The multivariate analysis, taking into account parameters that differed between groups, showed that PICI% was the only independent parameter differentiating study groups.

Increased risk of variceal bleeding in patients with hypercoagulability, as shown by the assay for the functional evaluation of the Protein C anticoagulant pathway, may be a consequence of faster progression of liver disease in these patients. In practice this means that in patients with thrombotic tendency, liver cirrhosis has more severe, complicated course. There are several potential explanations of the relationship between increased procoagulant activity and progression of chronic liver disease. The first, classic proposed mechanism is the possibility of parenchymal extinction as an effect of thrombi formation and deposition of fibrin-fibrinogen complexes in microcirculation causing tissue ischemia and fibrosis [19]. The second potential mechanism is the direct thrombin-mediated stellate cell activation by Protease Activated Receptors-1 (PAR-1) [20]. Thrombin is a serine protease and can signal to various cells including stellate cells and fibroblasts by PAR-1. Thrombin-mediated activation of these cells leads to progression of liver fibrosis.

There are also potential, important clinical implications of our research. Currently recommendations regarding management of coagulopathy and thrombocytopenia cannot be made based on available data [8]. An individual patient based meta-analysis of two randomized controlled studies including 497 cases, showed a beneficial effect of recombinant factor VIIs (rFVIIa) on the primary composite endpoint of control of acute bleeding, prevention of rebleeding days 1–5 and 5-day mortality in patients with advanced liver cirrhosis and active bleeding from esophageal varices at endoscopy [9]. However, a major drawback of the treatment was a potential increased risk of arterial thromboembolic events (5 thromboembolic events in rFVIIa treated patients vs none in placebo treated patients). Theoretically, the hypercoagulability demonstrated in our study by means of ThromboPath® test in patients with variceal bleeding history can contribute to the occurrence of thromboembolic events. Awareness of an increased thrombotic tendency, as evidenced by an assay for the functional evaluation of the Protein C anticoagulant pathway, should lead to a more restrictive strategy for correcting coagulopathy in patients with history of variceal bleeding.

Our study has several limitations. First, the groups differed in terms of the severity of liver failure, as reflected by the MELD score, bilirubin level and INR. Differences in the severity of cirrhosis are also most likely the cause of differences in procoagulant and anticoagulant imbalance between groups. Therefore, the results of our study may rather indicate hypercoagulability associated with more severe hepatic insufficiency, instead of hypercoagulability in patients with variceal bleeding history. The relationship between procoagulant imbalance and the severity of liver cirrhosis have already been studied before. Tripodi et al. showed that median values of PICI% ThromboPath® for cirrhotic patients population, subdivided according to the Child-Pugh score, decreased progressively from Child-Pugh Children A to C, with Child-Pugh C displaying slightly lower median value than that for patients with factor V Leiden [6]. The occurrence of variceal bleeding in our study seemed primarily dependent on portal hypertension associated with more severe cirrhosis and independent of coagulation disorders. Moreover, we were able to detect a hypercoagulability with a simple chromogenic assay in patients with more severe cirrhosis, despite the occurrence of variceal bleeding episodes. We did not assess the relationship between our results and the occurrence of thrombotic events. Thus, the clinical significance of this laboratory finding is unclear. However, we can speculate that this hypercoagulability detected by means of ThromboPath® assay is insufficient to prevent variceal hemorrhage.

Second, the clinical usefulness of the ThromboPath® test to assess the thrombotic risk in patients with liver cirrhosis has not been evaluated so far. Therefore, the results of our study should be interpreted with caution, especially regarding anticoagulant therapy strategies.

In conclusion, we have confirmed the lack of influence of coagulation disorders on the occurrence of variceal bleeding. Moreover, the results of simple chromogenic assay for the functional evaluation of the Protein C anticoagulant pathway, paradoxically indicate hypercoagulability in patients with a history of variceal bleeding and more severe liver cirrhosis, compared to patients who have never bled.

Conflict-of-interest statement

The authors declare no conflicts of interest.

Declarations of interest

None.

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