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Review Article

Ascitic Fluid Calprotectin as an Accurate Diagnostic Marker for Spontaneous Bacterial Peritonitis

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Abstract

Background: Calprotectin is an acute-phase inflammatory protein secreted by polymorphonuclear leucocytes (PMNL). It is frequently employed in clinical practice for the diagnosis and monitoring of inflammatory bowel diseases. The objective of this work was to assess the usage of calprotectin that found in ascitic fluid as a diagnostic indicator for spontaneous bacterial peritonitis (SBP).

Methods: This cross-sectional laboratory-based work was conducted on 50 individuals, diagnosed with cirrhotic-induced ascites with no laboratory or clinical findings of SBP for group 1 and diagnosed with cirrhotic-induced ascites with SBP for group 2. SBP was identified depending on a positive bacterial ascitic fluid culture, an elevated count of PMNLs in the ascites (>250 cells/mm3), and the lack of any infection originating from inside the abdomen.

Results: Protein, serum ascites albumin gradient (SAAG), WBCs, PMNL, calprotectin, color of ascitic fluid, number of individuals with yellow, whitish color ascitic fluid, culture, number of patients with klebsiella, pseudomonas, E-coli, streptococcus, total leukocytic count (TLC), Alanine transaminase (ALT), International normalized ratio (INR), and C-reactive protein (CRP)had been substantially elevated in the SBP group contrasted to the group without SBP ($P < 0.001$). Platelets had been substantially reduced in the SBP group contrasted to non-SBP group (P-value = 0.013). The cut off point value of calprotectin 5.045 showed 89% sensitivity and 86% specificity (P-value <0.001). In multilinear regression, PMNL, and calprotectin were significant predictors of SBP ($P \leq 0.05$).

Conclusions: The levels of ascitic calprotectin were markedly raised in the group with SBP contrasted to the group without SBP. Furthermore, there was a strong association between ascitic calprotectin levels and the established diagnostic criterion for SBP, which is the presence of PMNLs of 250 cells/mm3 or higher. Consequently, it may serve as a dependable and satisfactory diagnostic indicator for accurately identifying SBP. Furthermore, there is a direct correlation between the amount of ascitic calprotectin and the severity of liver damage.

Keywords: Spontaneous Bacterial Peritonitis, Ascitic Fluid, Calprotectin, Cirrhosis, Marker

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Introduction:

Spontaneous bacterial peritonitis (SBP) refers to the infection of the fluid that accumulates in the abdomen of individuals with cirrhosis of the liver and portal hypertension. No apparent surgical etiology as a perforation or an abscess or other intra-abdominal inflammatory focus was existed. Approximately 30% of individuals with ascites may develop SBP. [1]

SBP is caused by immune system malfunction, the movement of bacteria from the gut into the bloodstream, problems with blood circulation, and an inflammatory state. The diagnosis of SBP is confirmed by the examination of ascitic fluid. [2]

SBP was characterized as an ascitic fluid PMNL more than 250/mm3. [3] Not every instance is correlated with positive cultures of ascitic fluid.

There are many types of infections in ascitic fluid, including monomicrobial non-neutrocytic bacterascites, culture-negative neutrocyte ascites, polymicrobial bacterascites, and 2ry bacterial peritonitis.[4]

The advent of the SBP is associated with a poor prognosis, with hospital death rates ranging from 10 to 50%. Therefore, it is essential to evaluate every patient with SBP for liver transplantation. It is necessary to promptly begin therapy with antibiotics and intravenous (IV) albumin. **[3]**

Research was carried out to investigate other methods for measuring the ascitic PMNL count, including serum procalcitonin, high sensitivity C-reactive protein (hsCRP), urine lipocalin, homocysteine, ascitic lactoferrin, and ascitic or fecal calprotectin.[5]

Analysis ascitic fluid with detection of the PMNL is the definitive diagnostic test for SBP. It has a practical application in the identification and ongoing assessment of medical interventions. If the ascitic fluid culture was performed accurately, it may provide an effective outcome. **[6]**

A type of SBP exists, defined as culture-negative neutrocytic ascites. It is distinguished by an increased presence of PMNL in the ascitic fluid, yet the culture yields no evidence of microorganisms. It is administered in the same manner as classical SBP. These instances would go unnoticed if cultures hadn't been carried out. [4]

The process of manually counting PMNLs is characterized by being time-consuming, requiring a significant amount of effort, and necessitating a certain level of expertise in order to minimize discrepancies between different observers and within the same observer. Therefore, a straightforward and efficient bedside diagnostic test would have clinical value. [7]

Calprotectin is an inflammatory protein that is secreted by the PMNL during the acute-phase response. Calprotectin has both anti-microbial and anti-proliferative characteristics. Calprotectin is commonly employed in clinical practice for the diagnosis and therapeutic observation of inflammatory bowel diseases. [7]

The purpose of this work was to assess the value of ascitic calprotectin as a diagnostic indicator of SBP.

Patients and Methods:

This cross-sectional laboratory-based work was performed on 50 individuals, diagnosed with cirrhosis-induced ascites with no laboratory or clinical findings of SBP for group 1 and diagnosed with cirrhotic-induced ascites with SBP for group 2. The identification of SBP was determined by a positive bacterial culture of the ascitic fluid, an elevated count of PMNLs in the ascites (>250 cells/mm3), and the absence of any infection originating from inside the abdomen. The work was performed with clearance from the Ethical Committee of Sohag University Hospitals, Sohag, Egypt. The patients provided their informed written permission.

The criteria for exclusion included patients with cirrhotic conditions, both with and without SBP, who had received antibiotics within the past week. Additionally, patients who had undergone abdominal surgery within the last three months, had abdominal malignancies (including hepatocellular carcinoma, colorectal carcinoma, cancer of stomach, cancer of pancreas, or cholangiocarcinoma), had intra-abdominal infected damage (such as appendicitis, abscess, cholecystitis, or pancreatitis), had a history of inflammatory bowel diseases (which might be as ulcerative colitis or Crohn's disease), or had heart failure (HF), hematological disorders, or autoimmune diseases were also excluded.

All patients had been exposed to comprehensive taking of history, clinical assessment, abdominal ultrasound (To evaluate the quantity of ascites, dimensions of the spleen and liver, echogenicity of the liver, and presence of localized lesions) and lab tests [Full blood picture, international normalized ratio (INR) and prothrombin time (PT), liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (Alb), total proteins (TP), and total and direct bilirubin, kidney function tests (uric acid, creatinine, and urea), C- reactive protein (CRP)] and bacterial examination of ascitic fluid

Diagnostic abdominal paracentesis:
This work was carried out on ascitic individuals with cirrhosis at admission, who subsequently exhibited manifestations of SBP throughout their hospital stay, including fever, abdominal discomfort, and alterations in motility of GIT (including diarrhea, vomiting, or ileus). A sample of ascitic fluid, measuring 20 mL, was collected using paracentesis. Subsequently, 10 mL of the sample was introduced into aerobic blood culture bottles (Egyptian Diagnostic Media, Cairo, Egypt) at the patient's bedside. A portion of the material was promptly sent to the laboratory for the purpose of conducting an analysis on the differential leukocyte counts (PMNLs) as well as the red blood cells.

Isolation of bacteria:
The specimens had been preserved at 37°C for 24-48 hours on blood agar, nutrition agar, and MacConkey agar. The pH was set to 7.4 and all media received sterilization by autoclaving at a temperature of 121°C for a duration of 20 minutes unless otherwise was mentioned .[8]

Macroscopic appearance of colonies: MacConkey can differentiate the enteric bacteria into lactose fermenter and non-fermenter, while blood agar can differentiate organisms according to the type of hemolysis. [10]

Microscopic examination of Gram-stained colonies: Examination of unstained preparation (wet mount) helped in demonstrating motility, while examination of Gram-stained preparation detected the staining reaction of the organism; whether gram positive or negative, their morphology (cocci, bacilli, etc.), size and arrangement [11], The VITEK2 Compact identification kits are utilized to validate the identification of the isolates and conduct antibiotic susceptibility testing. [12]

Evaluation of calprotectin of ascitic fluid:
To evaluate calprotectin, The specimen was obtained from every patient and preserved at a temperature of -20 C until the assessment of calprotectin, that is quantified utilizing the Human Calprotectin Enzyme-linked immunosorbent assay (ELISA) kit, Bioassay Technology Laboratory, China. Thermo Multiscan EX device, Singapore used for calprotectin ELISA kit.

Reagent preparation:
Prior to use, it is necessary to ensure that all reagents are equilibrated to room temperature. To create a stock solution of the standard at a concentration of 320ng/ml, mix 120µl of the standard (640ng/ml) with 120µl of standard diluent. Before producing dilutions, it is recommended to let the standard rest for 15 minutes while gently stirring. Create duplicate standard points by sequentially diluting the standard stock solution (320ng/ml) at a 1:2 ratio with the standard diluent. This will produce solutions with concentrations of 160ng/ml, 80ng/ml, 40ng/ml, and 20ng/ml. The conventional diluent functions as the baseline standard with a concentration of 0 ng/ml. The residual solution should be stored at a temperature of -20 ºC and must be utilized within a period of one month. Standard solution dilution. Cleansing solution Prepare a solution by adding 20ml of Wash Buffer to a larger volume of solvent to reduce its concentration. Concentrate 25 times in deionized or distilled water to get 500ml of 1x Wash Buffer. If crystals have precipitated in the concentrate, agitate the mixture delicately until the crystals have fully dissolved.

Statistical analysis
The statistical analysis was conducted utilizing SPSS v26 software (IBM Inc., Chicago, IL, USA). The normality of the data distribution had been evaluated utilizing the Shapiro-Wilks test and histograms. The mean and standard deviation (SD) of quantitative parametric parameters had been reported and contrasted among both groups utilising an unpaired Student's t-test. The quantitative non-parametric data had been reported utilising the median and interquartile range (IQR) and were assessed utilising the Mann Whitney-test. The qualitative parameters were expressed as frequencies and percentages (%) and were evaluated using the Chi-square test or Fisher's exact test, as appropriate. The area under the curve (AUC) evaluates the overall test performance. Multivariate regression was also utilised to determine the relationship among a dependent parameter and more independent parameters. A two-tailed P value < 0.05 was deemed statistically significant.

Results:

Regarding age, gender, RBCs, MCV, MCH, MCHC, HB, AST, Albumin, TP, bilirubin, creatinine, and urea, there was insignificant variation among both groups under the study. TLC, ALT, INR, and CRP were significantly raised in serum in SBP group contrasted to non-SBP group (P-value < 0.05), while platelets had been substantially reduced in SBP group contrasted to non-SBP group (P-value = 0.013). **Table 1**

Table 1: Demographic data and routine laboratory test of the studied patients

		SBP (n = 25)	Non SBP (n = 25)	p
Age		61.68 ± 8.25	58.2 ± 8.83	0.157[(b)]
Sex	**Male**	12 (48.0 %)	13 (52.0 %)	0.777[(a)]
	Female	13 (52.0 %)	12 (48.0 %)	
Routine Laboratory Test				
TLC (cells/mm^3)		13.02 (5.89)	5.81 (5.29)	**<0.001*[(a)]**
RBCs		3.36 (1.09)	3.63 (1.68)	0.838[(a)]
MCV		81.4(14.2)	81.6 (18.6)	0.675[(a)]
MCH		27.6 (5.55)	27.4 (7.55)	0.734[(b)]
MCHC		33.9 (3.8)	33 (4.1)	0.079[(a)]
HB		8.5 (3.29)	7.3 (4)	0.542[(a)]
Platelets		77 (34)	107 (61)	**0.013*[(b)]**
ALT		73 (40)	52 (35)	**0.008*[(b)]**
AST		63 (39)	58 (33)	0.132[(b)]
Albumin		2.8 (0.44)	2.68 (0.7)	0.245[(b)]
TP		5.5 (0.75)	5.8 (0.95)	0.188[(a)]
Bilirubin		2.6 (1.47)	1.96 (1.46)	0.086[(b)]
Creatinine		1.54 (1.11)	1.22 (0.74)	0.573[(b)]
Urea		54 (40)	73 (55)	0.277[(b)]
INR		2.48 (1.14)	1.62 (0.5)	**<0.001*[(b)]**
CRP		48 (72)	6 (6)	**<0.001*[(b)]**

Data are presented as mean ± SD or frequency (%) or median (IQR). *Significant p value <0.05, (a) Chi square test, (b) Independent t test, SBP: Spontaneous bacterial peritonitis, RBCs: red blood cells, TLC: Total Leukocyte Count, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, Hb: hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, AST: Aspartate aminotransferase, ALT: Alanine transaminase, TP: Total protein, CRP: C-reactive protein, INR: International normalized ratio.

Regarding color of ascitic fluid, number of patients with yellow, whitish color ascitic fluid, culture, number of patients with klebsiella, pseudomonas, E-coli and streptococcus were significantly greater in SBP group contrasted to non-SBP group (P-value < 0.001). Regarding aspect of ascitic fluid, all SBP patients had turbid ascitic fluid (P-value < 0.001). All non-SBP patients had negative culture. **Table 2**

Table 2: Microbiological aspects of the ascitic fluid of the studied patients

		SBP (n = 25)	Non SBP (n = 25)	P
Color of ascitic fluid	**Pale yellow**	0 (0.0 %)	25 (100.0 %)	**<0.001*[(a)]**
	Yellow	16 (64.0 %)	0 (0.0 %)	
	Whitish	9 (36.0 %)	0 (0.0 %)	
Aspect of ascitic fluid	**Clear**	0 (0.0 %)	25 (100.0 %)	**<0.001*[(b)]**
	Turbid	25 (100.0 %)	0 (0.0 %)	
Culture	**Negative**	0 (0.0 %)	25 (100.0 %)	**<0.001*[(a)]**
	Gram negative culture	20(80.0%)	0(0.0%)	
	Klebsiella	4 (16.0 %)	0 (0.0 %)	
	Pseudomonas	2 (8.0 %)	0 (0.0 %)	
	E coli	14 (56.0 %)	0 (0.0%)	
	Gram positive culture	5(20.0%)	0(0.0%)	
	Streptococcus	5 (20.0 %)	0 (0.0 %)	

Data are presented as frequency (%), *significant p value <0.05, (a) Chi square test, (b) Independent t test, SBP: Spontaneous bacterial peritonitis.

Protein, SAAG, WBCs, PMNL and calprotectin had been substantially elevated in SBP group contrasted to non-SBP group (P < 0.001). Glucose and RBCs had insignificantly different among both studied groups. **Table 3**

Table 3: Laboratory findings of ascitic fluid among the studied patients

	SBP (n = 25)	Non SBP (n = 25)	P
Protein	1.7 (0.4)	0.9 (0.5)	**<0.001*[(b)]**
Glucose	75 (27)	87 (34)	0.631[(a)]
SAAG	1.7 (0.3)	1.4 (0.2)	**0.001*[(b)]**
WBCs	1680 (4985)	60 (40)	**<0.001*[(b)]**
PMNL	90% (5%)	10% (10%)	**<0.001*[(b)]**
RBCs	2 (4)	2 (4)	0.838[(a)]
Calprotectin	8 (1.96)	2.25 (1)	**<0.001*[(b)]**

Data are presented as median (IQR), *significant p value <0.05, (a) Chi square test, (b) Independent t test, SAAG: Serum ascites albumin gradient, SBP: Spontaneous bacterial peritonitis, RBCs: Red blood corpuscles, WBCs: White blood cells, PMNL: Polymorphonuclear Leukocytes White Blood Cells.

In multilinear regression, $PMNL_s$ and calprotectin were significant predictors of SBP ($P \leq 0.05$). Other parameters (platelet, ALT, protein, SAAG, WBCs, INR, and CRP) were insignificant predictors for SBP. **Table 4**

Table 4: Multilinear regression of significant predictor factors for prediction of spontaneous bacterial peritonitis

	B	95% CI		B	t	P
Platelet	0.00099	000	000	0.020	1.266	0.213
ALT	0.000	-0.001	0.000	-0.024	-1.341	0.188
Protein	0.032	-0.014	0.078	0.029	1.399	0.169
SAAG	0.044	-0.025	0.113	0.022	1.281	0.208
WBCs	-0.00011	000	000	-0.032	-0.813	0.421
PMNL	0.011	0.010	0.012	0.829	16.580	**<0.001***
Calprotectin	-0.028	0.009	0.048	0.184	2.883	**0.006***
INR	0.007	-0.035	0.022	-0.010	-0.463	0.646
CRP	-0.000033	-0.001	0.000	-0.003	-0.141	0.889

Data are presented as numbers, *significant p value <0.05, R2 ad = 0.99 (N=50, p=0.000*), CI= Confidence interval for B, INR: International normalized ratio, ALT: Alanine transaminase, CRP: C-reactive protein, SAAG: Serum ascites albumin gradient, RBCs: Red blood corpuscles, WBCs: White blood cells, PMNL: Polymorphonuclear Leukocytes White Blood Cells.

Calprotectin can significantly diagnose SBP with AUC of 0.937, at cut off point value of 5.045, with 89% sensitivity and 86% specificity (P-value <0.001). **Figure 1**

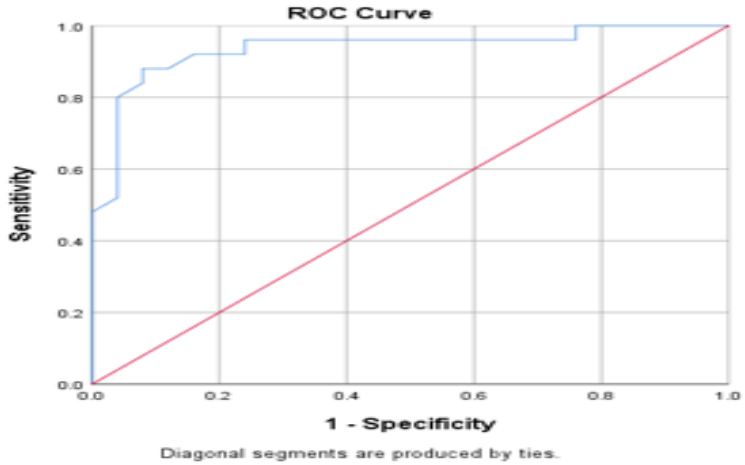

Figure 1: Sensitivity and specificity of ascitic fluid calprotectin as a diagnostic marker for spontaneous bacterial peritonitis

Discussion

Calprotectin is a protein that binds to calcium and zinc. It is mostly found in neutrophils and its levels in bodily fluids are directly related to the number of neutrophils present. [7]

The calprotectin in the ascitic fluid is a dependable indicator for predicting the existence of PMNLs count over 250/mm3. This may be a valuable tool in diagnosing SBP, particularly when using a conveniently accessible bedside testing instrument. [14]

In the present study, it was found that TLC, ALT, INR, and CRP were substantially raised in serum in SBP group contrasted to the group without SBP (P-value < 0.05), while platelets were substantially reduced in SBP group contrasted to the group without SBP (P-value = 0.013). In agreement with our findings, Rasmy et al.[15] highlighted that TLC, ALT, INR and CRP were substantially raised in serum in SBP group contrasted to the group without SBP (P-value < 0.05), while platelets were substantially reduced in SBP group contrasted to the group without SBP (P< 0.005). In agreement with our findings, Abd Ellatif Afifi et al[16] revealed that TLC, ALT, INR and CRP were substantially raised in serum in SBP group contrasted to the group without SBP (P-value < 0.05), while platelets were substantially reduced in SBP group contrasted to the group without SBP. Moreover, Ali and Mohamed. [17] showed that TLC, ALT, INR, and CRP were substantially raised in serum in SBP group contrasted to the group without SBP (P-value < 0.05), while platelets were substantially reduced in SBP group contrasted to the group without SBP.

In the present study, it was found that (HB, AST, Albumin, TP, bilirubin, creatinine, and urea) were insignificantly different among both studied groups. In agreement with our findings, Abd Ellatif Afifi et al. [16] highlighted that no substantial variation was existed among SBP and non SBP group as regard (HB, AST, Albumin, TP, bilirubin creatinine, and urea). In line with our findings, Rasmy et al. [15] highlighted that (HB, AST, Albumin, TP, bilirubin, creatinine, and urea) were insignificantly different between both studied groups. Parallel to our results, Abdel Rahman et al. [18] noted that no substantial variation was existed among SBP group and the group without SBP regarding (HB, AST, Albumin, TP, bilirubin, and creatinine).

In the present study, it was found that number of patients with klebsiella, pseudomonas, E-coli and streptococcus were substantially greater in SBP group contrasted to non-SBP group (P-value < 0.001). All non-SBP patients had negative culture. Parallel to our results, Rasmy et al. [15] highlighted that The positive culture cases consisted of the following: 13 (72.2%) cases of E coli, 2 (11.1%) cases of Streptococcus pneumoniae, 1 (5.5%) case of pseudomonas species, and 1 (5.5%) case each of Staphylococcus species and Enterococcus species. In agreement with our results, Ali and Mohamed. [17] highlighted that number of patients E-coli was substantially greater in SBP group contrasted to the group without SBP (P < 0.001). All non-SBP patients had negative culture. Moreover, Selim et al. [19] highlighted that the culture findings across the participants in the work of with SBP revealed the presence of Escherichia coli (12 cases), then Streptococcus viridans (6), Klebsiella pneumoniae (2), and Staphylococcus aureus (2) in the SBP group (P <0.001). All non-SBP patients had negative culture.

In the present study, it was found that protein, SAAG, WBCs, differential and calprotectin were substantially raised in SBP group contrasted to the group without SBP (P-value < 0.001). However, Glucose and RBCs were insignificantly different between both studied groups. In agreement with our findings, Abd Ellatif Afifi et al. [16] highlighted that calprotectin was substantially greater in SBP group contrasted to the group without SBP. In agreement with our results, Rasmy et al. [15] highlighted that calprotectin were substantially elevated in SBP group contrasted to the group without SBP (P-value < 0.001). Also, Honar et al. [20] revealed that calprotectin was substantially raised (P-value < 0.001). Also, protein was substantially elevated (P-value < 0.001). However, Glucose and RBCs were insignificantly different between both studied groups. Furthermore, Ali and Mohamed [17] highlighted that that calprotectin was substantially elevated in SBP group contrasted to the group without SBP (P-value < 0.001). Also, protein, WBCs and differential leucocytes had been substantially raised in SBP group contrasted to the group without SBP (P-value < 0.001). However,

Glucose and RBCs were insignificantly different between both studied groups. In line with our results, Heikl et al. [21] highlighted that calprotectin was substantially high in SBP group contrasted to the group without SBP (P value < 0.001).

In the present study, it was found that calprotectin can significantly diagnose SBP with AUC of 0.937, at cut off point value of 5.045, with 89% sensitivity and 86% specificity (P <0.001).

In accordance with our results, Patel et al. [22] showed that The overall sensitivity, specificity, and LDOR for calprotectin were 0.942 (95% confidence interval [CI], 0.916, 0.967), 0.860 (95% CI, 0.799, 0.935), and 4.250 (95% CI, 3.504, 4.990), correspondingly. The AUC for calprotectin was 0.91.

Consistent with our findings, Rasmy et al. [15] emphasized that when the ascitic calprotectin level exceeds 320 ug/l, it might potentially serve as an indicator for the onset of SBP in cirrhotic-individuals with ascites, with a sensitivity of 95% and specificity of 90%. Consistent with our findings, Heikl et al. [21] emphasized that the threshold value of ascitic calprotectin for detecting SBP was 783 ng/ml. This cutoff value demonstrated a sensitivity of 90% and a specificity of 100%, resulting in an area under the curve of 0.980 (P<0.001).

In multilinear regression, our results showed that differential and calprotectin were significant predictors of SBP (P-value ≤ 0.05) while other parameters (platelet, ALT, protein, SAAG, WBCs, INR, and CRP) were insignificant predictors for SBP. In accordance with our results, Abdel Rahman et al. [18] highlighted that only ascitic calprotectin was the independent predictor of SBP. Parallel to our results, Heikl et al. [21] highlighted that calprotectin was significant predictor of SBP (P ≤ 0.05).

Conclusions:

The levels of ascitic calprotectin were markedly elevated in the group with SBP compared to the group without SBP. Furthermore, a strong association was existed among ascitic calprotectin levels and the established diagnostic criteria for SBP, which includes the presence of PMNLs of 250 cells/mm3 or higher. Consequently, it may serve as a dependable and satisfactory diagnostic indicator for accurately identifying SBP. Furthermore, there is a direct correlation between the amount of ascitic calprotectin and the severity of liver disease.

Limitations of our study: The sample size was relatively small. The study was in a single center and the results may differ elsewhere. Lack of following the therapy measuring calprotectin and so the recurrence.

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Conflict of Interest: Nil

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