The value of ascitic fluid calprotectin and calprotectin-to-albumin ratio in the diagnosis and prognosis of spontaneous bacterial peritonitis

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Introduction Spontaneous bacterial peritonitis (SBP) is a potentially fatal condition, characterized by infection of ascitic fluid (AF) in the absence of any intra-abdominal surgically treatable source of infection. It is the most frequent and severe complication of cirrhotic ascites. SBP is a condition that requires a high index of suspicion, rapid and accurate diagnosis, in addition to prompt and effective therapy.

Aim The aim of this study was to evaluate AF calprotectin as a diagnostic marker in detecting SBP. In addition, we have evaluated AF calprotectin-to-albumin ratio in the diagnosis and prognosis of SBP.

Patients and methods A total of 72 patients with cirrhotic ascites were included in this study. They were divided into two groups: SBP group included 50 patients with cirrhotic ascites and SBP diagnosed by presence of polymorphonuclear leukocyte count at least 250 cells/mm^3 in AF with or without positive AF culture, and non-SBP group included 22 patients with cirrhotic ascites without evidence of SBP. All patients were subjected to complete clinical evaluation, laboratory investigations, diagnostic abdominal paracentesis, serum and AF C-reactive protein levels, which were assessed quantitatively, and AF calprotectin levels, which were measured by quantitative sandwich enzyme-linked immunosorbent assay.

Results AF calprotectin was significantly elevated in patients with SBP in comparison with non-SBP patients ($P<0.001$), with the best cutoff value for the detection of SBP (372 ng/ml) with a sensitivity, specificity, positive predictive value, negative predictive value, and an accuracy of 100% for each. Moreover, there was a positive correlation with total leukocytic count, polymorphonuclear leukocyte, and C-reactive protein in serum and AF. Moreover, calprotectin-to-albumin ratio was increased in SBP group versus non-SBP group ($P<0.001$).

Conclusion AF calprotectin can be used as a valuable marker in rapid diagnosis of SBP. Moreover, calprotectin-to-albumin ratio in ascites is useful in the diagnosis of SBP, as well as it provides prognostic information on short-term survival of patients with SBP with follow-up treatment.

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Introduction

Patients with liver cirrhosis, who have an impaired immune status, are often highly susceptible to infections such as spontaneous bacterial peritonitis (SBP), urinary tract infection, pneumonia, and skin infection [1]. Ascites is the most common complication, and ~60% of patients with compensated liver cirrhosis develop ascites within 10 years of disease onset [2]. Up to one-third of patients with cirrhosis and ascites will develop SBP [3]. The mortality rate after an episode of SBP may be as high as 70%, and it increases with each subsequent episode [4]. Current American [5] and European guidelines [6] recommend diagnostic paracentesis in all patients with decompensated liver cirrhosis and ascites to assess ascitic fluid (AF) polymorphonuclear (PMN) cell count and exclude SBP. SBP is estimated to affect 10–30% of cirrhotic patients hospitalized with ascites, with a mortality rate approaching 30% [7]. Many of these patients are asymptomatic, and it is therefore recommended that all patients with ascites undergo paracentesis at the time of admission to confirm the SBP status [4].

The diagnosis of SBP is based upon the polymorphonuclear leukocytes (PMNL) cell count exceeding 250 cells/mm^3 in AF [6]. Currently, differential cell count is usually performed by a manual method using light microscopy and counting chambers. Moreover, the diagnosis is often delayed when laboratory personnel are not readily available. However, this procedure is time consuming as well as subjective. This is a major drawback, as rapid diagnosis of SBP and immediate initiation of antibiotic treatment is of paramount importance [8]. Therefore, the detection and evaluation of new

biomarkers can support the rapid diagnosis and management of SBP [9].

C-reactive protein (CRP) is an acute-phase B globulin. Although the precise in-vivo functions of CRP during the inflammation state is not known, there is considerable evidence indicating a role in the recognition and elimination of foreign pathogens. CRP, as an acute-phase reactant, binds to different substrates. It activates the complement system, takes part in cytokine secretion, and increases the phagocytosis of leukocytes [10]. CRP is synthesized by the liver, mainly in response to interleukin-6, which is produced not only during infection but also in many types of inflammation [11]. CRP has been reported to be a reliable predictor of SBP and an index of therapeutic effectiveness in adults [12].

Calprotectin is a 36 kDa calcium and zinc-binding protein that is detected almost exclusively in neutrophils, which are released in response to inflammatory conditions [13], and its presence in the body fluids (serum, spinal fluid, synovial fluid, urine, saliva, and AF) and feces is directly proportional to the influx of neutrophils [14]. Moreover, calprotectin is a multipotent biologically active molecule. It has been suggested that calprotectin plays an important role in myeloid cell metabolism. When externalized to cells, it has immunomodulatory and antimicrobial effects [15].

Calprotectin is primarily expressed in neutrophils and macrophages, and it rarely appears in lymphocytes [16]. AF calprotectin reliably predicts PMNLs count of at least 250 cells/mm^3, which may provide a useful marker for diagnosis of SBP, especially with a readily available bedside testing device [17]. High AF calprotectin levels in cirrhotic patients appear to be predictive of high mortality [15].

There are many factors that influence the accuracy of traditional methods used in AF infection such as false-positive and false-negative result in addition to delayed diagnosis. Therefore, the investigators propose to use an enzyme-linked immunosorbent assay (ELISA) method that is standardized, rapid, automated and applicable in diagnosis of SBP.

The main objective of this study was to evaluate AF calprotectin as a diagnostic marker in detecting SBP in Egyptian cirrhotic patients. In addition, we have evaluated the role of AF calprotectin-to-albumin ratio in the diagnosis and prognosis of SBP.

Patients and methods

This study was carried out on 72 (49 males and 23 females) Egyptian patients with liver cirrhosis and ascites age ranged between 45 and 65 years admitted to Internal Medicine Department, Al-Zahra Hospital, Al-Azhar University, Cairo, Egypt, during the period from May 2017 to April 2018.

The patients were divided into two groups:

(1) SBP group included 50 patients with cirrhotic ascites and SBP diagnosed by presence of PMNL count at least 250 cells/mm^3 in AF with or without positive AF culture.
(2) Non-SBP group included 22 patients with cirrhotic ascites without clinical or laboratory evidence of SBP and with AF PMNLs count less than 250 cells/mm^3 and negative culture.

Exclusion criteria

(1) Patients with ascites owing to other causes than chronic liver disease, for example, secondary bacterial peritonitis, tuberculosis peritonitis, malignant ascites and ascites owing to cardiac and renal causes were excluded on the basis of history, laboratory, and radiobiological findings.
(2) Patients on antibiotic treatment 2 weeks before paracentesis as it could alter the result.
(3) Patients with history of inflammatory bowel disease (Crohn's disease and ulcerative colitis).

Informed written consent was obtained from each patient before enrollment in this study. The study protocol was approved by the Research Ethical Committee of Faculty of Medicine, Al-Azhar University.

All patients included in the study were subjected to the following:

(1) Full medical history taking including episodes of gastrointestinal bleeding suggesting portal hypertension, paracentesis, episodes of fever, abdominal pain, history of drug intake including antibiotics, and history of viral hepatitis infection.
(2) Clinical examination with special stress on presence or absence of fever, jaundice, ascites, spleen and liver enlargement, and the presence of abdominal tenderness.
(3) Abdominal ultrasonography was performed 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) Laboratory investigations:
   (a) Complete blood count was determined using Sysmex KX-21 (Sysmex America Inc, Mundelein, Illinois, USA).
   (b) Liver function tests: alanine aminotransferase, aspartate aminotransferase, serum total protein and albumin, serum bilirubin, prothrombin time, prothrombin concentration, and international normalized ratio.
   (c) Lactate dehydrogenase (LDH) and kidney function tests (serum creatinine and urea) were performed using Cobas c311 autoanalyzer (Roche, Grenzach-Wyhlen, Germany).
   (d) CRP was assessed quantitatively in serum and AF by immunoturbidimetric method.
   (e) AF calprotectin was measured by an ELISA using immuno diagnostic AG, Stubenwald-Alee 8a, Benshein, Germany.

Diagnostic abdominal paracentesis

It was performed under complete aseptic conditions. Samples were withdrawn from each patient and divided as follows: samples for examining total leukocytic count (TLC) on automatic cell counter Sysmex KX-21 and differential PMN count by Leishman stain. Samples for glucose, total protein, albumin, and LDH levels were measured, and serum-ascites albumin gradient was estimated as serum albumin – AF albumin. Samples for microbiological culture were done by inoculating 10 ml of AF at the bedside in two blood culture bottles, one for aerobic and the other for anaerobic media under complete aseptic condition and were inoculated for 3 days at 37°C with continuous shaking of bottles. The growth is detected through blind subcultures or turbidity of the media. Subcultures were done on blood, MacConkey, and chocolate agar plates [18]. Antimicrobial susceptibility testing and bacterial identification were carried out using standard procedures [19]. A 5 ml of AF was collected from each patient and stored at −20°C until the analysis of calprotectin, which was measured using a sandwich ELISA (the kit was supplied by Epitope Diagnostics Inc., San Diego, California, USA).

All patients were given empirical antibiotics which started immediately after taking samples for cultures, and this empirical antibiotic regimen was modulated according to the results of culture and antibiotic sensitivity tests.

Statistical analysis

Data were coded and entered using the statistical package SPSS version 25. Data were summarized using mean and SD for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired *t*-test in normally distributed quantitative variables, whereas nonparametric Mann–Whitney test was used for non-normally distributed quantitative variables [20]. For comparing categorical data, χ^2-test was performed. Exact test was used instead when the expected frequency is less than 5 [21]. Correlations between quantitative variables were done using Spearman correlation coefficient [22]. Receiver operating characteristic (ROC) curve was constructed with area under the curve (AUC) analysis performed to detect the best cutoff values of calprotectin, calprotectin/albumin, calprotectin/protein, and CRP for detection of SBP. *P* values less than 0.05 were considered as statistically significant.

Results

This study included 72 Egyptian cirrhotic patients with ascites, comprising 49 (68.1%) male patients and 23 (31.9%) female patients. Their ages ranged from 45 to 65 years, with mean age of 52.41±5.17 years. There were 65 (90.3%) patients with chronic hepatitis C-related cirrhosis and seven (9.7%) patients with chronic hepatitis B-related cirrhosis.

According to the clinical data and AF analysis, patients were divided into 50 (37 males and 13 females) patients who were defined as SBP group with mean age of 53.22 ±5.59 years and 22 (12 males and 10 females) patients who were defined as non-SBP group, with mean age of 52.32±3.09 years (Table 1).

Table 1 Comparison of demographic characteristic, clinical presentation, and disease severity of the studied groups

Parameters	SBP group (*n*=50)	Non-SBP group (*n*=22)	*P* value
Age (years)	53.22±5.59	52.32±3.09	0.384
Sex			0.103
Male	37 (74)	12 (54.5)	
Female	13 (26)	10 (45.5)	
Fever	44 (88)	9 (40.9)	<0.001*
Abdominal pain	33 (66)	7 (31.8)	0.007*
Abdominal tenderness	28 (56)	5 (22.72)	0.009*
Altered mental status	32 (64)	12 (54.5)	0.448
Child–Pugh class			
A	0	0	0.768
B	12 (24)	6 (27.3)	
C	38 (76)	16 (72.7)	
MELD score	11.3 (10.5–18)	11.1 (10–17)	0.841

MELD, model for end-stage liver disease; SBP, spontaneous bacterial peritonitis. *Significant.

Table 2 Comparison between laboratory investigations of the two studied groups

Parameters	SBP group (*n*=50)	Non-SBP group (*n*=22)	*P* value
WBCs (×10^3/mm^3)	16.49±3.39	7.83±1.95	<0.001*
Platelets (×10^3/mm^3)	105.86±20.20	120.23±15.56	0.004*
Hb (g/dl)	9.45±1.49	8.36±1.58	0.007*
ALT (IU/l)	46.46±6.52	48.09±6.12	0.323
AST (IU/l)	51.20±12.34	73.82±15.02	<0.001*
Total serum protein (g/dl)	6.18±0.85	6.31±0.74	0.551
Serum albumin (g/dl)	2.35±0.56	2.40±0.41	0.619
Serum bilirubin (mg/dl)	3.12±0.48	2.45±0.75	0.001*
PT (s)	18.85±1.10	18.10±1.28	0.013*
PC (%)	50.51±6.09	54.47±7.23	0.019*
INR	1.85±0.17	1.76±0.18	0.031*
LDH (IU/l)	338.50 ±144.75	121±30.20	<0.001*
Urea (mg/dl)	100.08±28.30	73.55±9.39	<0.001*
Creatinine (mg/dl)	2.34±0.38	1.79±0.17	<0.001*
CRP (mg/dl)	5.32±2.69	1.68±0.67	<0.001*

ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; Hb, hemoglobin; INR, international normalized ratio; LDH, lactate dehydrogenase; PC, prothrombin Concentration; PT, prothrombin time; SBP, spontaneous bacterial peritonitis; WBCs, white blood count. *Significant.

There was no statistically significant difference in the age and sex distribution between the two groups (*P*=0.384 and 0.103). The results of clinical examination are summarized in Table 1, which showed a significant difference regarding fever, abdominal pain, and abdominal tenderness (*P*<0.001, 0.007, and 0.009, respectively), and there was no significantly difference in hepatic encephalopathy between both groups (*P*=0.448). The majority of both groups were child C (76% in SBP group and 72.7% in non-SBP group) but without statistically significant difference with respect to Child–Pugh score (*P*=0.768). Moreover, MELD score mean values were not statistically significant in both groups (*P*=0.841).

The comparison between the SBP group and non-SBP group for serum total leukocytic count, platelets, hemoglobin%, aspartate aminotransferase, total bilirubin, prothrombin time, prothrombin concentration, international normalized ratio, serum LDH, creatinine, urea, and serum CRP was statistically significant (*P*<0.001, 0.004, 0.007, <0.001, <0.001, 0.013, 0.019, 0.031, <0.001, <0.001, <0.001, and <0.001, respectively). However, the comparison between both groups for alanine aminotransferase, total serum protein, and serum albumin was not statistically significant (Table 2).

Table 3 Comparison of ascitic fluid biochemical analysis of the studied groups

AF parameters	SBP group (*n*=50)	Non-SBP group (*n*=22)	*P* value
AF TLC (cells/mm^3)	1379.26 ±1120.64	341.05±266.22	<0.001*
AF PMN (cells/mm^3)	802.94 ±746.84	59.55±39.61	<0.001*
AF total protein (g/dl)	1.90±1.18	1.43±0.78	0.253
AF total albumin (g/dl)	0.86±0.44	0.66±0.25	0.108
AF LDH (IU/l)	256.80 ±180.36	132.32±81.45	0.001*
AF glucose (mg/dl)	110.03 ±22.01	116±13.26	0.243
AF CRP (mg/dl)	7.99±1.60	3.42±1.53	<0.001*
AF calprotectin (ng/ml)	569.15 ±80.98	237.64±61.88	<0.001*
AF calprotectin–albumin ratio	825.56 ±401.28	440.86±300.10	<0.001*
AF calprotectin–protein ratio	448.29 ±290.10	208.60±104.22	0.001*

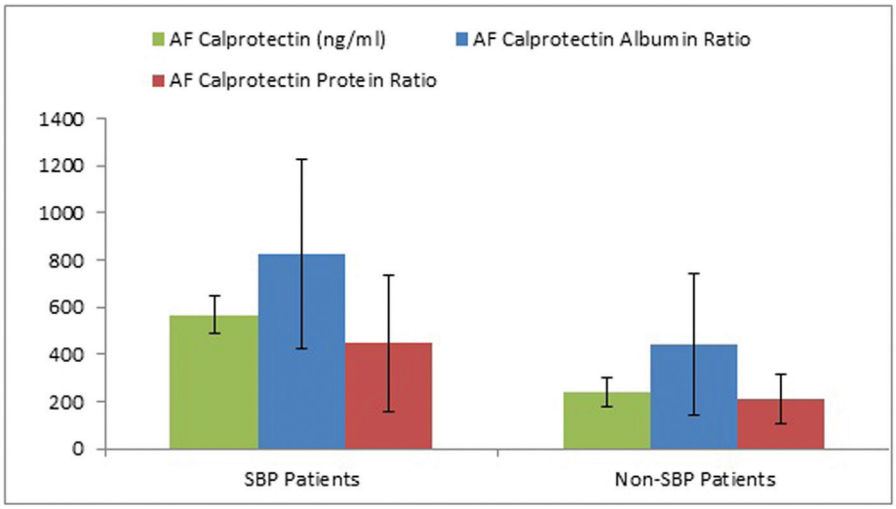
AF, ascitic fluid; CRP, C-reactive protein; LDH, lactate dehydrogenase; PMN, polymorphonuclear leukocyte; SBP, spontaneous bacterial peritonitis; TLC, total leukocyte count. *Significant.

Table 3 shows the AF chemical analysis. There was a statistically significant difference between the two groups regarding TLC, PMN cell count, LDH, AF, and CRP, as they were elevated in SBP group more than non-SBP group (*P*<0.001 for each). However, AF total protein, AF albumin, and AF glucose were not statistically significant. There was a statistically significant increase in AF calprotectin (ng/ml) in SBP group versus non-SBP group (569.15±80.98 vs. 237.64 ±61.88) (*P*<0.001). Moreover, the calculated ratios between calprotectin and AF total protein and AF albumin were statistically significantly higher in SBP group than non-SBP group (*P*<0.001; Table 3 and Fig. 1).

Table 4 showed correlation between AF calprotectin and different parameters among the studied groups. There was a positive correlation between AF calprotectin and main parameters [AF TLC (Fig. 2), AF PMN (Fig. 3), AF calprotectin–albumin ratio (Fig. 6), AF calprotectin–protein ratio, serum and AF LDH, serum and AF CRP (Figs 4 and 5), and serum total bilirubin], and there was a negative correlation between AF calprotectin and prothrombin concentration and serum albumin (Table 4).

Regarding AF culture, only 17 patients of 50 patients with AF PMNLs equal to or more than 250 cells/mm^3 were cultured positive mainly for *Escherichia coli* and 33

Figure 1



AF calprotectin, AF calprotectin–albumin ratio and AF calprotectin–protein ratio concentrations in both groups. AF, ascitic fluid.

Table 4 Correlation between ascitic fluid calprotectin and different parameters among the studied groups

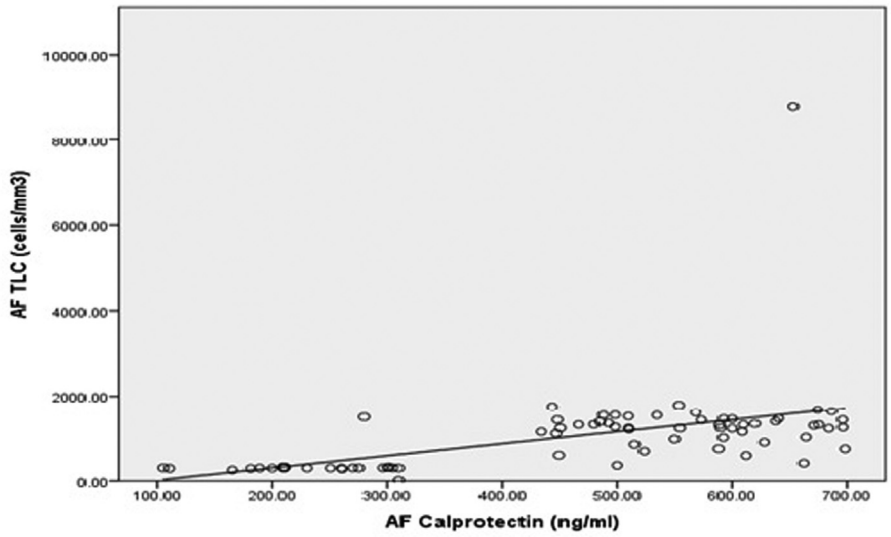
Parameters	SBP group (*n*=50)		Non-SBP group (*n*=22)	
	R	*P* value	*R*	*P* value
Age (years)	–0.030	0.836	–0.173	0.440
WBCs (×10^3/mm^3)	0.117	0.418	–0.132	0.559
Platelets (×10^3/mm^3)	0.054	0.708	–0.027	0.907
Hb (g/dl)	–0.114	0.430	0.295	0.182
ALT (IU/l)	0.045	0.756	0.075	0.741
AST (IU/l)	0.008	0.954	0.058	0.797
Total serum protein (g/dl)	0.282	0.047*	–0.048	0.831
Serum albumin (g/dl)	–0.017	0.886	0.409	0.059
Serum bilirubin (mg/dl)	0.075	0.605	0.383	0.079
PT (s)	0.039	0.789	0.213	0.342
PC (%)	–0.031	0.831	–0.124	0.584
INR	0.065	0.654	0.009	0.969
LDH (IU/l)	0.074	0.609	0.186	0.407
Urea (mg/dl)	0.029	0.841	–0.389	0.074
Creatinine (mg/dl)	–0.054	0.708	–0.463	0.030*
CRP (mg/dl)	0.364	0.009*	–0.125	0.578
AF TLC (cells/mm^3)	0.003	0.985	0.261	0.240
AF PMN (cells/mm^3)	0.633	0.001*	–0.257	0.249
AF total protein (g/dl)	–0.209	0.146	–0.232	0.298
AF total albumin (g/dl)	0.052	0.718	–0.146	0.517
AF LDH (IU/l)	0.185	0.120	–0.307	0.164
AF glucose (mg/dl)	0.350	0.013*	–0.197	0.381
AF CRP (mg/dl)	0.634	0.001*	–0.051	0.822
AF calprotectin–albumin ratio	0.249	0.081	0.490	0.021*
AF calprotectin–protein ratio	0.398	0.004*	0.554	0.008*

AF, ascitic fluid; ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; Hb, hemoglobin; INR, international normalized ratio; LDH, lactate dehydrogenase; PC, prothrombin concentration; PMN, polymorphonuclear leukocyte; PT, prothrombin time; SBP, spontaneous bacterial peritonitis; TLC, total leukocyte count; WBCs, white blood count. *Significant.

patients were cultured negative. Overall, 22 patients with AF PMNLs less than 250 cells/mm^3 were cultured negative.

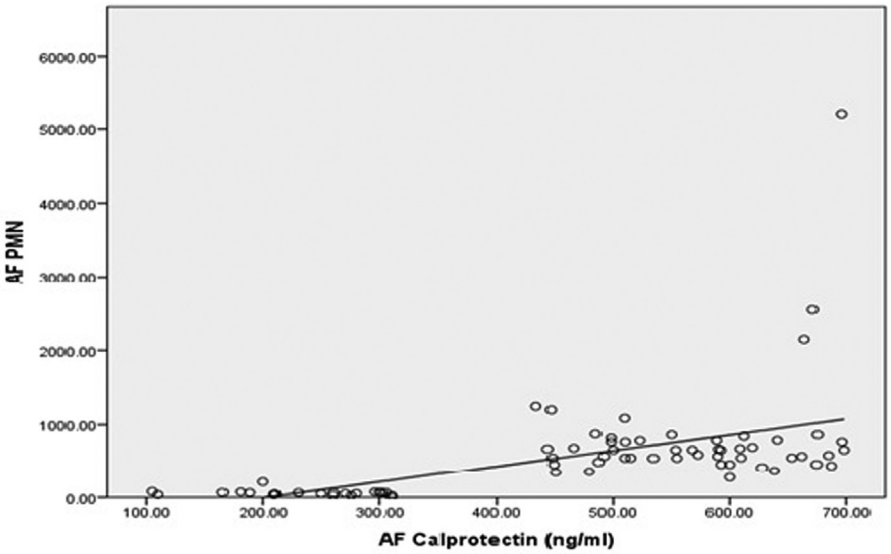
From the ROC curve, the optimum calprotectin level cutoff point for the diagnosis of SBP was 372 ng/ml with a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy of 100% for each, with an AUC of 1.000 (P<0.001). Moreover, ROC curve analysis suggested that the optimum calprotectin-to-albumin ratio cutoff points for SBP in cirrhotic patients was 535.25 ng/ml,

Figure 2



Correlation between AF calprotectin and AF TLC (r=0.003, P=0.985). AF, ascitic fluid; TLC, total leukocytic count.

Figure 3



Correlation between AF calprotectin and AF PMNL (r=0.633, P<0.001). AF, ascitic fluid; PMNL, polymorphonuclear leukocytes.

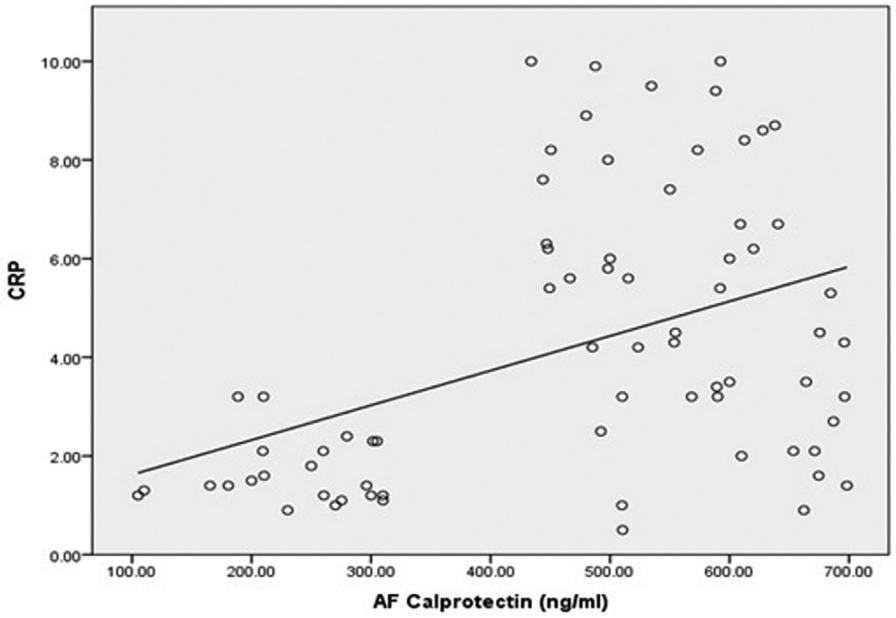
with a sensitivity, specificity, PPV, NPV, and overall accuracy of 78, 77.3, 88.64, 60.71, and 77.78%, respectively, with an AUC of 0.814 (P<0.001). In addition, the ROC curve showed that the cutoff value of the ratio between AF calprotectin and AF total protein was 352.67 ng/ml, with a sensitivity, specificity, PPV, NPV, and overall accuracy of 54, 90.9, 93.10, 46.51, and 65.28%, respectively, with an AUC of 0.755 (P<0.001). The ROC analysis for AF CRP showed that the cutoff value was 6.05 mg/dl, with a sensitivity, specificity, PPV, NPV, and overall accuracy of 96, 100, 100, 91.67, and 97.22%, respectively, with an AUC of 0.999 (P<0.001; Table 5 and Fig. 7).

Discussion

Liver cirrhosis is the clinical end stage of different entities of chronic liver diseases. Ascites is a common complication in patients with liver cirrhosis [23]. SBP is a bacterial infection of AF in the absence of any intra-abdominal source of infection [24]. 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 the mortality in this group approaches 30% [25].

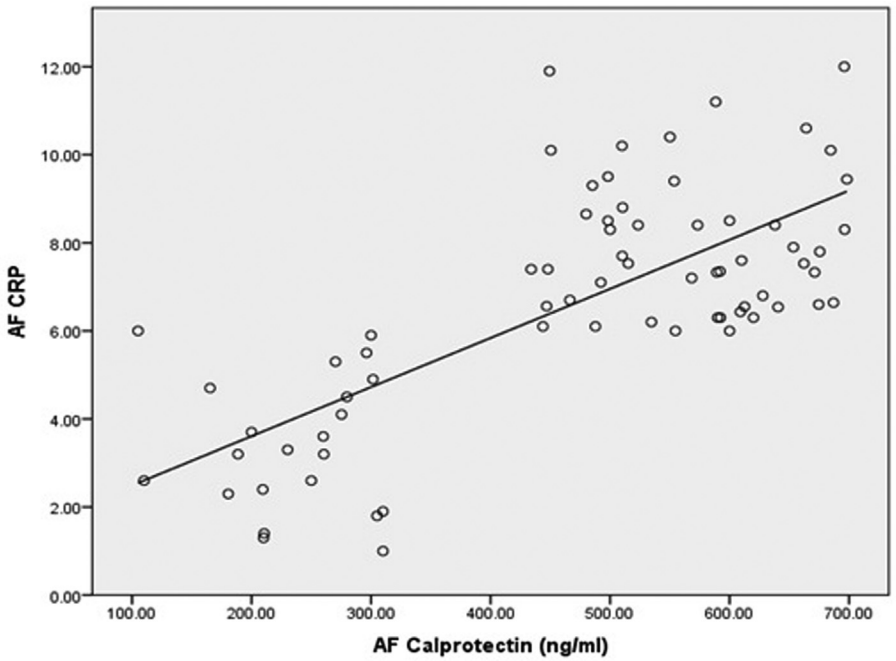
The diagnosis of SBP still relies on PMNLs in AF of 250 cells/mm^3 or more in the absence of a contiguous

Figure 4



Correlation between AF calprotectin and serum CRP (r=0.364, P=0.009). AF, ascitic fluid; CRP, C-reactive protein.

Figure 5

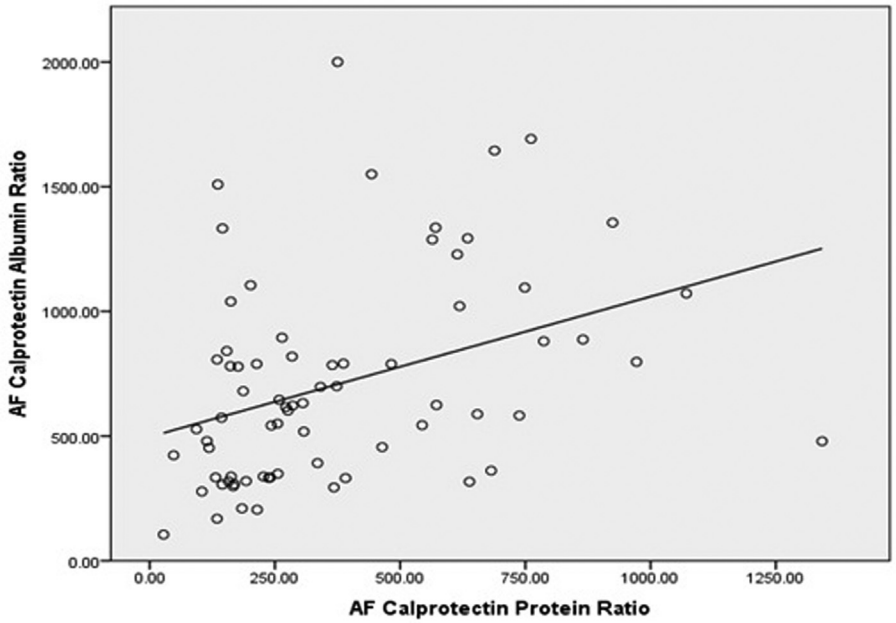


Correlation between AF calprotectin and AF CRP (r=0.634, P<0.001). AF, ascitic fluid; CRP, C-reactive protein.

source of intra-abdominal infection with or without a positive culture [26]. However, TLC and PMNLs counts in AF are not always readily available [27]. Many markers in AF such as tumor necrosis factor-α, interleukin-6, ascitic procalcitonin, AF lactoferrin, high-sensitive CRP, white blood count (WBCs), and mean platelets volume have been studied and may play a promising role in supporting the diagnosis of SBP [28,29].

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 of SBP in the setting of liver cirrhosis [25]. So, this study was conducted to assess the role of AF calprotectin level for the diagnosis of SBP as a rapid bedside test and follow-up treatment. In this study, SBP was found

Figure 6



Correlation between AF calprotectin–albumin ratio and AF calprotectin-to-protein ratio (r=0.249, P=0.081 and r=0.398, P=0.004 respectively). AF, ascitic fluid.

Table 5 Receiver operating characteristic curve, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy for ascitic fluid C-reactive protein, ascitic fluid calprotectin, and the ratio of calprotectin to ascitic fluid total protein and ascitic fluid albumin in diagnosis and prognosis of spontaneous bacterial peritonitis

	Area under the curve	*P* value	95% confidence interval		Cutoff value	Sensitivity (%)	Specificity (%)	PPV	NPV	Accuracy
			Lower bound	Upper bound						
AF CRP	0.999	<0.001*	0.996	1.000	6.05	96	100	100.00	91.67	97.22
AF calprotectin	1.000	<0.001*	1.000	1.000	372	100	100	100.00	100.00	100.00
AF calprotectin–albumin ratio	0.814	<0.001*	0.702	0.926	535.25	78	77.3	88.64	60.71	77.78
AF calprotectin–protein ratio	0.755	0.001*	0.646	0.865	352.67	54	90.9	93.10	46.51	65.28

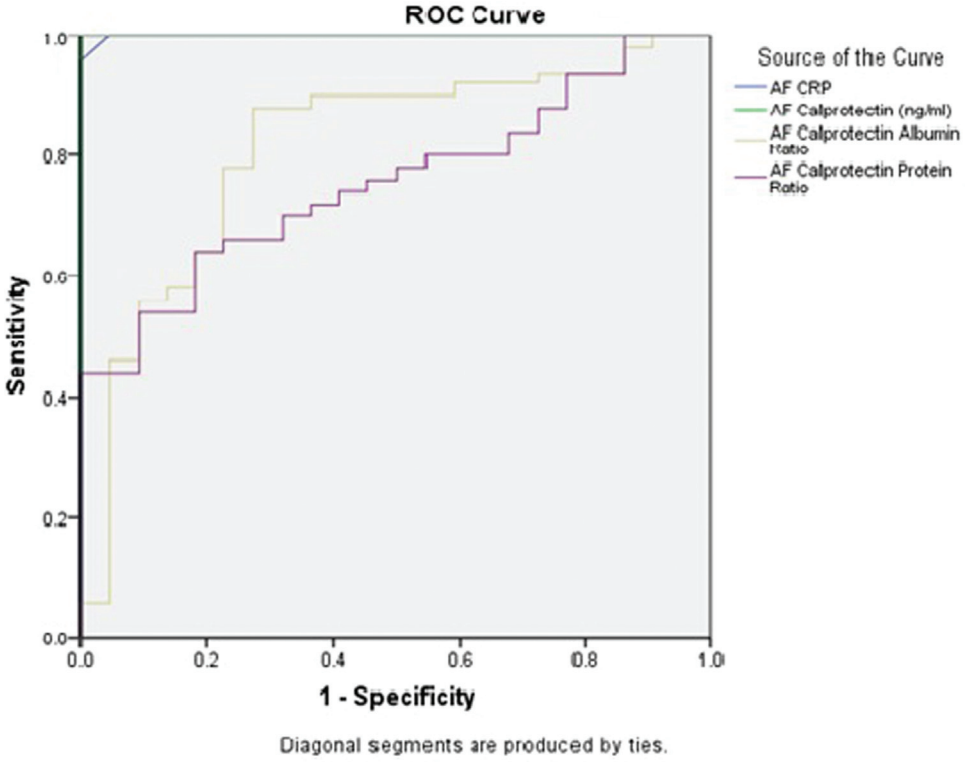
AF, ascitic fluid; CRP, C-reactive protein; NPV, negative predictive value; PPV, positive predictive value. *Highly significant.

to be more common in males than females, which goes in agreement with the result obtained by Reiperger *et al.* [30] who reported that SBP was increased in males. The male predominance in this study may be owing to higher incidence of bilharziasis and HCV infection.

In this study, the most common clinical presentations in patients with SBP were fever (88%) and abdominal pain (66%). These results were consistent with Chang *et al.* [31] and Paul *et al.* [32], who detected that most patients of SBP have clearly suggestive signs of peritoneal infection especially fever and abdominal pain. The majority of our patients (90.3%) had chronic hepatitis C-related cirrhosis. These results were consistent with Mohamoud *et al.* [33], who concluded that the most common cause of cirrhosis in Egypt is hepatitis C virus infection.

The isolated organism in SBP group in the current study was the *E. coli*. This was in agreement with the results of Liovet *et al.* [34] and Noomann *et al.* [35], who stated that single Gram-negative organism especially *E. coli* causes most episodes of SB,P and this can be explained by the fact that the organisms predominantly causing AF infection are normally found in gastrointestinal tract [36]. We found that most patients with SBP were Child–Pugh class C (76%), and this result was in agreement with the result of Cirera *et al.* [37] who reported that Child–Pugh class C was reported in 70% of the patients who had SBP.

Figure 7



Receiver operating characteristic (ROC) curve for ascitic calprotectin, ascitic calprotectin-to-albumin ratio, ascitic calprotectin-to-protein ratio, and AF CRP. AF, ascitic fluid; CRP, C-reactive protein.

In this study, AF calprotectin showed a statistically significant positive correlation with AF PMNL in SBP group ($P<0.001$), and it was proven that AF calprotectin is a reliable surrogate for PMNL. AF calprotectin was detected in both groups. There was a highly statistically significant increase in AF calprotectin in SBP group when compared with non-SBP group (569.15±80.98 and 237.64±61.88, respectively; $P<0.001$). This result is in agreement with those reported by Burri *et al.* [17], Elbanna *et al.* [38], Ali *et al.* [39], and Abdel-Razik *et al.* [40]. We found that the best cutoff value of AF calprotectin as a diagnostic marker for SBP was 372 ng/ml, with a sensitivity, specificity, PPV, NPV and overall accuracy of 100% each. These results are in agreement with those demonstrated in the studies of Elbanna *et al.* [38], Burri *et al.* [17], and Ali *et al.* [39]. Moreover, and Abdel-Razik *et al.* [40] showed that AF calprotectin was significantly higher in patients with SBP.

Moreover, Abdel-Razik *et al.* [28] showed that at cutoff value of 270 mg/dl, ascitic calprotectin demonstrated 86% specificity and 97.5% sensitivity for diagnosis of SBP. Abdel-Razik *et al.* [40] reported that the cutoff value of calprotectin in AF of 445 ng/ml had 95.4 and 82.2% for specificity and sensitivity, respectively, for the diagnosis of SBP. Burri *et al.* [17] detected that the best cutoff value of AF calprotectin measured by ELISA method for the diagnosis of SBP was 630 ng/ml, with a sensitivity, specificity, PPV, NPV, and overall accuracy of 94.8, 89.2, 60, 99, and 90%, respectively.

This results were superior to those of previous studies, as we used a lower cut-off value compared with other studies (372 ng/ml). We found that the ratio between AF calprotectin and AF total protein was statistically significantly higher in SBP group than non-SBP group ($P<0.001$). Lutz *et al.* [26] studied the ratio of AF calprotectin to AF total protein, and it was significantly higher in SBP group than non-SBP group, and this means that these results were not superior to calprotectin alone for the diagnosis of SBP but can be used as a good negative screening test. This study was not superior to the study carried out by Lutz *et al.* [26], as we found that the ratio between AF calprotectin and AF albumin was statistically significantly higher among SBP group than non-SBP group ($P<0.001$).

This study suggested that the ratio of calprotectin-to-albumin in ascites could be a useful test for the diagnosis and prognosis of SBP. Moreover, in this study, we investigated the role of other parameters in supporting the diagnosis of SBP as there was an

increase in WBCs count, PMNL count, and serum and AF CRP. Preto-Zamperlini *et al.* [12] demonstrated that patients with SBP had an elevated serum CRP, and they concluded that CRP is an independent predictor and development and follow-up of SBP. In this study, we have similar results, where CRP was significantly increased in the serum and AF of the SBP group versus non-SBP group (5.32±2.69 and 7.99±1.60 vs. 1.68±0.67 and 3.42±1.53).

In this study, there was a positive correlation between AF calprotectin and AF TLC, AF PMNLs, AF calprotectin–albumin ratio, AF calprotectin–protein ratio, serum and AF LDH, serum and AF CRP, and serum total bilirubin, whereas there was a negative correlation between AF calprotectin and prothrombin concentration and serum albumin. These results are in agreement with, Abdel-Razik *et al.* [40], and Ghweil *et al.* [41]. From these results, we can conclude the elevated AF calprotectin is correlated with the severity of liver cirrhosis.

Conclusion

Calprotectin in AF was significantly higher in SBP group than non-SBP group and positively correlated with AF WBCs count, PMNLs count, and CRP in serum and AF. AF calprotectin may be used as a valuable diagnostic test for detecting SBP in patients with cirrhotic ascites. Moreover, the level of AF calprotectin is correlated with the severity of liver function test. In addition, our findings suggested that calprotectin-to-albumin ratio in ascites could be a useful diagnostic test for SBP and provide prognostic information.

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Conflicts of interest

There are no conflicts of interest.

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