# Ascitic calprotectin as a diagnostic marker for spontaneous bacterial peritonitis in hepatitis C virus cirrhotic Egyptian patients Ahmed A. Heikl<sup>a</sup>, Mahmoud M. El-Nokeety<sup>a</sup>, Eman Roshdy<sup>a</sup>, Abeer Mohey<sup>b</sup>

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#### Background

The gold standard for the diagnosis of spontaneous bacterial peritonitis (SBP) is a polymorphonuclear leukocyte (PMNL) count of 250/mm<sup>3</sup>or more. Accurate and early diagnosis of SBP is important to decrease the mortality and complications in patients with cirrhosis.

#### Aims

The aim of this study was to evaluate the accuracy of ascitic fluid calprotectin as a diagnostic marker for the detection of SBP.

#### Patients and methods

Seventy Egyptian patients with liver cirrhosis and ascites were enrolled; these patients were divided into two groups: 50 patients with SBP and 20 patients with no SBP on the basis of an elevated ascitic PMNL count of 250 cells/mm<sup>3</sup> or more. Ascitic samples were examined for PMNL count, culture, chemistry, and calprotectin concentrations in all patients.

# Results

Calprotectin levels in ascitic fluid were correlated significantly with PMNLs and significantly higher in patients with SBP than non-SBP (P<0.001), with the best cutoff value for the detection of SBP of 783 ng/ml with a sensitivity, a specificity, a positive predictive value and negative predictive value, and an accuracy of 90, 100, 100, 80, and 92.9%, respectively.

#### Conclusion

Elevated ascitic calprotectin levels in cirrhotic patients are a diagnostic and reliable marker for the detection of SBP and considered a surrogate marker for PMNL.

#### Keywords:

ascites, ascitic calprotectin, polymorphonuclear leukocyte, spontaneous bacterial peritonitis

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# Introduction

Spontaneous bacterial peritonitis (SBP) is an important cause of morbidity and mortality in cirrhotic patients with ascites. SBP is estimated to affect 10–30% of cirrhotic patients hospitalized with ascites, with a mortality rate approaching 30% [1].

Calprotectin is calcium-binding and zinc-binding protein that is detected almost exclusively in neutrophils, and its presence in body fluids is proportional to the influx of neutrophils [2].

Ascitic fluid (AF) calprotectin reliably predicts ascitic polymorphonuclear leukocyte (PMNL), which may provide a useful marker for the diagnosis of SBP [3].

This work was planned with the aim of evaluating AF calprotectin as an accurate diagnostic marker for SBP.

# Patients and methods

This study was an analytical cross sectional study that included 70 Egyptian ascitic patients with liver

cirrhosis recruited from the inpatient ward and outpatient clinic of Internal Medicine at Cairo University Hospital during the period from June 2016 to October 2016. The protocol was approved by the ethical committee of Cairo University and informed consent was obtained.

The patients were divided into two groups.

- Group A included 50 patients with SBP on the basis of the clinical picture and AF PMNL of 250/mm<sup>3</sup> or more, in whom antibiotic treatment for SBP had not yet been started.
- (2) Group B included 20 patients without SBP on the basis of the clinical picture and AF polymorphonuclear cells less than 250/mm<sup>3</sup>.

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# **Exclusion criteria**

- (1) Patients with ascites because of any other cause (malignancy, cardiac, or tuberculosis) excluded on the basis of history, and laboratory and radiological findings.
- (2) Ascitic patients receiving antibiotics 2 weeks before paracentesis as it could alter the result.
- (3) Ascitic patients with hepatitis B virus.

All participants were subjected to the following:

- (1) Full detailed assessment of medical history including age, occupation, and area of residence, history of drug intake, and associated disease.
- (2) Clinical examination.
- (3) Abdominal ultrasonography for the assessment of liver and spleen size, presence or absence of hepatic focal lesions, hepatic, portal, and splenic vein diameter, and the degree of ascites and echogenicity.
- (4) The following laboratory investigations were carried out including complete blood picture by (Cell Dyn 3500; Spectra Group, California, USA). Aspartate aminotransaminase, alanine aminotransaminase, bilirubin, serum albumin, alkaline phosphatase,  $\gamma$ -glutamyltransferase, urea, creatinine, and lactate dehydrogenase (LDH) were measured using (AU 480; Beckman Coulter) using its commercially available reagents. Prothrombin time, concentration, and international normalized ratio were also determined.

Paracentesis was performed under strict sterile conditions in the supine position guided by abdominal ultrasonography. Samples of AF were withdrawn and divided as follows: samples for PMNL were collected in a heparin anticoagulant tube. Differential cell count and cytology were examined using a conventional optical microscope. A manual cell count with a differential study was performed for all samples by experienced technicians. Samples for glucose, protein, albumin, LDH, and serum ascites albumin gradient were obtained for each sample by calculating the difference between serum albumin and ascitic albumin. Culture samples were seeded at bedside with the inoculation of AF in aerobic and anaerobic media blood culture bottles (Bactec; Gerresheimer Moulded Glass Gmbh, Germany). A 5 ml ascitic sample was collected from each patient and stored at -20°C until analysis of calprotectin, which was measured using a sandwich enzyme-linked immunosorbent assay (the kit was supplied by Epitope Diagnostics Inc., San Diego, California, USA).

## Statistical methods

Data were coded and entered using the statistical package for the social sciences, version 23. Data were summarized as mean, SD, median, minimum, and maximum for quantitative data and as frequency and percentage for categorical data. A P value less than 0.05 indicated statistical significance. Differences between the groups were evaluated using an independent-sample *t*-test and a  $\chi^2$ -test. A receiver operating characteristic curve was constructed, with an area under curve analysis carried out to detect the best cutoff value of calprotectin and calprotectin/AF total protein (TP) for the detection of SBP.

# Results

The present study included 70 Egyptian cirrhotic patients with ascites: 41 (58.6%) male patients and 29 (41.4%) female patients. Their ages ranged from 45 to 62 years, with an average age of  $52.41\pm5.17$  years, divided into 50 patients with SBP (group A) and 20 patients without SBP (group B). The non-SBP group included 20 (28.6%) patients with a mean age of 52.65  $\pm5.4$  years, ranging from 45 to 62 years of age, including seven (35.0%) women and 13 (65.0%) men. The SBP group included 50 (71.4%) patients with a mean age of 52.32 $\pm5.13$  years, ranging in age from 45 to 62 years, including 22 (44.0%) women and 28 (56.0%) men (Table 1).

There was no statistically significant difference in the age and sex distributions between the two groups (P=0.509 and 0.728).

Clinical data analysis showed that none of the non-SBP patients had any recurrent hepatic encephalopathy (100%), none of the 20 (100%) patients had fever, 15 (75%) patients had no abdominal pain, five (25%) patients had abdominal pain, and only one (5%) patient had abdominal tenderness on examination, whereas 19 (95%) patients were free. In the SBP group, on the basis of history, all 50 (100%) patients had abdominal pain and abdominal tenderness, 47 (94.0%) patients had fever, three (6.0%) patients had no fever, 24 (48.0%) patients had recurrent hepatic

Table 1 Descriptive data of the patients

Patients (n)	70
Age (mean±SD) (years)	52.41±5.17
Male sex [n (%)]	41 (58.6)
SBP patients [n (%)]	50 (71.4)
Male SBP patients [n (%)]	28 (56)
Non-SBP patients [n (%)]	20 (28.6)
Male non-SBP patients $[n (\%)]$	13 (65)

SBP, spontaneous bacterial peritonitis.

encephalopathy, and 26 (52%) patients had no recurrent hepatic encephalopathy. The comparison between the SBP group and the non-SBP group was statistically significant for clinical presentation (P<0.001).

The comparisons between the SBP group and the non-SBP group for serum total leukocyte count, platelets, alkaline phosphatase,  $\gamma$ -glutamyltransferase, creatinine, urea, serum LDH, total bilirubin, direct bilirubin, prothrombin time, prothrombin concentration, and international normalized ratio were statistically significant (*P*<0.001, 0.021, <0.001, 0.002, 0.004, 0.002, <0.001, <0.001, 0.002, 0.004, 0.002, <0.001, <0.001, 0.002, 0.006, 0.010, and 0.004, respectively). However, the comparisons between both groups for hemoglobin, alanine aminotransaminase, aspartate aminotransaminase, serum albumin, and serum TP were not statistically significant (Table 2).

The comparison between the SBP group and the non-SBP group was statistically significant for AF PMNLs, AF TP, AF albumin, and AF LDH (P<0.001, 0.031, 0.012, and <0.001, respectively), whereas AF glucose was not statistically significant. Serum albumin ascitic gradient (SAAG) in the entire non-SBP group was less than 1.1, whereas in the SBP group, three patients had SAAG less than 1.1 and 47 patients had SAAG more than 1.1. The comparison between both groups was not statistically significant (Table 3).

On the basis of the AF culture results from 70 patients, 41 patients were culture positive mainly for *Escherichia coli* and 29 patients were culture negative. All 41 patients with a positive culture had SBP with PMNLs exceeding 250 cells/mm<sup>3</sup>, whereas of the 29 patients with a negative culture, 20 patients did not have SBP with PMNLs less than 250 cells/mm<sup>3</sup> and

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		ients ( <i>n</i> =50)		Group B non-SBP patients (n=20)					P value		
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
НВ	9.60	1.77	9.80	3.60	13.80	8.96	1.38	9.10	6.20	11.30	0.115
TLC	16.48	6.61	16	1.6	32	6.08	1.95	6.33	2.3	8.9	<0.001
PLT	139.56	96.47	103.50	47.00	519.00	91.85	41.24	82.00	30.00	205.00	0.021
ALT	42.56	26.29	34.00	10.00	107.00	32.90	19.24	24.50	13.00	73.00	0.093
AST	84.10	92.42	57.50	12.00	552.00	61.30	20.01	62.50	20.00	93.00	0.745
Albumin	2.19	0.46	2.20	1.40	3.60	2.21	0.46	2.10	1.60	3.40	0.948
ALP	211.60	90.62	200.00	41.00	477.00	108.35	63.54	100.00	34.00	337.00	<0.001
GGT	172.10	104.77	150.00	18.00	477.00	113.05	89.47	92.50	14.00	407.00	0.002
Creatine	2.39	1.54	1.90	0.50	6.57	1.53	1.33	1.05	0.50	5.80	0.004
Urea	114.16	80.39	101.50	15.00	345.00	73.85	88.58	30.00	16.00	285.00	0.002
Total protein	5.84	0.74	5.60	3.80	7.50	5.84	0.70	5.75	4.50	7.00	0.917
LDH	347.58	130.52	350.00	130.00	940.00	123.55	31.24	120.00	80.00	200.00	<0.001
Total bilirubin	6.11	5.45	4.30	0.43	30.40	2.57	2.31	1.80	0.80	10.00	<0.001
Direct bilirubin	3.23	3.90	1.70	0.09	22.13	1.27	1.18	0.80	0.20	5.00	0.002
PT	19.67	3.63	18.45	14.00	27.00	16.63	3.11	17.00	12.00	22.50	0.006
PC	44.81	11.28	45.00	20.00	67.00	58.48	19.12	50.85	35.00	95.00	0.010
INR	2.25	1.58	1.95	1.14	12.00	1.59	0.50	1.48	0.90	2.60	0.004

ALP, alkaline phosphatase; ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; GGT, γ-glutamyltransferase; HB, hemoglobin; INR, international normalized ratio; LDH, lactate dehydrogenase; PC, prothrombin concentration; PLT, platelet; PT, prothrombin time; SBP, spontaneous bacterial peritonitis; TLC, total leukocyte count. Italic values are statistically significance.

Table 3 Comparison between spontaneous bacterial peritonitis and nonspontaneous bacterial peritonitis groups of ascitic fluid analysis

		Group /	A SBP patie	nts ( <i>n</i> =50)		Group B non-SBP patients (n=20)					P value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
AF PMNLs	1866.82	1308.71	1630.00	320.00	5200.00	81.50	65.80	60.00	10.00	240.00	< 0.001
AF TP	1.83	1.00	1.55	0.30	4.30	1.32	0.82	1.05	0.50	3.50	0.031
AF Alb	0.62	0.47	0.50	0.10	1.97	0.37	0.27	0.30	0.10	1.00	0.012
AF glucose	129.14	71.72	120.00	53.00	518.00	81.50	65.80	60.00	10.00	240.00	0.754
AF LDH	267.58	182.40	230.00	39.00	920.00	1.32	0.82	1.05	0.50	3.50	< 0.001
Culture	1.59	0.48	1.50	0.54	2.70	0.37	0.27	0.30	0.10	1.00	0.096

AF, ascitic fluid; Alb, albumin; LDH, lactate dehydrogenase; PMNL, polymorphonuclear leukocyte; SBP, spontaneous bacterial peritonitis; TP, total protein.

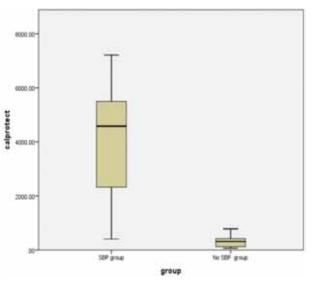
nine patients had SBP. There was a statistically significant difference in AF culture between the SBP group and the non-SBP group (P<0.001) as shown in Tables 4 and 5.

Our results showed that AF calprotectin was statistically significantly higher in the SBP group (P<0.001), than the non-SBP group (Fig. 1).

Also, the calculated ratio between calprotectin and TP in AF was statistically significantly higher in the SBP group than the non-SBP group (P<0.001) (Fig. 2).

The ROC curve showed that the cutoff value of ascitic calprotectin for the diagnosis of SBP was 783 ng/ml with a sensitivity of 90% and a specificity of 100%, with an area under the curve of 0.980 (P<0.001).





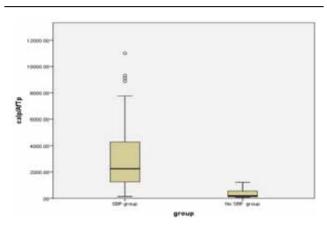


Also, the receiver operating characteristic curve showed that the cutoff value of the ratio between AF calprotectin and AF TP was 688.75 ng/ml with a sensitivity of 86% and a specificity of 95%, with an area under the curve of 0.943 (P<0.001). However, it was not superior on AF calprotectin alone for diagnosing SBP (Fig. 3).

The positive predictivity, negative predictivity, and accuracy of AF calprotectin were 100, 80, and 92.9%, respectively, whereas the positive predictive value (PPV), the negative predictive value (NPV), and the accuracy of ascitic calprotectin to AF TP ratio were 97.7, 73.1, and 88.6%, respectively.

There was a positive correlation between ascitic calprotectin and ascitic PMNLs, AF TP, white blood cells (WBCs), serum TP, and ascitic LDH with coefficient r were 0.754, 0.177, 0.606, 0.460, and 0.607, respectively. There was a statistically

### Figure 2



comparison between SBP and non SBP regarding ascitic calprotectin to ascitic fluid total protein (AF TP) ratio.

Table 4 Comparison between ascitic fluid culture in the spontaneous bacterial peritonitis group and nonspontaneous bacterial peritonitis patients

	Group A SBP patients (n=50) [n (%)]	Group B non-SBP patients (n=20) [n (%)]	
Positive	41 (82.0)	0 (0.0)	<0.001
Negative	9 (18.0)	20 (100.0)	

SBP, spontaneous bacterial peritonitis.

# Table 5 Sensitivity, specificity, positive predictivity, negative predictivity, and accuracy for calprotectin in ascitic fluid and the ratio of calprotectin to ascitic fluid total protein in diagnosing spontaneous bacterial peritonitis

Test result variable(s)	Area	P value	95% CI		Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
		-	Lower bound	Upper bound						
Calprotectin	0.980	<0.001	0.955	1.000	783	90	100	100	80	92.9
Calprotectin/AF TP	0.943	< 0.001	0.894	0.992	688.75	86	95	97.7	73.1	88.6

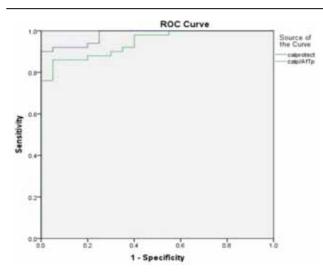
AF, ascitic fluid; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; TP, total protein.

significant correlation between ascitic calprotectin and AF PMNLs, serum WBCs, ascitic LDH, and total bilirubin (P<0.001), whereas the correlation between ascitic calprotectin and AF TP was not statistically significant (Figs. 4–7).

There was a negative correlation between ascitic calprotectin and both serum albumin and prothrombin concentration with coefficient r was -0.063 and -0.391, respectively. There was a statistically significantly negative correlation between ascitic calprotectin and prothrombin concentration (P=0.001) (Fig. 8), whereas the negative correlation between ascitic calprotectin and serum albumin was not statistically significant.

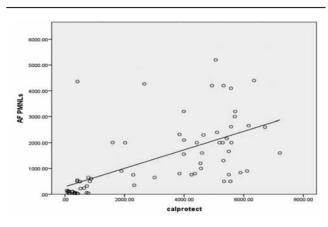
There was a positive correlation between AF PMNLs and the ratio between ascitic calprotectin to AF TP with coefficient r of 0.698; this was statistically significant (P<0.001) (Fig. 9).

#### Figure 3



Roc curve for ascitic Calprotectin and ascitic Calprotectin to ascitic fluid total protein Ratio.

#### Figure 4



Correlation between calprotectin in ascitic fluid and ascitic fluid PMNL.

# Discussion

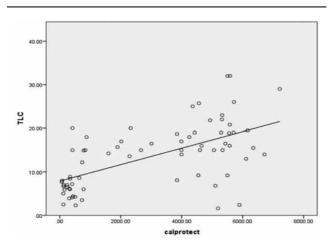
Liver cirrhosis is the clinical end-stage of different entities of chronic liver disease when patients suffer from considerable mortality and morbidity, both of which are correlated positively with disease severity [4].

SBP is a life-threatening complication in cirrhotic patients with ascites. Late or misdiagnosed SBP can lead to increased mortality; SBP is estimated to affect 10–30% of cirrhotic patients hospitalized with ascites, and mortality in this group approaches 30% [5].

The diagnosis of SBP still relies on PMNL in AF of 250 cells/mm<sup>3</sup> or higher in the absence of a contiguous source of intra-abdominal infection with or without a positive culture [6].

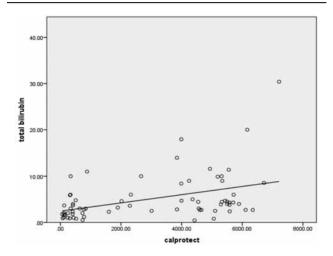
Many markers in AF such as tumor necrosis factor- $\alpha$ , interleukin-6, ascitic procalcitonin, and serum





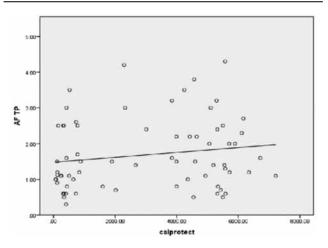
Correlation between ascitic fluid calprotectin and serum TLC.

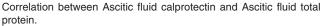




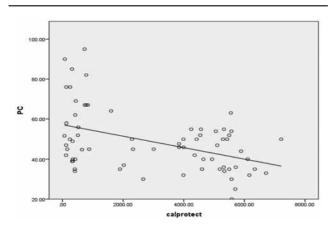
Correlation between ascitic fluid calprotectin and serum total bilirubin.







#### Figure 8



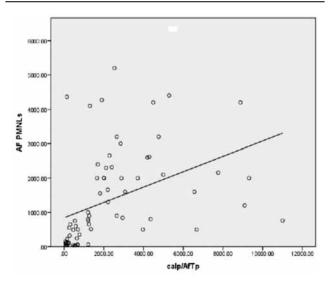
Correlation between ascitic fluid calprotectin and prothrombin concentration.

high-sensitive C-reactive protein have been investigated for the diagnosis of SBP and their diagnostic usefulness is still limited and large-scale studies are needed [7].

Calprotectin is a neutrophil-derived protein found in both plasma and stool that is elevated in infectious and inflammatory conditions. Previous studies have suggested that ascitic calprotectin may be useful in diagnosing SBP in the setting of liver cirrhosis [5].

In the study, AF calprotectin showed a statistically significant correlation with PMNL (P<0.001) and it was proven that ascitic calprotectin is a reliable surrogate for PMNL. Calprotectin levels were increased significantly in patients with SBP than those without SBP (P<0.001). This result is agreement with those reported by Burri *et al.* [3],





Correlation between ascitic fluid Calprotectin/AF TP ratio and Ascitic fluid PMNL.

Fernandes et al. [5], Elbanna et al. [8], Ali et al. [9], Ghweil et al. [10], and Abdel-Razzik et al. [11].

We found that the best cutoff value of AF Calprotectin as a diagnostic marker for SBP was 783 ng/ml with a sensitivity, a specificity, a PPV, an NPV, and an accuracy of 90, 100, 100, 80, and 92.9%, respectively.

Fernandes *et al.* [5] found that the patients with evidence of SBP had significantly higher levels of ascitic calprotectin than patients without SBP with a sensitivity, a specificity, a PPV, and an NPV of 87.8, 97.9, 97.3, and 90.2%, respectively; our results were slightly similar, except for NPV, in which they were superior to us.

Also, Abdel-Razzik *et al.* [11] had tested AF calprotectin for the diagnosis of SBP in 79 patients with ascites and liver cirrhosis because of different etiologies unlike our patients who only had HCV, and found that the ascitic calprotectin level was significantly higher; using a cutoff value of 470 ng/ml, the sensitivity, specificity, PPV, and NPV were 95.4, 85.2, 71, and 93%, respectively.

Burri *et al.* [3], detected that the best cutoff value of ascitic calprotectin measured by the enzyme-linked immunosorbent assay method for the diagnosis of SBP was 630 ng/ml with a sensitivity, a specificity, a PPV, an NPV, and an accuracy of 94.8, 89.2, 60, 99, and 90%, respectively.

To identify all patients with a PMNL count of  $250/\mu$ l or higher and to obtain 100% test sensitivity, a slightly

lower cutoff value is necessary. However, the use of this lower value yields lower specificity and lower PPV [3].

This explains why our results were superior to those of previous studies in terms of specificity and PPV and lower for sensitivity and NPV as we used a higher cutoff value compared with other studies (783 ng/ml).

In the study, there was a statistically significant positive correlation between ascitic calprotectin and ascitic PMNLs, ascitic LDH, serum WBCs, and serum total bilirubin (P<0.001), and a positive correlation with AF TP, but not statistically significant. Also, there was a statistically significantly negative correlation between ascitic calprotectin and prothrombin concentration (P<0.001) and a negative correlation with serum albumin.

Fernandes et al. [5] detected only a statistically significant positive correlation between ascitic calprotectin and ascitic LDH. Also, Rizk et al. [12] detected a statistically significant positive correlation between ascitic calprotectin and serum WBCs. Ghweil et al. [10] found a statistically significant positive correlation between ascitic calprotectin and serum bilirubin (P=0.000) and a statistically significant negative correlation between ascitic calprotectin and both serum albumin and prothrombin concentration; thus, we can conclude that the elevated calprotectin is correlated with the severity of liver cirrhosis as there was a significant positive correlation with serum bilirubin and an inverse correlation with serum albumin and prothrombin concentration and these correlations with the severity of liver cirrhosis can be explained by impaired reticuloendothelial function.

We found that the ratio between ascitic calprotectin and AF TP was statistically significantly higher in the SBP group than the non-SBP group (P<0.001) with a sensitivity, a specificity, a PPV, a NPV, and an accuracy of 86, 95, 97.7, 73.1, and 88.6%, respectively, but it was not superior to calprotectin alone for the diagnosis of SBP. Lutz *et al.* [6] studied the ratio of ascitic calprotectin to ascitic TP and it was significantly higher in the SBP group than the non-SBP group with a sensitivity, a specificity, a PPV, and an NPV of 93, 79, 60, and 97%, respectively, and this means that this ratio can be used as a good negative screening test. Our study was superior to the study carried out by Lutz *et al.* [6] in terms of specificity and PPV, which means that it can be used as a good positive diagnostic test.

# Conclusion

Calprotectin in AF was significantly higher in the SBP group than the non-SBP group and correlates well with the gold standard for diagnosing SBP, which is PMNLs of 250 cells/mm<sup>3</sup> or more. This means that it can be used as a reliable and satisfactory diagnostic marker for diagnosing SBP with high accuracy. Also, the level of ascitic calprotectin is correlated with the severity of liver disease. In addition, our findings suggest that the ratio of ascitic calprotectin to ascitic TP could be a useful diagnostic test for SBP.

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Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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