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Original article

IMPAIRED BALANCE OF CLOTTING FACTORS IN LIVER CIRRHOSIS

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Abstract

Liver cirrhosis (LC) has been accepted as prototype of a disease with acquired prohemorrhagic diathesis. However, as the synthesis of all coagulant factors is affected, a rebalanced but fragile hemostasis is maintained. With increasing disease severity, a disproportion between levels of certain coagulants occurs that can lead to prothrombotic tendency. We aimed to evaluate the levels of factor VIII (FVIII), protein C (PC) and their ratio (FVIII/PC) as the main determinants of thrombin generation in patients with LC at different stages of disease.

Fifty patients with LC were divided in three groups according to LC severity using the Child-Turcotte-Pugh Score (CTP-A, CTP-B, CTP-C). The levels of FVIII and protein C were measured in sodium citrate plasma on Siemens, BCS XP Blood Coagulometer. The levels of FVIII, PC and FVIII/PC were compared between the groups and a correlation of their values to MELD score was performed.

Plasma levels of FVIII increased with severity of the disease, with concurrent statistically significant decrease of plasma PC levels (p=0.0008). This was accompanied with statistically significant increase of the FVIII/PC ratio (p=0.0004) indicating hypercoagulable state in advanced stage of the disease. A significant correlation to MELD score was identified for PC in group CTP-B and CTP-C and for FVIII/PC ratio in group CTP-C.

As liver cirrhosis severity increases, a disproportion in plasma levels of the most powerful determinants of thrombin generation occurs. This could be the explanation for the observed increased risk of venous thromboembolism in patients with liver cirrhosis.

Keywords: liver cirrhosis, hemostasis, prothrombotic state

Introduction

Coagulation is a tightly regulated process modulated by two opposing mechanisms. The first mechanism includes procoagulant factors that are activated when tissue damage occurs resulting in generation of thrombin and conversion of fibrinogen to fibrin[1]. The second mechanism represents the anticoagulant factors, which activation follows after

interaction of thrombin with its endothelial receptor, thrombomodulin[1]. The most potent of the anticoagulants is protein C (PC), which together with its cofactor, protein S, inhibits activated forms of factor VIII (FVIII) and factor V, thereby reducing the production of thrombin[2]. In this precisely regulated process, the balance between procoagulant and anticoagulant factors is essential for limiting excessive thrombin production under physiological conditions. Liver cirrhosis (LC) is characterized by impaired coagulation factor synthesis resulting in impaired values of conventional global coagulation tests, and decompensated LC has traditionally been accepted as a prototype of disease with acquired prohemorrhagic diathesis[3]. However, recent studies have found that levels of both procoagulant and anticoagulant factors are affected resulting in rebalanced hemostasis that keeps homeostasis in a narrower range[4,5]. Studies in patients with stable LC, or compensated LC, show that the level of thrombin production is maintained[6]. But this rebalanced hemostasis is fragile and can easily lead to thrombosis or bleeding in certain complications of the disease.

Although most procoagulant and anticoagulant factors have decreased levels, certain procoagulant factors, such as FVIII and von Willebrand factor (vWF) may have increased levels[6]. Several mechanisms contributing to elevated FVIII levels have been described such as increased vWF levels and decreased synthesis of ADAMTS 13 metalloproteinase[4,7]. Unlike other procoagulant factors, FVIII has an additional source of synthesis, the endothelial cells, and acts as an acute-phase reactant in the presence of inflammation[8].

Not only increased levels, but also decreased levels of FVIII with concurrent decreased levels of anticoagulant PC may promote prothrombotic condition. An impaired relation of procoagulants to anticoagulants with predominance of procoagulant factors (such as FVIII) is described as a possible mechanism for limited PC activation in patients with cirrhosis resulting in a state of hypercoagulability[4]. The impaired ratio of FVIII to PC (FVIII/PC ratio) is cited as an index of procoagulant imbalance [9]. Results from studies show that with progression of LC or decompensation, FVIII values increase and the FVIII/PC ratio is impaired in addition to hypercoagulability[4]. These changes could explain the epidemiological studies indicating an increased risk of venous thromboembolism (VTE) in LC as well as an increased risk compared to healthy population[10]. In our study we aimed to determine the levels of FVIII, PC and their ratio as the most important components in the regulation process of thrombin production in patients with LC at different stages of the disease. Additionally, we evaluated the possible correlation between these factors and disease activity using the Model for End-Stage Liver Disease (MELD) score for appropriate groups.

Materials and methods

This cross-sectional study was conducted at the University Clinic for Gastroenterohepatology in Skopje. In total, 50 consecutive patients with LC, both inpatients and outpatients, meeting the inclusion criteria were enrolled in the study. The diagnosis of LC was based on clinical and laboratory evaluation and transabdominal ultrasound examination. Patients were divided into 3 groups according to their LC severity and expected survival using the Child-Turcotte-Pugh (CTP) score (CTP A - 5-6 points, CTP B – 7-9 points and CTP C – 10-15 points).

The study protocol was approved by the Ethics Committee at the Faculty of Medicine, Ss. Cyril and Methodius University in Skopje. Eligible patients were patients with LC of different etiology at the age of 18 years and older, and a voluntary signed informed consent by the patient or its family. Exclusion criteria were: presence of sepsis; acute bleeding; use of vitamin K antagonists, oral therapy with direct anticoagulants or antithrombotics and presence of malignant disease (hepatocellular carcinoma included). Blood samples for laboratory and hemostatic assessment were retrieved before initiation of therapy for the

hospitalized patients and at a regular follow-up for outpatients using vacutainers containing 3.2% buffered sodium citrate. The samples were processed within 30 minutes of assembly on Siemens' fully automated coagulometer, the Dade Behring BCS® XP System according to the manufacturer's protocols at the Institute for Transfusion Medicine.

The Kruskal-Wallis test was used to compare the values of FVIII, PC and FVIII/PC ratio between groups and *p*-values < 0.05 were taken as statistically significant. The Spearman Rank Order Correlations test was used to assess the correlation between the values of all groups with appropriate MELD scores, where significant relations had R values < 0.05.

Results

Patients' clinical characteristics are summarized in Table 1. The mean age in group CTP-A was 56.9±10.4 years with 38.9% being men, in group CTP-B the mean age was 54.0±16.7 years with 50% being men, and in group CTP-C the mean age was 51.6±14.4 years with 81.3% being men.

Table 1. Demographic and clinical characteristics of patients

	CTP A	CTP B	CTP C	*P*
Number of patients (n)	18	16	16	
Age (mean± SD)	56.9±10.4	54.0±16.7	51.6±14.4	
Sex, %				
Male	7/38.9	8/50.0	13/81.3	
Female	11/61.1	8/50.0	3/18.7	
Etiology (n)				
Virus (HBV, HCV)	6	3	4	
Alcohol	2	5	8	
Autoimmune diseases	6	3	/	
MAFLD*	3	2	/	
Others	1	3	4	
Albumine (mean ± SD)	42.1±5.9	34.0±5.9	26.6±4.4	.0000
Bilirubine (mean ± SD)	15.5±8.6	43.3±28.8	143.2±139.5	.0000
PT (mean ± SD)	12.2±0.9	14.3±2.3	20.0±4.0	.0000
INR (mean ± SD)	1.1±0.1	1.3±0.2	1.8±0.4	.0000
Ascites, %	4/22.22	12 /75	16 /100	
Hepatic encephalopathy, %	0	2/12.5	10/62.5	
CRP‡ (mean ± SD)	7.1±9.6	9.1±8.7	50.8±46.5	.0000
MELD score† (mean ± SD)	8.7±3.1	12.3±3.6	24.6±7.4	.0000

*MAFLD: metabolic associated fatty liver disease; ‡CRP: C-reactive protein; †MELD score: Model for End-Stage Liver Disease Score

The median [interquartile range (IQR)] FVIII and PC levels in group CTP-A were 121.5% (95.0-153.0%) and 62% (51.0-73.0%), respectively, with a mean MELD score of 8.7±3.1. In group CTP-B, the median (IQR) FVIII and PC levels were 150.0% (67.5-153.0%) and 39.5% (32.5-52.5%), respectively, with a mean MELD score of 12.3±3.6. The median (IQR) FVIII and PC levels in group CTP-C were 147.7% (100.0-153.0%) and 27.0% (18.5-43.0%) respectively, with a mean MELD score of 24.6±7.4. The differences in FVIII (p=0.297) between the groups had no statistical significance (Table 2), while the differences in PC (p=0.0008) levels were statistically significant (p< 0.05).

The FVIII levels in all groups showed a weak correlation with MELD score (Table 3), which did not reach significance. A weak and negative correlation of PC with MELD scores was seen in CTP-A and CTP-C groups, while a weak and positive correlation was seen in CTP-B, with a significance for groups CTP-B and CTP-C.

The mean values for PT and INR in group CTP-A were 12.2±0.9 and 1.1±0.1, respectively. In group CTP-B the mean values for PT and INR were 14.3±2.3 and 1.3±0.2,

respectively. The mean values for PT and INR in group CTP-C were 20.0±4.0 and 1.8±0.4 respectively. A statistically significant difference was registered for both parameters between the groups (p<0.0001).

Table 2. Plasma levels of coagulation factors, MELD Score and CRP

	CTP A (N=18)	**CTP B (N=16)**	**CTP (N=16)**	***P***
Factor VIII (median with IQR)	121.5(95.0-121.55)	150.0(67.5-153.0)	147.7(100.0-153.0)	.2976
Protein C (median with IQR)	62.0(51.0-73.0)	39.5(32.5-52.5)	27.0(18.5-43.0)	.0008
FVIII/PC	1.9	2.8	4.5	.0004
MELD score† (mean ± SD)	8.7±3.1	12.3±3.6	24.6±7.4	.0000
CRP‡ (mean ± SD)	7.1±9.6	9.1±8.7	50.8±46.5	.0000

‡CRP: C-reactive protein; †MELD score: Model for End-Stage Liver Disease Score

In addition to the above parameters, the FVIII/PC ratio was calculated as an index of procoagulant tendency in all groups and a statistically significant difference (p<0.05) was observed. The FVIII/PC ratio in CTP-A showed a weak positive correlation with the MELD scores that was insignificant, while the ratio in CTP-B and CTP-C showed a weak negative and positive correlation with the MELD score respectively, which was significant for the CTP-C group (R -.013).

Table 3. Correlations coefficients with MELD score for FVIII, PC and FVIII/PC ratio

	CTP-A R	**CTP-B R**	**CTP-C R**
Factor VIII	.121	-.302	.260
Protein C	-.315	.032	-.311
FVIII/PC	.1653	.056	-.013

Furthermore, in all groups C-reactive protein (CRP) levels were examined as reflection of the presence of inflammation. Elevated levels were detected in all groups (reference value < 6 mg/L) (Table 2) and a statistical significance among the groups was detected (p<0.0001).

Discussion

A preserved liver function is essential for maintaining efficient coagulation because the liver is the main organ for synthesis of almost all coagulation factors and proteins involved in fibrinolysis. Consequently, acute or chronic liver damage affects clotting[3].

In LC, a late-stage liver disease characterized by the replacement of the functional liver parenchyma with fibrous tissue due to long-term liver damage, coagulation disorders result in abnormal values of commonly used conventional coagulation tests (prothrombin time - PT and international normalized ratio - INR) and thrombocytopenia[3]. This constellation of abnormalities most commonly accompanies decompensated LC and indicates hemorrhagic diathesis. Therefore, the widely accepted concept is that decompensated LC is a prototype of acquired coagulation disorder with prohemorrhagic diathesis. This means in routine clinical practice frequent administration of blood products (freshly frozen plasma, platelet concentrate) aimed at correcting abnormal hemostatic values and thus reducing the risk of bleeding[11]. However, this practice has recently been reassessed and specific target outcomes are not recommended[12].

Particularly, in patients with LC the risk of bleeding is not correlated with the degree of PT and INR prolongation[12]. *In vitro* studies demonstrate that in patients with LC thrombin generation is preserved despite prolonged PT and INR[6]. This observation allowed

for a different interpretation of the mechanisms that regulate the balance between procoagulant and anticoagulant factors, suggesting that patients with stable LC behave essentially like normal individuals[13]. This is as a consequence of the simultaneous decrease in the synthesis of anticoagulant factors such as PC and antithrombin III and the increased level of FVIII (which is also synthesized by endothelial cells)[4]. The results of recent studies have shown that patients with LC have a rebalanced hemostasis that keeps homeostasis in a narrower range, thus, is more fragile and in certain conditions it can more easily lead to thrombosis or bleeding[4,5].

Case control retrospective studies in patients with LC have shown a significantly increased risk of VTE and an increased risk of thrombosis when compared to healthy population[10,14]. In regard to this finding is the study of Tripodi *et al.*[4] in which resistance to thrombomodulin activity was detected in plasma from patients with LC, and thus less efficient *in vitro* activation of PC compared to plasma from healthy subjects. Resistance may be due to the presence of a factor V Leiden mutation[4], but given the frequency of this mutation in general population, it is unlikely that in LC it has a different frequency. Therefore, the authors believe that resistance to thrombomodulin in LC is due to higher levels of procoagulant factors such as FVIII and decreased levels of anticoagulant factors such as PC, i.e., their imbalance.

Evaluation for risk of bleeding or thrombosis in patients with LC is not straightforward despite the globally available conventional laboratory tests indicating impaired hemostasis. In addition to the changes caused by the liver dysfunction itself, other associated pathological conditions occurring in LC additionally contribute to prohemorrhagic (gastroesophageal varices, volume overload, abnormal endothelial function, bacterial infections, renal insufficiency) or prothrombotic risks (portal vein stasis, peripheral edema, endothelial inflammation)[9,15,16].

The widely used conventional test, PT, reflects the activity of factors I, II, V, VII and X, while the INR value is a result of patients' PT values and control PT (mean PT value of 20 healthy noncoagulated individuals of both sexes). Although INR was initially designed to monitor and titrate anticoagulant therapy with warfarin, it has been incorporated into clinical scoring systems (MELD score)[17] to appropriately prioritize patients for liver transplantation and in clinical scoring systems to assess mortality from LC (CTP score)[18], although it has not been adapted for this category of patients. Prolonged values of PT and INR in LC patients give insight only into the decreased levels of the abovementioned procoagulants and do not reflect decreases in anticoagulant factors such as PC. In our study, we demonstrated a progressive increase in PT and INR values as disease severity increased, with a progressive decrease in PC values. Accordingly, these conventional clotting tests do not assess patients' overall clotting status[9].

In LC the synthesis of most procoagulant and anticoagulant factors is affected, but certain procoagulant factors such as FVIII and vWF may have elevated values[4]. Several studies have confirmed that FVIII, the most potent factor determining thrombin generation, has significantly increased levels in LC[4,19,20]. Mechanisms contributing to increased FVIII are increased vWF levels and decreased synthesis of ADAMTS 13 metalloproteinase, as well as increased endothelial release of FVIII[5,9]. Considering the inhibitory action of PC to FVIII[2], the concurrent increases in FVIII and vWF levels and decrease in PC levels contribute to procoagulant status in chronic liver disease.

In our study, we recorded elevated levels of factor VIII in all groups, with the highest detected values in the CTP-B and C groups. A limiting factor in the evaluation of FVIII levels was the FVIII maximum detectable value for the automated coagulometer, which was 153%. Hence, higher levels could not be detected, and this may account for the lack of statistical significance between groups, especially between CTP-B and CTP-C. High levels of

FVIII are a major risk factor for venous thrombosis[21] and may be a promoter of thrombosis in chronic liver disease, especially in decompensated LC. Factor VIII is released from endothelial cells as an acute-phase reactant in response to inflammation. Accordingly, treatment of inflammation and infections can reduce the thrombotic tendency. In correlation with FVIII levels among the groups, the highest CRP levels were also detected in CTP-C group (50.8±46.5 mg/dL). Protein C is synthesized solely by the liver, and in chronic liver damage levels decrease. In our study, we demonstrated a progressive decrease in PC levels as LC severity increased. The difference in PC levels between the groups was statistically significant (p=0.0008). All groups had a correlation of PC with the MELD score, with significant values for groups CTP-B and CTP-C (Table 3).

The imbalance between FVIII and PC, referred to as the index of procoagulant imbalance, has been shown to progressively increase with disease severity and results in an increased procoagulant status in patients with advanced LC[4,9]. In our study patients with advanced LC (CTP-B and CTP-C) had higher values of FVIII/PC ratio and a statistical significance was demonstrated between the groups (p=0.0004). Although a correlation between FVIII/PC ratio and MELD score was recorded in all groups, it was significant only for CTP-C group. Nonetheless, in patients with decompensated LC, additional causes for disorders in hemostasis other than chronic liver damage can contribute to more complex and heterogeneous coagulopathies and impair the correlation with MELD score[22,23]. The impaired FVIII/PC ratio could explain the observation of increasing incidence of thrombotic events as LC severity increases, which is 1% in patients with stable compensated LC reaching to 8-25% in decompensated LC and candidates for liver transplantation[24].

The results of recent studies imply to the presence of impaired balance of procoagulant and anticoagulant factors in LC, which was also seen in our study. Although in our study we demonstrated trends in coagulation factor levels that contribute to this imbalance as disease severity progresses, the lack of statistical significance for certain parameters in the study may be due to several factors which are also limitations to the study: the small sample of subjects *versus* studies from the literature; the maximum detectable value for FVIII set to 153%; and extended inclusion criteria (patients with portal vein thrombosis and Budd-Chiari syndrome were included). Therefore, it is not possible to compare nor to dispute the findings and results that have been confirmed by multiple studies. The results from studies favor a disproportionate increase in FVIII and a decrease in PC as the main factors determining thrombin formation and inhibition, with a consequent disruption of their balance. The magnitude of this imbalance could be a predictive marker of the severity of liver disease.

In the future, prospective studies with end-clinical targets in patients with LC of all categories and at high-risk patients are needed to confirm whether the hypercoagulability detected by this disrupted balance of procoagulant and anticoagulant factors is of value in identifying LC patients at increased risk for thrombosis and decompensation.

Conflict of interest statement. None declared.

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