THE CORRELATION BETWEEN CONVENTIONAL COAGULATION TESTS AND THE STUDY OF THROMBIN GENERATION IN PATIENTS WITH LIVER CIRRHOSIS

LILIANA VECERZAN[1], ADRIAN BOICEAN[2], CĂLIN REMUS CIPĂIAN[3], ARIELA OLTEANU[4], ROMEO GABRIEL MIHĂILĂ[5]

[1]PhD candidate "Lucian Blaga" University of Sibiu, [2,3,5] "Lucian Blaga" University of Sibiu, [2,3,4,5]Emergency County Clinical Hospital, Sibiu

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Abstract: *The knowledge about coagulation disorders in patients with liver cirrhosis changed in the last decade. The conventional coagulation tests (the prothrombin time – PT, activated partial thromboplastin time – APTT) do not accurately reflect the complex modifications of the hemostasis in cirrhotic patients, as opposed to the thrombin generation test which can offer more precise information regarding the coagulation status. Thrombin generation could be a superior test compared to conventional coagulation tests in assessing the bleeding risk in cirrhotic patients. Patients with cirrhosis and relatively high levels of thrombin generation have a hypercoagulable state and this may pose a risk of thrombotic events, at the same time, patients with relatively low levels of thrombin generation, may present a hemorrhagic risk. The purpose of this study is to assessing the thrombosis and hemorrhagic risk in 41 patients with liver cirrhosis. The study of thrombin generation in patients with liver cirrhosis reveals that this test evaluates more accurately the risk of hemorrhage and thrombosis compared to the conventional tests, because it takes into account both the pro- and the anticoagulation factors.*

INTRODUCTION

The liver plays a central role in the hemostatic system because it synthesizes most of the coagulation factors as well as the proteins involved in the process of fibrinolysis.(1) The cirrhotic patients have a dysfunction in the hemostatic system due to the law plasma levels of the pro and anticoagulant factors that are synthetized at the hepatic level, which is associated with the risk of thrombotic and hemorrhagic complications. The conventional coagulation tests (the prothrombin time – PT, activated partial thromboplastin time – APTT) do not accurately reflect the complex modifications of the hemostasis in cirrhotic patients, as opposed to the thrombin generation test which can offer more precise information regarding the coagulation status. It evaluates the balance between the procoagulant and anticoagulant factors, offering new perspectives in understanding the coagulation cascade.(2)

PURPOSE

Objectives: assessing the thrombosis and hemorrhagic risk in patients with liver cirrhosis.

MATERIALS AND METHODS

We conducted a prospective observational study from January 2017 until March 2018, in which we included 41 patients with liver cirrhosis and different etiologies who were consecutively admitted in the Gastroenterology wards, sections I and II of Sibiu County Emergency Hospital. The patients agreed to sign the information consent and presented no altered state of consciousness affecting their discernment.

Forty one patients with cirrhosis, 24 men and 17 women, were included in the study, which was approved by the Ethic Committee of the Sibiu County Emergency Hospital. The diagnosis of cirrhosis was established based on the clinical data, laboratory analyses and abdominal ultrasound.

The exclusion criteria were: the use of anticoagulation medication, platelet antiaggregants, bacterial infections and neoplasia.

A blood sample was collected from each control subject and each patient in a 4.5 ml CTAD glass tube (Vacutainers® citrate-theophylline-adenosine-dipyridamole, Beckton Dickinson), containing 3,2% sodium citrate 0,109M. Thrombin generation was performed from platelet-poor plasma by using the Technothrombin® TGA kit (Technoclone, Vienna, Austria) for fully automated Ceveron® alfa. It is based on monitoring the fluorescence generated by the cleavage of a fluorogenic substrate by thrombin, after activating the coagulation cascade by different concentrations of tissue factor and the negative cha rged phospholipids in plasma. From the changes in fluorescence in time, the concentration of thrombin (nM) in the sample can be calculated using the respective thrombin calibration curve.(3) The results were displayed on five parameters: lag time (phase) (min), peak time (min), peak thrombin (nM), velocity index (nM/min), and endogenous thrombin potential (nM).

Other blood samples were collected from each patient: platelet count, activated partial thromboplastin time (APTT), prothrombin time (PT), international normalized ratio (INR). The platelets were determined with an automated hematology analyzer, Sysmex XT4000I, and the coagulation tests with Sysmex CS2000i.

The patient data were analyzed using the SPSS 20 program. Demographic characteristics were analyzed using descriptive statistics, while the frequency and percentages were generated using the table function.

RESULTS

Forty one cirrhotic patients were singed up in the study, of which twenty-four men (59%) and seventeen women

[1]Corresponding author: Liliana Vecerzan, Str Spitalului, Nr 29, Agnita, România, E-mail: vecerzan.liliana@yahoo.com, Phone: +40742 425065
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(41%), with an average age of 62 years, the age interval 44-87 years.

Liver cirrhosis was classified according to etiology and Child-Pugh score (table no. 1). The conventional coagulation tests were analyzed for each Child-Pugh score (table no. 2), as following : PT suffered changes in 59% of the cases of patients with A Child-Pugh score, 73% with B Child-Pugh score and 92% with C Child –Pugh score. APTT appeared modified in 31% of the cases with A Child –Pugh score, 50% in patients with B Child-Pugh score and 71 % in patients with C Child Pugh score. INR was modified for 56% of the patients in A Child-Pugh score, 80% of the patients with B Child-Pugh score and for 91 % of the patients with C Child Pugh. The platelet count was modified for 44% of the patients with A Child-Pugh score, 64% for B Child-Pugh score, and 92% for C Child-Pugh score.

The descriptive statistics (table no. 3) were calculated according to the specific test, on each stage of liver cirrhosis.

The statistical analysis of the correlation between the thrombin generation parameters and the conventional coagulation tests (table no.4) has demonstrated that Lag phase is directly related to INR; the Peak time is correlated to PT, APTT and INR; Peak thrombin is directly correlated to APTT, platelet count and INR; the Velocity index is correlated to PT, APTT and platelet count, and ETP is directly correlated to all the conventional tests parameters.

Table no. 1. Demographic characteristics of cirrhotic patients

Characteristics	All (n=41)	Child-Pugh A (n=16)	Child-Pugh B (n=14)	Child Pugh C (n=11)
Age	62(44-87)	60,5(44-71)	62,5(48-75)	67(43-87)
Etiology				
Alcohol(n)	20(49%)	4(20%)	9(45%)	7(35%)
HCV(n)	14(34%)	9 (64%)	4 (29%)	1(7%)
HBV(n)	3 (7%)	1(34%)	1(33%)	1(33%)
Others(n)	4 (10%)	2(50%)	0(0%)	2(50%)

Table no. 2. Modification of conventional coagulation tests according to Child-Pugh score

Test	Child – Pugh A		Child – Pugh B		Child-Pugh C	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
PT	8	59%	12	73%	11	92%
APTT	5	31%	6	50%	9	71%
Platelets	7	44%	9	64%	11	92%
INR	9	56%	12	80%	10	91%
Total	16		14		11	

Table no. 4. Statistical analysis regarding the correlation between the thrombin generation parameters and the conventional coagulation tests

Parameters	Test t Student resultant (p)				
	Lag phase	Peak time	Peak thrombin	Velocity index	Endogenous thrombin potential
PT	NS	0,026	NS	0,0006	0,005
APTT	NS	0,004	0,0004	0,002	0,034
Platelets	NS	NS	<0,001	0,017	0,025
INR	0,052	0,0082	0,008	NS	0,0005

DISCUSSIONS

From the present study it can be noticed that more men were included in the study and the average age was between 44-87 years. Within the studied group, the most frequent cases of cirrhosis was of alcoholic etiology (49%), followed by the cirrhosis caused by the C virus (34%).

PT/ INR and APTT are prolonged in most cirrhotic patients, which highlight a state of hypocoagulability and a predisposition to hemorrhagic events.

Thrombocytopenia occurs in most cirrhotic patients; this is due to splenic platelet sequestration in portal hypertension or due to the decrease of thrombopoietin production.

The prothrombin time (PT), the international normalized ratio (INR) and the activated partial thromboplastin time (aPTT) are widely used in clinical laboratories as routine screening tests of the coagulation system. They cannot accurately predict the risk of hemorrhage.(4) Evidence has recently been found that thrombin generation provides more useful information about the coagulation status in cirrhosis.

The hepatic disease is accompanied by disorders in the hemostatic system due to reduced plasma levels of pro- and anticoagulant factors which are synthesized by the liver.(5) Thus, the overall effect of the hepatic disease on hemostasis is very complex as patients with advanced liver cirrhosis may experience thrombotic and hemorrhagic events. In clinical practice, cirrhosis is accompanied by a prolonged PT / INR and aPTT due to impairment of the synthesis of most coagulation factors. The combination of thrombocytopenia, prolonged PT / INR and aPTT is suggestive for hemorrhagic dialysis, and it is assumed that cirrhotic patients have a predisposition for hemorrhagic events as a result of these hemostatic changes.(1)

The hereby study reveals a progressive decrease of the thrombin generation indices according to the Child-Pugh score. In Child-Pugh score, the Lag phase and tPeak indices are within normal range, while Peak, VI, ETP indices are slightly decreasing.

Table no. 3. Descriptive statistics

Test	N=41		Child-Pugh A (n=16)		Child-Pugh B (n=14)		Child Pugh C (n=11)	
	Standard deviation	Median	Standard deviation	Median	Standard deviation	Median	Standard deviation	Median
PT	4,024612	15,6	3,222177	12,6	2,360628	16,2	4,687483	19,5
APTT	10,83175	36,5	7,41018	33,9	4,865555	36,7	16,88784	45,4
Platelets	80,10938	111	85,81606	154,5	71,92304	92,5	56,99841	89
INR	0,337761	1,305	0,284418	1,06	0,206606	1,39	0,335799	1,71
Lag phase	1,096974	3,8	0,512482	3,8	0,812963	3,7	1,874214	4,35
tPeak	1,588924	7,6	0,959166	7,3	1,360214	7,2	2,405203	8,5
Peak	80,03248	188,8	90,37222	224,85	73,36617	187,8	55,76495	152,55
VI	30,38969	52,85	33,28748	63,5	31,30224	58,2	17,63315	32,65
ETP	525,5321	1922,3	533,7395	1993,8	552,8507	1961,45	552,3073	1864,8

This ratio is maintained in B Child-Pugh score as well and these particular patients present a risk of hemorrhagic events. Patients within Child-Pugh C class have increased values of Lag phase and tPeak indices, while the other three indices have low values. These patients can be subjected to both hemorrhagic and thrombotic events.

The study of the coagulation profile can help us assess the cellular function of the liver and detect cellular lesions. Prolonged PT/INR and APTT together with the progression of liver cirrhosis points to a deterioration of hepatic parenchyma and triggers the decrease of the production of the proteins involved in coagulation, thus entailing the risk of hemorrhage.

Based on current literature, the study of thrombin generation follows the global evaluation of hemostasis, providing information on the incipient, amplification or propagation and resolution phases. The results of this test better reflect the hemostatic phenotype compared to conventional tests and have great potential for assessing the risks of bleeding and thrombosis.(2)

Thrombin generation is a global coagulation test which measures the dynamics of thrombin production by using small amounts of tissue factors to trigger an interaction in the coagulation factors. Thrombin is one of the most important enzymes in the coagulation cascade. It cleaves the soluble fibrinogen and forms the fibrin thrombus. Taking this into account, thrombin generation could be a superior test compared to conventional coagulation tests in assessing the bleeding risk in cirrhotic patients.(6) When thrombin generation is evaluated in cirrhotic patients, they have the potential to generate more thrombin, just as healthy subjects, after the addition of thrombomodulin.(7,8) Patients with cirrhosis and relatively high levels of thrombin generation have a hypercoagulable state in vitro (9) and may pose a risk of thrombotic events. At the same time, patients with relatively low levels may present a hemorrhagic risk.(10)

CONCLUSIONS

To conclude, the study of thrombin generation in patients with liver cirrhosis reveals that this test evaluates more accurately the risk of hemorrhage and thrombosis compared to the conventional tests, because it takes into account both the pro- and the anticoagulation factors. Thus, studying the profile of coagulation in cirrhotic patients may be useful in the prevention of hemorrhagic and thrombotic complications which may be life-threatening.

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