Serum and ascitic procalcitonin as a marker for early diagnosis of spontaneous bacterial peritonitis

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Background and aim
of the work Spontaneous bacterial peritonitis (SBP) is a serious complication in patients with decompensated cirrhosis. SBP is an inflammation of the peritoneum by micro-organisms such as gram-negative bacilli. Early diagnosis of SBP is essential which may be a challenge for clinicians owing to lack of symptoms in early stage of SBP. The aim of this study is to evaluate procalcitonin (PCT) level in the serum and ascitic fluid of patients with cirrhosis for early diagnosis of SBP.
Patients and methods
This study was carried out on 45 patients with decompensated liver cirrhosis. They were classified into two groups: group 1 included 15 patients free of SBP and group 2 with SBP based on ascetic polymprphnuclear leucocytes (PNLs) more than 250/3 mm and ascitic fluid culture. Evaluation of C-reactive protein, ascetic fluid polymorphs count, and serum and ascetic PCT levels was done for all patients.
Results
No significant difference between SBP group and non-SBP group regarding ascetic PCT level, with *P* value more than 0.05. Serum PCT in patients with SBP shows high statistically significant difference, with *P* value less than 0.005, in comparison with patients without SBP.
Conclusion
Serum PCT is a good predictor marker for early diagnosis of SBP in patients with decompensated cirrhosis.

Keywords:
liver cirrhosis, serum and ascetic procalcitonin, spontaneous bacterial peritonitis

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Introduction

Liver cirrhosis is a serious and irreversible disease. It is a consequence of chronic liver disease characterized by replacement of liver tissue by fibrotic scar tissue as well as regenerative nodules, leading to progressive loss of liver function [1]. Cirrhosis is an increasing cause of morbidity and mortality in more developed countries. The main causes in more developed countries are infection with hepatitis C virus, alcohol misuse, and increasingly, nonalcoholic liver disease; infection with hepatitis B virus is the most common cause in sub-Saharan Africa and most parts of Asia [2]. Infection increases mortality in cirrhosis four times and has a poor prognosis, with 30% of patients dying within a month of infection and another 30% within a year [3]. Most frequently diagnosed infections are spontaneous bacterial peritonitis (SBP), urinary-tract infections, pneumonia, and skin infections; their incidences increase with worsening liver function [4]. SBP is the infection of the ascitic fluid that occurs in the absence of a visceral perforation and in the absence of an intra-abdominal inflammatory focus such as abscess, acute pancreatitis, or cholecystitis. For SBP diagnosis, the number of polymorphonuclear leukocytes (PMNLs) from the ascetic fluid obtained by paracentesis must exceed 250 cells/mm^3, and from bacteriological cultures, only one germ must be isolated [5,6]. As SBP in most cases is a mono-microbial infection, the presence of more micro-organisms in the culture (>1) must raise the suspicion of secondary peritonitis [7]. Clinical manifestations of SBP are nonspecific. The most frequently encountered symptoms and signs are fever (69%), abdominal pain (59%), signs of hepatic encephalopathy, abdominal tenderness (very rare), diarrhea, ileus, shock, and hypothermia. Approximately 10% of the patients with SBP are asymptomatic [7]. The SBP diagnosis is established by analysis of the ascetic fluid obtained at paracentesis. The main indications for paracentesis in a patient with hepatic cirrhosis include the following: unexplained clinical deterioration, the onset of complications (hepatic encephalopathy and gastrointestinal bleeding), and new-onset ascites in

hospitalized patients. Paracentesis should be avoided only in case of a suspicion of fibrinolysis or disseminating intravascular coagulopathy [8]. Therefore, new studies for early diagnosis, prevention, and treatment are needed to improve clinical outcomes. Many studies have shown that serum procalcitonin (PCT) is a sensitive biomarker that can be used to monitor bacterial infections, and measurements of PCT levels may guide the clinical use of antibiotics [9]. However, the diagnostic value of serum PCT levels in decompensated cirrhotic patients (DCPs) with infections [10], especially SBP, remains unclear, and several studies provide conflicting results [11–15]. The aim of this study was to assess the potential role of serum and ascetic PCT in diagnosis of SBP.

Patients and methods

The present experimental study was conducted at Gastroenterology Department of Menoufia University from June 2016 to December 2017 after obtaining Menoufia ethical committee approval. An oral and written consent from Each patient participated in the study already done according to the ethical committee of menoufia university hospital. A total of 45 patients were selected and divided to two groups. Fifteen patients with ascetic DCPs without SBP were included as a control group (group 1), comprising 13 female patients and two male patients, with mean age of 55.67±7.45 years. Thirty patients with ascetic DCPs with SBP were included as a case group (group 2), comprising 20 female patients and 10 male patients, with a mean age of 58.03±8.97 years. All patients were subjected to history taking; full general and local examination; laboratory investigation, including complete blood count (CBC), liver function tests, kidney function tests, C-reactive protein (CRP), total leukocytic count (TLC) of ascetic fluid, and serum and ascetic PCT level by enzyme-linked immunosorbent assay; and abdominal ultrasound.

Overall, 5 ml of venous blood was withdrawn from every patient by sterile vein-puncture and divided into two tubes, where 2 ml of blood was transferred into an EDTA tube for determination of CBC [16], and 3 ml of blood was transferred into a plain tube and allowed to clot at 37°C, centrifuged for 10 min at 4000 rpm, and stored at −80°C for serum determination of liver function tests [17], kidney function tests [18], CRP [19], and serum PCT. Moreover, 2 ml of ascetic fluid was taken and centrifuged to remove precipitate, and store at −80°C until determination of ascetic Procalcitonin (PCL). Serum PCT and ascetic PCL were measured using the PCT enzyme-linked immunosorbent assay Kit for the in-vitro determination of PCT in serum and body fluid (Chongqing Biospes Co. Ltd, Chongqing city, China; www.biospes.com).

Statistical analysis

Statistical analysis of the present study was carried out using statistical package for the social sciences, version 20 (SPSS Inc., California, USA). Data were divided into two phases: a descriptive and an analytical study including the *t* test, the Mann–Whitney test, and Fisher's exact test. *P* value was set to be significantly different if less than or equal to 0.05.

Results

Our result shows highly significant difference in bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and international normalization ratio (INR), and there is no significant difference in CBC, urea, creatinine, and albumin, as in Table 1. There was a highly significant increase in mean value of serum PCT, CRP, and PMNLs of ascetic fluid between two groups, whereas there was no significant difference of mean value of ascetic PCT between two groups, as shown in Table 2. There was a highly significant positive correlation between creatinine, bilirubin, CRP, and serum PCT, as shown in Table 3. According to area under the curve (AUC)- receiver operating characteristic (ROC) curve, the serum PCT level shows AUC of 67.8, with *P* value of 0.045 and 95% confidence interval of 0.524–0.832, which means good predictor for SBP, with sensitivity of 67% and specificity of 46% at cutoff point of 0.382 ng/ml, as shown in Table 4 and Fig. 1. According to AUC-ROC curve, the ascetic PCT shows AUC of 58.9, *P* value of 0.336, 95% confidence interval of 0.420–0.758, which means moderate predictor of SBP with sensitivity of 67% and specificity of 40% at a cutoff point of 320 pg/ml (0.320 ng/ml), as shown in Table 5 and Fig. 2.

Discussion

This work was carried out to evaluate the role of PCT in diagnosis of patients with SBP.

In the term of hemoglobin, platelets, and TLC, there was an insignificant difference between patients with SBP and patients without SBP, which was in agreement with Magdalena *et al.* [20]. However, Le-Yong *et al.* [21] reported significant increase in TLC in patients with SBP, and this may be owing to the pathogenesis of abnormal hematological indices in cirrhosis, which is multifactorial and includes portal

Table 1 Laboratory finding in complete blood count, liver function test, and kidney function test between case and control groups

	CBC	Control LC (*N*=15) (mean±SD)	Study LC+SBP (*N*=30) (mean±SD)	*t* test	*P* value
CBC	Hemoglobin (g/dl)	9.83±1.23	10.61±2.25	1.249	0.218 (NS)
	WBCS×10^3/μl	8.25±4.46	12.23±11.65	1.272	0.210 (NS)
	PLT×10^3/μl	96.27±38.28	120.96±110.72	835	0.408 (NS)
Liver function test	Bilirubin total (mg/dl)	2.91±3.83	5.09±4.48	1.607	0.015 (HS)
	Bilirubin direct (mg/dl)	1.52±2.50	3.66±3.95	1.904	0.033 (HS)
	Bilirubin indirect (mg/dl)	1.37±1.38	1.43±0.93	165	0.870 (NS)
	Prothrombin concentration %	71.16±19.57	62.39±17.22	1.540	0.131 (NS)
	INR	1.46±0.26	1.74±0.55	1.867	0.025 (HS)
	ALT (U/l)	33.67±15.40	74.33±48.39	3.160	0.003 (HS)
	AST (U/l)	52.67±33.22	90.47±64.81	2.116	0.040 (HS)
	Albumin (g/dl)	2.86±0.50	2.56±0.52	1.857	0.070 (NS)
Kidney function	Urea (mg/dl)	93.67±50.90	96.50±74.45	132	0.895 (NS)
	Creatinine (mg/dl)	1.96±1.13	1.87±1.36	0.200	0.843 (NS)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBC, complete blood count; HS, highly significant; INR, international normalization ratio; LC, liver cirrhosis; PLT, platelet; SBP, spontaneous bacterial peritonitis; WBC, white blood cell. *P* value of *t* test.

Table 2 Polymorphonuclear leukocytes in ascetic fluid, C-reactive protein, and procalcitonin level in serum and ascetic fluid between the two groups

Groups	Control LC (*N*=15) (mean±SD)	IQR	Study LC+SBP (*N*=30) (mean±SD)	IQR	*t* test	*P* value
PMNLs in ascetic fluid	31.33±18.75	30	4365.03±8705.7	2245	1.9	0.04 (HS)
CRP (mg/l)	7.20±9.41	12.00	94.67±83.81	113.02	4.007	0.000 (HS)
Serum procalcitonin (pg/ml)		658.00		3113.90	2.633	0.012 (HS)
Mean±SD	659.00±610.01		1846.48±1684.31			
Minimum–maximum	–		30–5301.7			
Ascetic procalcitonin (pg/ml)						
Mean±SD	480.47±346.28	630.00	1909.56±5615.45	991.25	979	0.333 (NS)
Minimum–maximum	–		32–31 179.1			

CRP, C-reactive protein; HS, highly significant; IQR, interquartile range; LC, liver cirrhosis; PMNL, polymorphonuclear leukocyte; SBP, spontaneous bacterial peritonitis.

Table 3 Correlation between laboratory data finding and procalcitonin level in serum and ascites

	Serum procalcitonin		Ascetic procalcitonin	
	r	*P* value	*r*	*P* value
Hb (g/dl)	–0.196	0.299 (NS)	–0.018	0.924 (NS)
WBCs×10^3/μl	–0.087	0.648 (NS)	–0.120	0.529 (NS)
PLT×10^3/μl	0.028	0.883 (NS)	–0.100	0.598 (NS)
ALT (U/l)	0.081	0.669 (NS)	–0.144	0.447 (NS)
AST (U/l)	0.191	0.312(NS)	–0.155	0.414(NS)
Urea (mg/dl)	0.33	0.075 (NS)	0.045	0.812 (NS)
Creatinine (mg/dl)	0.472	0.008 (HS)	0.053	0.78 (NS)
Bilirubin total (mg/dl)	0.433	0.003 (HS)	–0.123	0.518 (NS)
Bilirubin direct (mg/dl)	0. 541	0.015 (HS)	–0.124	0.512 (NS)
Bilirubin indirect (mg/dl)	–0.073	0.7 (NS)	–0.063	0.743 (NS)
CRP (mg/l)	–0. 451	0.002 (HS)	–0.144	0.448(NS)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; Hb, hemoglobin; HS, highly significant; PLT, platelet; WBC, white blood cell.

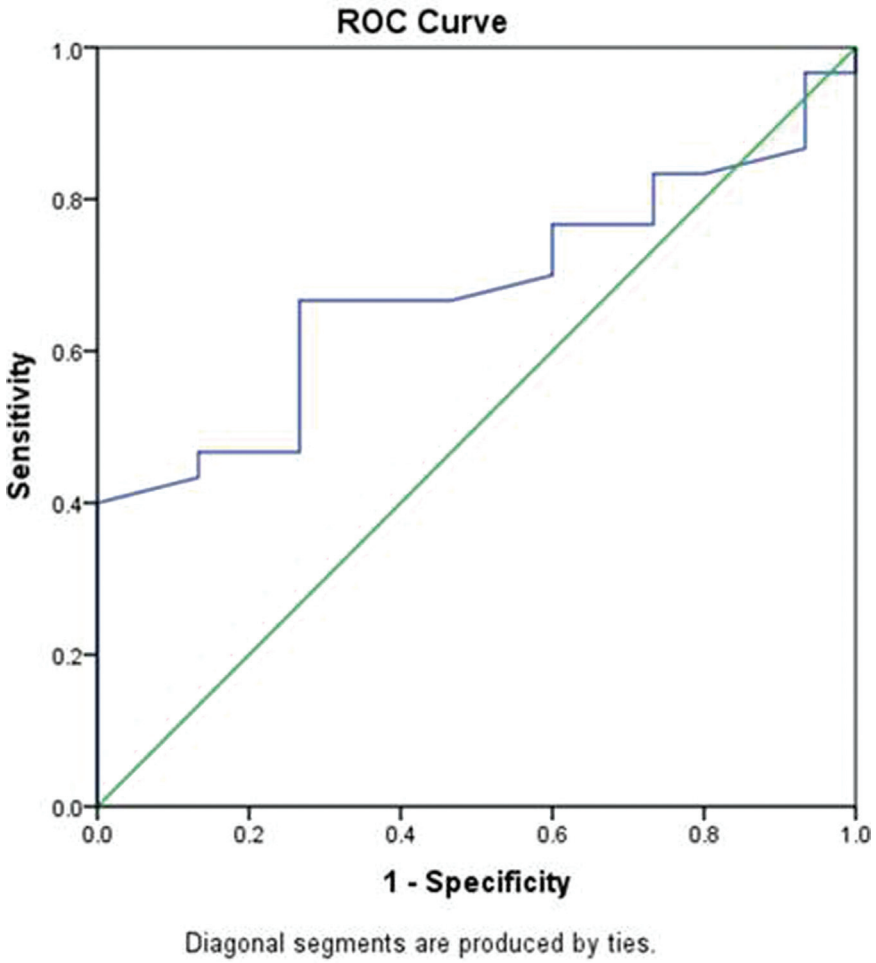
hypertension-induced sequestration, alterations in bone marrow stimulating factors, and viral-induced and toxin-induced bone marrow suppression and consumption or loss [22]. Regarding ALT and AST, there was a significant increase in mean value in patients with SBP more than patients without SBP between the two groups, which was in agreement with Viallon *et al.* [10]; moreover, Magdalena *et al.* [20] reported significant increase in ALT and AST in patients with SBP, which is owing to both aminotransferases are highly concentrated in the liver and injury to the liver, whether acute or chronic,

Table 4 Procalcitonin in serum

AUC	SE	*P* value	Cutoff point	Sensitivity	Specificity	Asymptotic 95% confidence interval	
						Lower bound	Upper bound
67.8	0.079	0.045 (HS)	382.5	67%	46%	0.524	0.832

AUC, area under the curve; HS, highly significant.

Figure 1



The ROC of serum procalcitonin regarding diagnosis of SBP. ROC, receiver operating characteristic; SBP, spontaneous bacterial peritonitis.

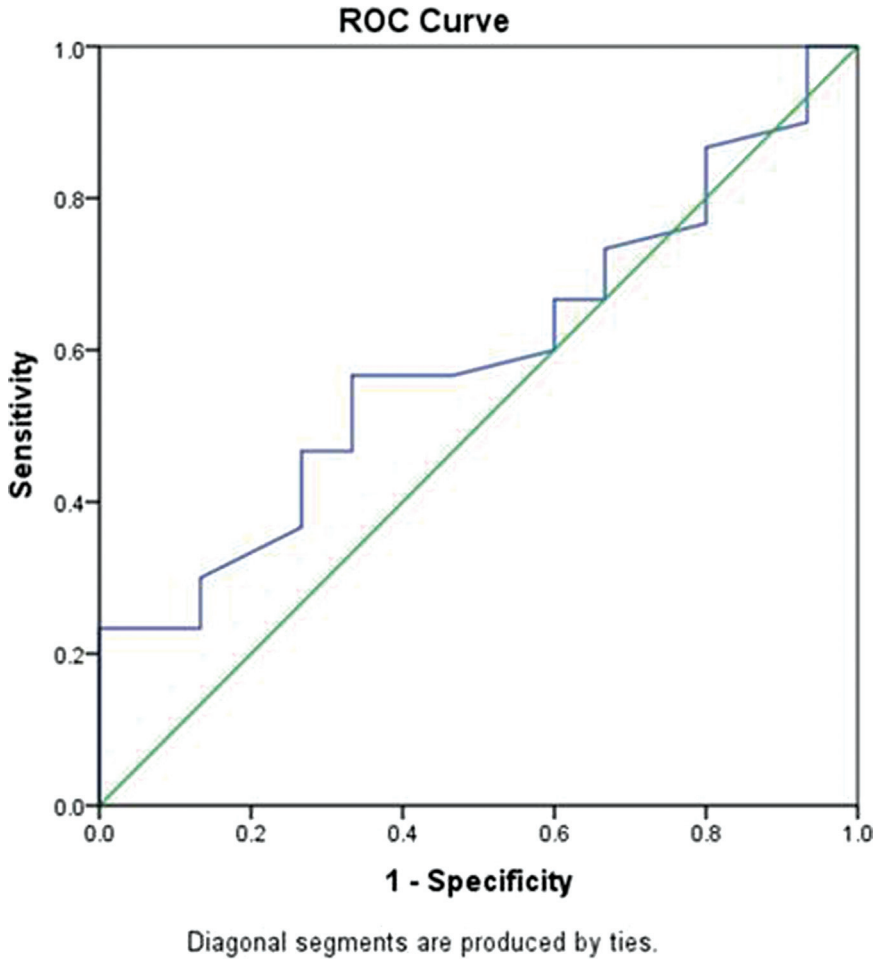
eventually resulting in an increase in serum concentrations of aminotransferases [23]. However, total and direct bilirubin levels show highly significant difference between the two groups; in contrast, indirect bilirubin shows insignificant difference, which is in agreement with Le-Yong *et al.* [21] who show increase in level of bilirubin in patients with SBP, and is in disagreement with Magdalena *et al.* [20]. On the contrary, regarding prothrombin concentration and INR, there was a significant increase in level of INR in SBP group and a nonsignificant difference in mean value of prothrombin concentration, which was in agreement with Viallon *et al.* [10] and in disagreement with Magdalena *et al.* [20] In our study, there was no statistically significant difference in serum albumin between our two groups. It is well known that low concentration of ascetic albumin is associated with increased rate of SBP regarding etiology and pathogenesis of SBP owing to low opsonic activity of ascetic fluid and predisposing more to bacterial infection. We studied PCL as predictor for early diagnosis of SBP, so we stressed its correlation with others predictors of SBP like ascetic PMNL count and CRP. There was no significant difference regarding serum albumin, creatinine, and blood urea between our two groups, which is in agreement with Magdalena *et al.* [20], who reported same findings and disagreement with Viallon *et al.* [10]. The difference can be assumed to the number of patients enrolled in each groups. In terms of PMNL and CRP, there is a highly significant increase between two groups, which is in agreement with Abd el Razik *et al.* [24] and

Table 5 Ascetic procalcitonin

AUC	SE	*P* value	Cutoff point	Sensitivity	Specificity	Asymptotic 95% confidence interval	
						Lower bound	Upper bound
58.9	0.086	0.336 (NS)	320	67	40	0.420	0.758

AUC, area under the curve.

Figure 2



The ROC of ascetic procalcitonin regarding diagnosis of SBP. ROC, receiver operating characteristic; SBP, spontaneous bacterial peritonitis

Hamed *et al.* [25]. On the contrary, Magdalena *et al.* [20] reported that there was insignificant difference of CRP between two groups, which can be explained by that CRP is well known but nonspecific systemic inflammatory biomarker synthesized in acute phase of inflammation.

The basic level of CRP in patients with cirrhosis is higher than in patients without cirrhosis, but when infection occurs, the more serious the potential liver dysfunction, the lower the increase in CRP [23]. Therefore, the predictive power of CRP for infection is weak in patients with advanced cirrhosis reported by Janum *et al.* [26] as the diagnosis of SBP is proved in the presence of an elevated ascetic fluid absolute PMNL count (i.e. ≥250 cells/mm^3, 0.25/l), without an evident intra-abdominal, surgically treatable source of infection.

Regarding serum and ascetic PCT, there is highly significant increase of mean value of serum PCT in SBP group, whereas there was no significant difference in level of ascetic PCT between two groups, which explained that PCT seemed to be a marker of infection. It increased during sepsis and correlated with the severity of sepsis. The mechanism, site of production, and clearance of PCT remain unknown, and also, there is no intraperitoneal synthesis. Viallon *et al.* [10] also reported no significant difference between patients with SBP and patients without

SBP regarding ascetic PCT, which means its poor predictor utility for SBP.

This current study shows significant correlation between serum PCT and serum CRP level (r=0.451 and P=0.002), total bilirubin (r=0.43 and P=0.003), direct bilirubin (r=0.54 and P=0.015) and creatinine level (r=0.472 and P=0.008), whereas there is insignificant correlation between ascetic PCL level and other laboratory finding such as CBC and other liver function test, which is in agreement with Le-Yong *et al.* [21] and Hamed *et al.* [25] who reported significant correlation between CRP and serum PCL level.According to AUC-ROC curve, the serum PCL level show AUC 67.8 and P value 0.045 and 95% confidence interval of 0.524–0.832, which means good predictor for SBP, with sensitivity of 67% and specificity of 46% at a cutoff point of 0.382 ng/ml.

Ascetic PCL AUC-ROC curve shows AUC of 58.9, P value of 0.336, and 95% confidence interval of 0.420–0.758, which means moderate predictor of SBP, with sensitivity of 67% and specificity of 40% at cutoff point of 320 pg/ml=0.320 ng/ml, and this is agreement with Hamed *et al.* [25] who indicate the importance of serum PCT as an early predictor of SBP. However, Wu *et al.* [27] found the optimal cutoff value for PCT was 0.78 ng/ml, with sensitivity of 77.5% and specificity of 60.4% (AUC: 0706, CI 95%: 0.576-0.798, p<0.01 confidence interval). This difference may be owing to different localities and samples volume. Moreover, close to our findings, Le-Yong *et al.* [21] stated the optimal cutoff value for PCT was 0.48 ng/ml, with sensitivity and specificity were 95 and 79%, respectively, in patients with chronic sever hepatitis B with SBP, and the variance in sensitivity and specificity may be owing to the different sample volumes.

In conclusion, serum PCT is a good predictor marker for early diagnosis of SBP in patients with LCF, whereas ascetic PCT is a poor diagnostic marker of SBP.

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Conflicts of interest
There are no conflicts of interest.

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