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DIAGNOSTIC AND PREDICTIVE POTENTIAL OF THE C-REACTIVE PROTEIN IN SERUM AND ASCITES FOR SPONTANEOUS BACTERIAL PERITONITIS IN PATIENTS WITH LIVER CIRRHOSIS AND ASCITES

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Abstract

Spontaneous bacterial peritonitis (SBP) in patients with liver cirrhosis is a spontaneous bacterial infection of sterile ascites fluid in the absence of intra-abdominal sources of infection or malignancy.

The purpose of the study is to determine the diagnostic and predictive potential of CRP in serum and ascites, as an inflammatory indicator of SBP in patients with liver cirrhosis and ascites and to compare the mean values of CRP in serum and ascites in patients with and without SBP.

In this prospective-observational study were included 70 patients with cirrhosis and ascites, divided into two groups, SBP and non-SBP group. Quantitative measurement of CRP in serum and ascites was determined by immunoturbidimetric method using latex particles. The average value of CRP in serum in SBP group was 35.4 ± 29.51 mg / L, and in control non-SBP group it was lower (18.6 ± 18.71 mg/L), and this difference was statistically significant for p = 0.006132. The average value of CRP in ascites in SBP group was 7.3 ± 7.2, and in non-SBP group it was lower (2.9 ± 3.1l), with statistically significant difference of p = 0.001604. ROC analysis indicates that CRP contributes to the diagnosis of SBP with 71.0% (p = 0.003) (good predictor). Univariate analysis showed that serum CRP> 6 mg / L and CRP in ascites> 6 mg / L significantly increased the chance of SBP by seven times ((Exp (B) = 7,319) and three times ((Exp (B) = 3,059), respectively.

Our research confirmed that serum CRP is a good predictor, significantly associated with the occurrence of SBP in patients with liver cirrhosis.

Keywords: SBP, C-reactive protein, ascites, liver cirrhosis

Introduction

Spontaneous bacterial peritonitis (SBP) in patients with cirrhosis of the liver is a new, spontaneous bacterial infection of sterile ascites fluid, in the absence of intra-abdominal sources of infection or malignancy. The most sensitive indicator for diagnosis is the number of polymorphonuclear cells (PMNK) ≥250 in 1 ml of ascites fluid (manual microscopic or automated counting) and / or when one bacterial species is isolated in the microbiological culture [1-5].

Although determining the number of PMN cells in ascites remains the gold standard for diagnosing SBP, in recent years research has focused on finding new diagnostic and prognostic markers.

Potential markers present in serum and ascites fluid include: proinflammatory cytokines (tumor necrotizing factor-TNFα and interleukin 6 IL-6), lactoferrin, procalcitonin, calprotectin, C-reactive protein (CRP) and tissue CRT collagen). Some of them are commercially unavailable, some with low sensitivity, or a high risk of false negative results, especially in patients with low neutrophil counts. So far, the value of any marker alone has not been proven to be optimal for the diagnosis of SBP, which indicates the need for additional studies [6].

C-Reactive Protein (CRP) is a glycoprotein that participates in the acute phase of inflammation. The influence of anti-inflammatory cytokines (interleukin-1 (IL-1), tumor necrotic factor-alpha (TNF-α) and interleukin-6 (IL-6) increases its concentration in the blood in the first 6 hours after the onset of inflammation, and its concentration increases by 10 to 100 times over the next

24 to 48 hours.CRP has long been thought to be produced exclusively by hepatocytes, but recent studies suggest other sites of production, including the smooth muscle cells of the coronary arteries , kidneys, neurons, alveolar macrophages, adipose tissue and external stimuli (smoking, drinking alcohol and coffee) [7-12].

Its role as an independent marker for SBP detection has been described in several studies [13-16].

In this context, although there were initially doubts about the competence of this inflammatory marker in patients with liver failure, it was found that CRP synthesis is preserved even in the context of advanced liver disease.

Starting from the relatively high prevalence of SBP in patients with cirrhosis and the poor prognosis associated with it, the question of the diagnostic and predictive potential of CRP as inflammatory indicators in this group of patients was gradually imposed.

Objectives: To determine the diagnostic and predictive potential of CRP in serum and ascites as an inflammatory indicator of SBP in patients with liver cirrhosis and ascites and to compare the mean values of CRP in serum and ascites in patients with SBP and non-SBP.

Materials and methods

In this prospective-analytical-observational study which was implemented at the University Clinic for Gastroenterohepatology in Skopje, were included 70 patients with cirrhosis and ascites, divided into two groups. The division into groups was made according to the number of polymorphonuclear cells (PMNC) in the ascites. The first group included 35 patients with a PMNC ≥250 in 1 ml of ascites fluid (SBP group) and the second group included 35 patients with a PMNC<250 in 1 ml ascites fluid (non SBP group).

Patients included in the study were aged > 18–70 years. Exclusion criteria for exclusion from the study were acute liver failure, abdominal surgery in the last 3 months, infectious pleural effusion, peritoneal carcinomatosis, haemorrhagic ascites, hepatocellular carcinoma and patients receiving antibiotics at least 2 weeks before enrollment.

After prior acquaintance with the structure, content and purpose of the study, the condition for participation in it was for the patients to sign the offered informed consent. The protocol of the study was in accordance with the ethical principles of the Declaration of Helsinki, and it was submitted, reviewed and approved by the Ethics Commission of the Medical Faculty at the University "St. Cyril and Methodius" in Skopje.

Paracentesis was performed under aseptic conditions, in a patient in a supine position, and a puncture in the left or right lower quadrant of the abdomen with ultrasound imaging (no patient had complications associated with diagnostic paracentesis). All diagnostic test specimens were immediately referred to the Central Clinical Laboratory. Out of a total of 10 ml of ascites, 5 ml were used for automatic counting of PMNK and 5 ml for biochemical analysis of ascites (C-reactive protein). At the same time, for the needs of biochemical blood analysis, venipuncture of 10 ml of blood was performed.

Quantitative measurement of C-reactive protein in serum and ascites was determined by immunoturbidimetric method using latex particles as facilitators-enhancers of the Architect apparatus reaction. 4100. Reference values: up to 6 mg / L.

Statistical processing

The collected data was processed using the statistical program SPSS 23 for Windows. Databases were created using specific computer programs designed for that purpose. Their processing was performed using standard descriptive and analytical bivariant and multivariate methods. Attributive statistical series were analyzed by determining the coefficient of relations, proportions, rates and by determining the statistical significance between the detected differences.

The numerical series were analyzed by central tendency measures and data dispersion measures. Statistical significance between the numerical parameters in both groups is analyzed by Mann-Whitney U Test. A P value of less than 0.05 showed a statistically significant difference.

Results

The average value of serum CRP in SBP group is 35.4 ± 29.51 mg / L, and in non-SBP group it is lower and is 18.6 ± 18.71 mg / L. Both average values are higher than the reference value (ref. Up to 6 mg / L). The difference between the mean values is statistically significant for p <0.05 (t = 2.83033; p = 0.006132) (Table 1 and fig 1).

Table 1. Average value of serum CRP and Student's t-test

	average – SBP	non-SBP	t-тест	p	S.D SBP	S.D Non-SBP
CRP serum	35,4	18,6	2,83033	0,006132	29,46989	18,72097

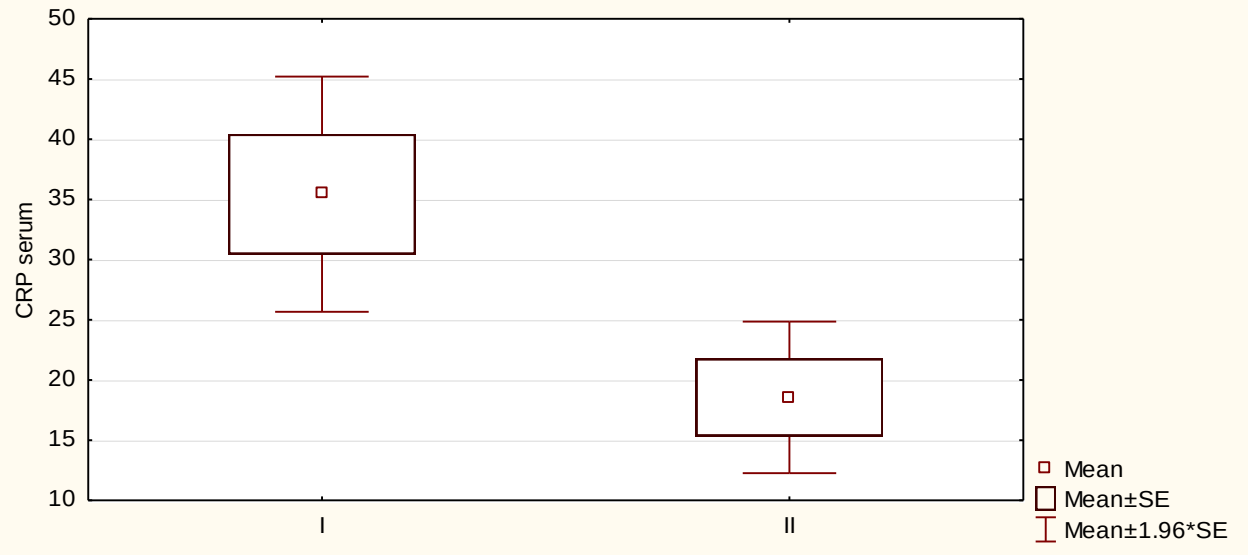

Fig 1. Average values of CRP

The average value of CRP in ascites in SBP group is 7.3 ± 7.2, and in non-SBP group it is lower and is 2.9 ± 3.1l. The difference between the mean values is statistically significant for p <0.05 (t-test = 3.28708; p = 0.001604) (Table 2 and fig 2).

Table 2. Average value of ascites CRP and Student's t-test

	average SBP	Average non SBP	t-тест	p	S.D SBP	S.D non SBP
CRP-ascites	7,3	2,9	3,28708	0,001604	7,23541	3,08887

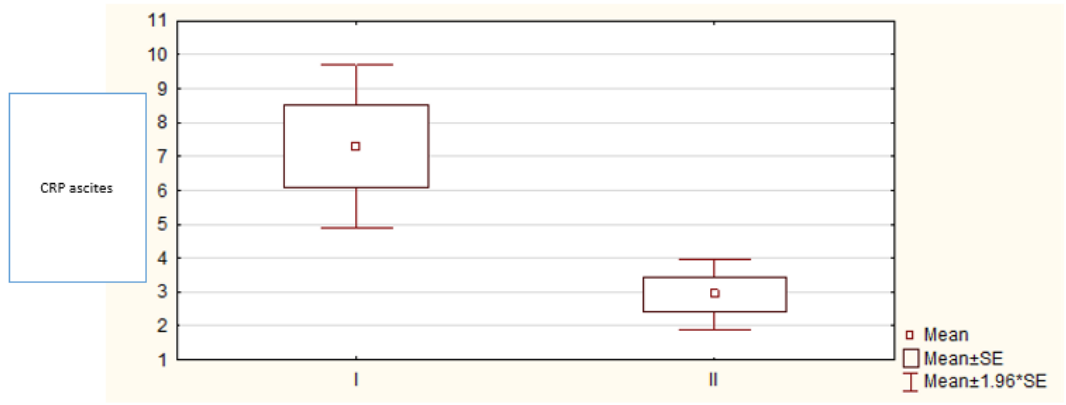

Fig 2. Average values of CRP in ascites.

ROC analysis indicates that CRP contributes to the diagnosis of SBP with 71.0% (p = 0.003) (good predictor), closer to the ideal value of 1.0 and above the worst value of 0.5 (tab. and fig 3) .

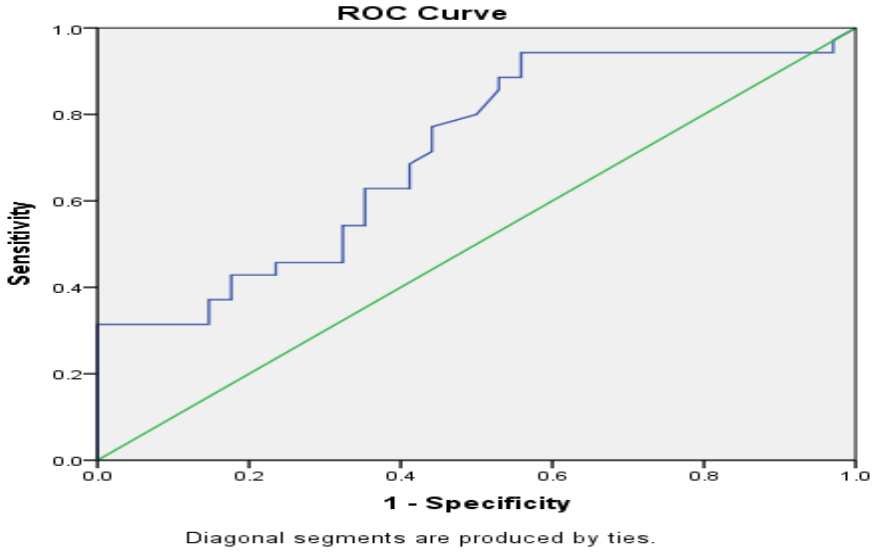

Fig 3. ROC-curve of CRP as a predictor of spontaneous bacterial peritonitis

Tabele. 3 Area Under the Curve

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0,710	0,062	0,003	0,588	0,832

Univariate analysis showed that serum CRP> 6 mg / L significantly increased the chance of SBP by seven times (Exp (B) = 7,319 and CRP in ascites> 6 mg / L significantly increased the chance of SBP by three times (Exp (B) = 3,059 (Table 4).

Table 4. Overview of Univariate analysis of CRP serum / ascites affecting the development of SBP.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	CRP in serum > 6 mg/L	1,991	0,630	9,990	1	0,002	7,319	2,130	25,151
	Constant	-0,544	0,296	3,365	1	0,067	0,581		

a. in relation to: CRP serum

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	CRP in ascites> 6 mg/L	1,118	0,514	4,736	1	0,030	3,059	1,117	8,373
	Constant	-0,693	0,408	2,883	1	0,090	0,500		

a. in relation to: CRP ascites

Discussion

The diagnostic capabilities and cut-off values of this marker for detecting infections in patients with decompensated cirrhosis have been described in several studies.

The systematic review of Pieri G et al. [17] analyzed patients with more advanced liver cirrhosis during bacterial infections and found that they had lower CRP values in the blood, which depends on the degree of hepatic dysfunction, meaning the worse the hepatic dysfunction, the lower the CRP value is.

For these reasons, the author believes that CRP has a weak predictive power for detecting infections in patients with cirrhosis of the liver and therefore recommends starting empirical antibiotic treatment even in conditions of its moderate increase. Serial CRP measurements can be useful in determining the resolution or persistence of sepsis or inflammation, helping clinicians make decisions in reassessing patients who have failed to improve clinically. The same study, regarding the optimal cut-off values of CRP for infection detection, found that there is a large inhomogeneity in the average values and in different studies it ranges from <10 mg / L to> 80.0 mg / L.

The mean values of CRP in the study of Preto-Zamperlini et al. [16] (6.5 ± 0.98 mg / dl versus 1.14 ± 8.98 mg / dl, p = 0.001) were higher than the results in our study. The authors proposed a cut-off value of this marker greater than 1.0 mg / dl to be used as an independent variable for predicting SBP. Similar results were reported in a prospective study in 2013 [18,] which aimed to

compare the diagnostic and predictive value of Procalcitonin (PCT) and leukocyte CRP in patients with SBP associated with B posthepatic cirrhosis. The optimal cross-sectional value of CRP was 16.15 mg / L (AUC, 0.86; 95% CI, 0.78-0.94; 64% / 95% at 16.15 mg / L) for SBP. The author concludes that serum PCT and CRP are better markers than leucocytes for diagnosing patients with SBP.

On the other hand, Ahmed Abdel-Razik et al.[19], in their study reported higher mean serum CRP values in patients with SBP versus patients with non-SBP (62.4 ± 28 vs. 9.81 ± 8.98, p 0.001). The cut-off value of serum CRP of 30 mg / dl has high specificity (96%) and sensitivity (90%) for the detection of SBP.

When analyzing the predictive value of CRP in serum and ascites for SBP, ROC analysis indicated that CRP is a good predictor of SBP with 71.0% (p = 0.003), closer to the ideal value of 1.0 and above the worst value of 0 , 5. Univariate logistic regression analysis showed that serum CRP is an independent risk factor for SBP, with a value> 6 mg / L and significantly increases the chance of SBP by seven times (Exp (B) = 7,319 (CI (2,130-25,151).

Several other studies have shown poor predictive power of this marker to detected SBP. Thus, Viallon A. and et al. [15] in their study analyzed several proteins from the acute phase of infection (procalcitonin, CRP, TNF-α and IL-6) in patients with SBP and concluded that CRP has a low sensitivity and specificity of 62/92% for detection of SBP with a sut-off of 80.0 mg / L of serum CRP, as opposed to the mean values presented in our study

A study by Bota et al. [20] also showed that there was a statistically insignificant difference in the concentrations of this marker with the severity of the disease calculated according to the Child-Pugh score. This protein in the acute phase of inflammation remains elevated even in the context of advanced liver failure, and its value reflects the degree of systemic inflammation, regardless of its cause. The CRP capacity to diagnose SBP is less relevant than the measurement of calprotectin in ascites.

In our study, a significantly increased value of CRP in ascites was recorded in patients with SBP (7.3. 7.2 mg / L versus 2.9 ± 3.1l mg / L, p = 0.001604).

A prospective study by Runyon B. A. [21] examined the diagnostic values of serum CRP and ascites in patients with SBP and non-SBP.

The analysis showed that the CRP values in ascites did not differ significantly between the two groups, but the serum CRP values were significantly higher in patients with peritonitis than in patients with sterile ascites associated with portal hypertension.

The author concluded that the determination of CRP in ascites is not a useful indicator of ascites fluid infection. It should be noted that this is a study with a small number of respondents with SBP, a total of 19.

A prospective study by Weil et al. [22] examined the correlation between the values of calprotectin in ascites and serum CRP in 119 hospitalized patients with cirrhosis and ascites and concluded that there was a positive correlation between them (r = 0.43, P <0.001). POCT was false negative in only one asymptomatic patient (calprotectin level was 0.45 μg / mL), but this patient had a high serum CRP (78 mg / L), which was a strong signal in favor of SBP.

The other four false negatives were "symptomatic" and standard cytology was performed to definitively rule out SBP. It is worth noting that the mean level of CRP (62.4 mg / L, range 20–89 mg / L) in false-negative cases did not differ significantly from true-positive cases (72.5 mg / L), but was higher than true negative (23.7 mg / L; P = 0.019) or false positive (37 mg / L; P = 0.24) cases.

This observation must encourage us to suspect SBP whenever POCT is negative but associated with a high level of CRP. This protein in the acute phase of inflammation remains elevated even in the context of advanced liver failure, and its value opens the degree of systemic inflammation, regardless of its cause, but the CRP's capacity to diagnose SBP was less relevant than the measurement of calprotectin in ascites in this study (AUC 0.83 vs. AUC 0.90 for calprotectin; P = 0.18).

Conclusion

Our research confirmed that serum CRP is a good predictor, significantly associated with the occurrence of SBP in patients with liver cirrhosis, with 71.0% (p = 0.003). Elevated serum CRP values> 6 mg / L significantly increase the chance of SBP by seven times (Exp (B) = 7,319 and CRP

in ascites> 6 mg / L significantly increase the chance of SBP by three times (Exp (B) Serum and ascites C-reactive protein did not show high values in patients with SBP, but still had a significant difference compared to patients with non-SBP.

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