5

The Bacterial Endotoxins Levels in the Blood of Cirrhotic Patients as Predictor of the Risk of Esophageal Varices Bleeding

Dmitry Garbuzenko, Alexandr Mikurov
and Dmitry Smirnov
Department of Surgical Diseases and Urology,
Chelyabinsk State Medical Academy,
Russia

1. Introduction

In cirrhotic patients the bleeding from esophageal varices is the most dangerous complication of portal hypertension and is attended with high rate of lethality. Endoscopic assessment of esophageal varices and the state of esophageal and stomach mucosa at the esophagogastroduodenoscopy presents high importance for the assessment of risk of their development (Coelho-Prabhu & Kamath, 2010). However, invasiveness as well as discomfort that are tolerated by patients during the given procedure, lead to the rejection from it and therefore they can't be subjected to examination in a number of cases. Besides, the research might be impossible to carry out in case if the state of patient is grave (de Franchis et al., 2008). The investigation of hepatic venous pressure gradient, that reflects the portal hypertension intensity best of all haven`t been realized in the clinical practice up to now (Groszmann et al., 2006). Considering the above mentioned disadvantages of main methods, the development of additional prognostic criteria of the risk of esophageal varices bleeding remains the urgent problem of the internal medicine (Cárdenas & Ginès, 2009).

Over the last years the greatest importance in the development of the given complication is placed to the endotoxemia as a result of the translocation of gram-negative bacteria from intestinal tube (Boursier et al., 2007). It was demonstrated that the latter presents the important part of the hyperdynamic circulatory status at the portal hypertension and also lies in the basis of hepatocellular insufficiency and brings about the haemostasis disorder in cirrhotic patients (Thalheimer et al., 2005). Besides, it was reported that bacteremia often accompanies esophageal varices bleeding (Lata et al., 2005), decreases the efficiency of the conservative or endoscopic therapy (Zhao et al., 2002) and bears the risk factor for recurrent bleeding (Brown et al., 2010).

The aim of the research is to illustrate the appropriateness of the assessment of the bacterial endotoxins levels in the blood of cirrhotic patients as the method to predict the risk of esophageal varices bleeding.

2. Patients and methods

The prospective incidence research has been carried out that covers 90 cirrhotic patients with portal hypertension between September 2008 and December 2010 at our clinic (The department of surgical diseases and urology, Chelyabinsk State Medical Academy, Chelyabinsk, Russia). Only patients with esophageal varices was included in the study. All patients underwent a detailed clinical evaluation, including blood tests, ultrasonography, esophagogastroduodenoscopy. Moreover the quality and quantity assessment of endotoxemia intensity was performed.

2.1 Blood tests

Hematological and biochemical work-up included measurement of hemoglobin, total leukocyte count, platelet count, serum concentrations of bilirubin (total and conjugated), protein, albumin, alanine aminotransferase and aspartate aminotrasferase. Furthermore the hemostatic factors of their peripheral blood, such as prothrombin time, International Normalized Ratio, activated partial thromboplastin time, activated recalcification time, soluble fibrin-monomer complexes, fibrinogen, Hageman factor dependent fibrinolysis, were examined. For each patient, a modified Child-Pugh score was calculated (Pugh et al., 1973). All patients were tested for HBV-DNA, HBeAg, HBsAg, anti-HBcAg, antibodies to hepatitis C virus, antimitochondrial antibodies to determine the cause of liver cirrhosis.

2.2 Ultrasound Doppler

All patients underwent ultrasonography and the follow details were recorded: Maximum vertical span of the liver, nodularity of liver surface; spleen size (length of its longest axis); diametr of the portal and splenic veins; presence of portal-systemic collaterals; and presence of ascites.

The portal vein was imaged longitudinally in the supine position, and the Doppler sample volume was set at the midpoint between the confluence of the splenic and superior mesenteric vein and the bifurcation of the portal vein at the hepatic hilus. When the sample point was adjusted to the center of the portal vein, the portal venous velocity was recorded in a quiet suspended expiration and was averaged over a few seconds. Portal venous flow was determined by the formula, cross-sectional area × mean velocity × 60 (Choi et al., 2003). The mean velocity of the splenic vein was determined using the same method as used for the portal vein.

2.3 Endoscopic evaluation

All patients underwent esophagogastroduodenoscopy for assessment of esophageal varices, the degree of which was defined according to the international classification offered by Japanese Research Society for Portal Hypertension (Japanese Research Society for Portal Hypertension, 1980): F0 – absent; F1 – straight; F2 – winding; F3 – nodule-beaded.

2.4 Assessment of endotoxemia intensity

To make the quality and quantity assessment of endotoxemia intensity their blood was collected into a sterile test tube without anticoagulants, it was placed into the thermostat for

30-40 minutes with the temperature of 37° C and then centrifugated during 15 minutes at 3000 revolution per minute. The obtained serosity was used for analysis during 2 hours. In a number of case it was possible to apply cold singly and store it.

Abacterial express-diagnostics of common endotoxin of gram-negative bacteria was conducted by the method of activated particles (MAP) with the test sets "MAP – Endotox spp.", developed in Research center of cardiovascular surgery named after A.N. Bakulev, Russian Academy of Medical Sciences and Research and Production Company "Rokhat" (Bokeriia et al., 2007). The given method is based on immobilization of polymer chemical microspheres with a size of particle equal to 0,62 – 0,68 micron of monoclonal antibodies, subclass IqG 3 and IqG 2a, 0:111 B4 J5. The reaction accounting was maintained considering the degree of particle activation (DPA) in the diagnostic titer 1:8 on point-based system ranging from 1 to 4.

The levels of endotoxemia is determined by means of turbidimetric test on the end point that serves as a variant of Limulus lysate amebocyte assay (LAL-assay), firstly described in 1968 by J. Levin and F.B. Bang (Levin J. & Bang F.B., 1968). Its basis is formed by the ability of Lymulus amebocyte lysate react with endotoxins (liposaccharides) of gram-negative bacteria in a specific way. The amount of bacterial endotoxin contained in the examined samples was determined according the pharmacopoeial techniques that were attached to the set of chemical reagents produced by the "LAL Center" (Moscow, Russia). The analysis was carried out on microtitration plates with the application of photoelectric colorimeter "Multi Scan". The measurement were made at the length of wave equal to 405 millimicron. Calibration curve was built using three-four known concentrations of control standard endotoxin.

2.5 Statistical analysis

The statistical analysis of the data received was conducted with the help of program package "Statistica 5.5". We calculated values M, their standard mistakes (m) and 95 % of confidence interval, and used non-parametric Mann and Whitney test. Besides, we applied Spirmen coefficient of rank correlation. The differences were considered to be accurate at $P < 0,05$.

3. Results

Ninety cirrhotic patients with portal hypertension were enrolled in this study, with a median age of 46,2 ± 20 years. There were 47 men and 43 women. Both the virus and alcohol were the aetiology of liver cirrhosis in 18 patients. Primary biliary cirrhosis was found in 3 patients. It was not possible to establish the cause of disease in 51 cases. Hepatocellular insufficiency according to Child-Pugh score was grade A in 18 patients, grade B in 52 patients, grade C in 20 patients.

Esophagogastroduodenoscopy demonstrated esophageal varices of first degree (F1) in 17 patients, of second degree (F2) in 52 patients, of third degree (F3) in 21 patients. Sixty-five had gastro-esophageal bleeding due to variceal rupture. Patients were divided into 3 groups: first group (25 people) was presented by the patients without esophageal varices bleeding, the second group (20 people) consisted of those who had tolerated it in the medical background, the third group (45 people) – was made by patients in the urgent order with esophageal varices bleeding.

In the framework of both the quality and quantity assessment of endotoxemia there were the statistically significant differences obtained between the groups put in contrast (P < 0,05). Thus, its intensity, determined by the method of particles activation turned out to be the most significant for the patients with esophageal varices bleeding, and appeared to be the minimal with people not suffering from such a complication (Table 1).

Groups of patients		Endotoxemia levels			
		1 DPA	2 DPA	3 DPA	4 DPA
I	Without esophageal varices bleeding	N = 20	N = 5	0	0
II	Esophageal varices bleeding in the medical history	N = 5	N = 10	N = 5	0
III	Acute esophageal varices bleeding	0	N = 5	N = 11	N = 29

Notice. Spirmen coefficient (R = 0,82; P < 0,05; N = 90).

DPA - degree of particle activation.

Table 1. Qualitative assessment of the endotoxemia intensity in cirrhotic patients with portal hypertension (MAP – Endotox test; DPA)

The levels of bacterial endotoxins in the blood of patients that had arrived in the order of emergency varied between 4,1 to 59,1 ng/ml and was authentically higher (p<0,05) than of patients without or with the variceal bleeding in the medical background: from 0 to 0,8 ng/ml and from 1,3 to 2,3 ng/ml, respectively (Table 2).

Groups of patients		
Without esophageal varices bleeding	Esophageal varices bleeding in the medical history	Acute esophageal varices bleeding
I	II	III
0,5 ± 0,1 N = 25	1,9 ± 0,1 N = 25 P(I – II)*	32,1 ± 3,0 N = 45 P(I - III)* P(II - III)*

Notice: P()* - difference between contrast groups are statistically significant.
U-criterion of Mann and Whitney applied; P < 0,05.
LAL-assay - Limulus lysate amebocyte assay.

Table 2. The bacterial endotoxins levels in the blood of cirrhotic patients with portal hypertension (ng/ml) (LAL-assay), M ± m

With 13 patients without the previous record of variceal bleeding, the latter appeared in a period of 3-4 days later from the moment of planned hospitalization. In all cases there was the increased levels of bacterial endotoxins in the blood recorded as early as the very moment of admittance to hospital (8,3 ± 3,9 ng/ml), at the time of bleeding it increased to 38,7 ± 4,6 ng/ml (p<0,05), and 5-7 days later after achieving the effective hemostasis it decreased to 3,2 ± 1,6 ng/ml (P < 0,05).

Among the patients admitted with acute esophageal varices bleeding, 16 of them had its early recurrence in the upcoming 24 hours after the primary hemostasia. They all had a high levels of bacterial endotoxins in blood (36,2 ± 6,7 ng/ml) which having decreased down to 6,8 ± 2,5 ng/ml ($P < 0,05$) against the background of conservative therapy, sharply rose to 39,3 ± 6,3 ng/ml at the blooding recurrence. When the stable hemostasis had been achieved its values decreased down to 3,1 ± 1,1 ng/ml ($P < 0,05$).

Presuming that the minimal concentration of bacterial endotoxins in the blood of those who suffer from the acute esophageal varices bleeding made 4,1 ng/ml and the maximal value among those who were admitted to hospital routinely comprised 2,3 ng/ml, we had the opportunity to determine the intervals of values endotoxemia according to the degree of esophageal varices bleeding risk. Considering the fact that the levels of bacterial endotoxins in the blood of the selected patients (90 people) ranging from 0 to 4,0 ng/ml determed the low possibility of complication occurrence and the values above 4,1 ng/ml confirmed the high risk, the diagnostic criterion 4,0 ng/ml turned out to be the best one. The sensitivity and specificity was 90 % and 85 % respectively.

From 45 patients that were admitted to hospitals with acute bleeding, only 2 patients had the esophageal varices of the first degree whereas 23 and 20 patients were found to suffer from the esophageal varices of the second and third degree correspondingly. On the opposite, among 45 patients that were hospitalized routinely the esophageal varices of the first degree was identified in 15 cases; of the second degree – found in 26 patients, and as for the third – only with 4 of them.

At the quality assessment of endotoxemia in case of all the patients involved in research, there is the credible correlation ($P < 0,05$) between its parameters, the degree of esophageal varices and the bleeding occurrence. Thus, if in case of patients with acute bleeding with esophageal varices mainly of 2-3 degrees (43 people), the intensity of endotoxemia seemed significant, its intensity was insignificant among the patients with esophageal varices of 1-2 degrees (41 people) as a rule, admitted without such complication (Table 3).

Esophageal varices			Endotoxemia levels			
			1 DPA	2 DPA	3 DPA	4 DPA
Acute esophageal varices bleeding	No	F1	10	4	1	0
		F2	15	10	1	0
		F3	0	1	3	0
	Yes	F1	0	1	1	0
		F2	0	4	10	9
		F3	0	0	0	20

Notice. Spirmen coefficient ($R = 0,87$; $P < 0,05$; $N = 90$).
MAP - method of activated particles.
DPA - degree of particle activation.

Table 3. The correlation between the intensity of endotoxemia in cirrhotic patients, the degree of esophageal varices and the availability of acute bleeding from them (MAP – Endotox test; DPA)

In order to carry out the correlation analysis of bacterial endotoxins levels in the blood, the degree of esophageal varices and the acute bleeding occurrence the patients were divided into four subgroups. The patients with esophageal varices of the first degree without acute bleeding made subgroup A. Since the intensity of endotoxemia in the patients with esophageal varices of the second and the third degree, admitted without the mentioned complication was similar and a number of the latter minor we combined into one subgroup B. For this very reason the patients were combined suffering from esophageal varices of the first and second degree, hospitalized with acute bleeding (subgroup C). Those who were admitted in the emergency order with the same diagnosis as well as the esophageal varices of the third degree made subgroup D.

The data received corresponded to the quality values and were statistically accurate ($P < 0,05$). There was also the direct correlation dependence observed between the levels of bacterial endotoxins in the blood, the degree of esophageal varices and the availability of acute bleeding from them. Thus, the endotoxemia was least pronounced in the patients of subgroup A: ranging from 0 to 0,8 ng/ml and mostly identified in subgroup D: from 29,4 to 59,1 ng/ml. In subgroups B and C: from 0,9 to 2,3 ng/ml and from 9,8 to 19,9 ng/ml, correspondingly (Table 4).

Groups of patients			
Without acute esophageal varices bleeding		With acute esophageal varices bleeding	
Subgroup A	Subgroup B	Subgroup C	Subgroup D
0,03 ± 0,01 N = 15	1,2 ± 0,8 N = 30 P(A-B)*	14,9 ± 5,0 N = 25 P(A-C)*; P(B-C)*	34,3 ± 4,7 N = 20 P(A-D)*; P(B-D)*; P(C-D)*

Notice: P()* - difference between the contrast subgroups are statistically significant. U-criterion of Mann and Whitney applied; $P < 0,05$.
LAL-assay - Limulus lysate amebocyte assay.

Table 4. The correlation between the bacterial endotoxins levels in the blood of cirrhotic patients, the degree of esophageal varices and the availability of acute bleeding from them (ng/ml) (LAL-assay), M ± m

4. Discussion

In cirrhotic patients the bleeding from esophageal varices is a serious and potentially life-threatening complication of portal hypertension. The risk of bleeding can be reduced nearly by half by appropriate prophylactic therapy (de Franchis, R. & Baveno V Faculty, 2010), which has prompted the identification of noninvasive indicators of esophageal varices, aimed to decrease the need for screening endoscopy, which is currently recommended in all cirrhotic patients at the time of diagnosis. Low platelet count, splenomegaly, a portal vein diametr on ultrasound ≥ 13 mm, a high Child-Pugh score, low ptothrombin activity, the presence of spider angiomas, and a low platelet to spleen ratio have been the parameters most frequently associated with presence varices and the risk of bleeding from them (Berzigotti A. et al., 2008). Endotoxaemia is frequently found in cirrhotic patients, even in the absence of any signs of sepsis. Thus higher endotoxins concentrations are found in peripheral blood of cirrhotics than in normal subjects with a statistically significant gradient

between portal and peripheral blood, highlighting the role of the bowel as the source of endotoxins. Both peripheral and portal levels of endotoxaemia are correlated with the severity of liver disease which is a more important predictor of high plasma endotoxins concentrations than portosystemic shunting or portal hypertension (Lin R.S. et al., 1995). Further, plasma levels of the bacterial endotoxins in cirrhotic patients were significantly positively associated with hepatic venous pressure gradient, wedge hepatic venous pressure, and hepatic sinusoid resistance (Lee K.C., 2010).

We evaluated of the bacterial endotoxins levels in the blood of cirrhotic patients as the method to predict the risk of esophageal varices bleeding. Our study detected that the highest manifestation of endotoxemia in cirrhotic patients is observed in case of acute bleeding from the esophageal varices. With the patients that had bleeding in the medical background, its values exceed the values of those who hadn`t this complication.

While the bacterial endotoxins levels in the blood of cirrhotic patients ranging from 0 to 4,0 ng/ml determines the low possibility of bleeding occurrence, the values exceeding 4,1 ng/ml demonstrate its high risk probability. Accordingly, diagnostics criterion of 4,0 ng/ml appears to be the most adequate and relevant one. The sensitivity and specificity was 90 % and 85 % respectively.

Moreover we found that the intensity of endotoxemia with cirrhotic patients adequately correlate with the degree of esophageal varices and the bleeding occurrence in them.

5. Conclusion

Notwithstanding the fact that the main predictor of the risk of esophageal varices bleeding in cirrhotic patients is endoscopic assessment of their intensity according to data of esophagogastroduodenoscopy, in case if its fulfillment appears to be impossible (if a patient rejects the research being made, if the condition of a patient is grave and so on) the detection of the bacterial endotoxins serum levels can serve as the alternative prognostic index. Further validation of the results will be achieved through long-term follow-up of the patients and a larger number of studied subjects.

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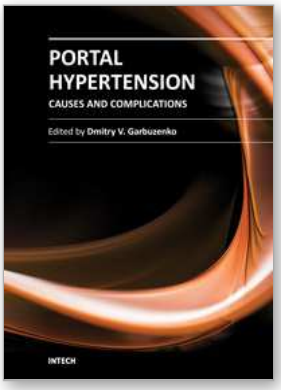

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Portal hypertension is a clinical syndrome defined by a portal venous pressure gradient, exceeding 5 mm Hg. In this book the causes of its development and complications are described. Authors have presented personal experiences on conducting patients with various displays of portal hypertension. Moreover, the book presents modern data about molecular mechanisms of pathogenesis of portal hypertension in liver cirrhosis, the information about the original predictor of risk of bleeding from gastro-esophageal varices and new methods for their conservative treatment.

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University Campus STeP Ri
Slavka Krautzeka 83/A
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Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821