

The ratio of calprotectin to total protein as a diagnostic marker for spontaneous bacterial peritonitis in patients with liver cirrhosis and ascites

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Abstract

Background: Spontaneous bacterial peritonitis (SBP) is a potentially fatal condition, characterized by infection of ascitic fluid in absence of any intra-abdominal surgically treatable source of infection. Diagnosis of SBP is based on a differential ascites leucocytic count. **Aim of the Work:** to assess the role of ascitic fluid calprotectin in diagnosis of SBP. **Patients and Methods:** A cross sectional study was conducted on 60 patients with decompensated liver disease were selected. They were divided into: 1) Non SBP Group: included 30 patients with cirrhotic ascites without clinical or laboratory evidence of spontaneous bacterial peritonitis. 2) SBP Group: included 30 patients with cirrhotic ascites and spontaneous bacterial peritonitis. **Results:** Ascitic fluid calprotectin level and the ratio of calprotectin to total protein was statistically significant higher in SBP group than non SBP group. There was a significant decrease in total protein and albumin in ascitic fluid in SBP group compared to non SBP group. A significant positive correlation was detected between ascitic fluid calprotectin and ascitic fluid TLC and PNLs among SBP group, A significant positive correlation between the ratio of calprotectin to total protein and ascitic fluid TLC and PNLs among SBP group. Ascitic fluid calprotectin at cut-off value 96 ng/ml, had a sensitivity 86.67 % and a specificity 76.67 % in diagnosis of SBP with positive predictive value 85.2 % and negative predictive value 78.8%. - The ratio of calprotectin to total protein at cut-off value 9.6, had a sensitivity 96.67 % and a specificity 90 % in diagnosis of SBP with positive predictive value 96.4 % and negative predictive value 90.6%. **Conclusion:** The ratio of calprotectin to total protein had high sensitivity and specificity in diagnosis of SBP and better than calprotectin alone. The ratio of calprotectin to total protein could be a useful diagnostic test for SBP.

Key words: calprotectin, total protein, spontaneous bacterial peritonitis, cirrhosis, ascites

Introduction

Spontaneous bacterial peritonitis (SBP) is a distinct form of infectious peritonitis occurring in patients with advanced liver cirrhosis and ascites ⁽¹⁾.

SBP is associated with a high one year mortality after the first episode of about 30% ⁽²⁾. While SBP has a low incidence in outpatients ⁽³⁾, approximately 50% of SBP episodes in hospitalized patients are diagnosed at the time of admission ⁽⁴⁾.

Symptoms of SBP include fever, chills, nausea, vomiting, abdominal pain and general malaise. Patients may complain of worsening of ascites ⁽⁵⁾.

Thirteen percent of patients have no symptoms and signs. Clinical diagnosis and systemic laboratory parameters are unreliable for diagnosis of SBP ⁽⁶⁾.

According to international guidelines, diagnosis of SBP is based on a polymorphonuclear (PMN) cell count of > 250/ μ L in the ascites in the absence of a surgically treatable intra-abdominal infection. However, a

differential cell count is not readily available in all clinical settings. Nevertheless, a delay in establishing the diagnosis is associated with a poor prognosis ⁽⁷⁾.

Attempts to establish alternative diagnostic tests are of limited success. In addition, no test provides prognostic information. A urinary test strip that detects leukocytes by their esterase activity performed well with a sensitivity of 100% and a specificity of 58%. A drawback is that this test cannot be applied to bloody or chylous ascites samples, so that 16% of the tests could not be interpreted. First results of a urinary test strip were promising but have to be confirmed ⁽⁸⁾.

An alternative approach is to detect proteins secreted by inflammatory cells into ascites. Lactoferrin, which is produced mainly by neutrophilic granulocytes, showed good test results ⁽⁹⁾, but has not been introduced into clinical practice due to the lack of commercially available diagnostic test kits. Recently, a study evaluated calprotectin levels in ascites due to different etiologies of liver cirrhosis and found a strong correlation to ascites polymorphonuclear (PMN) number ⁽¹⁰⁾.

Calprotectin is an acute phase inflammatory reaction protein originating mainly from polymorphonuclear (PMN) which exerts regulatory, antimicrobial and antiproliferative functions ⁽¹¹⁾.

Fecal calprotectin has been established as a diagnostic tool in inflammatory bowel disease. Calprotectin can be measured also in bloody and chylous ascites, so that all samples could be analyzed ⁽¹²⁾.

The ascitic fluid total protein concentration is lower in "spontaneously" infected ascitic fluid compared to sterile fluid obtained from different patients ⁽¹³⁾.

Aim of the work

Assessment of ratio of calprotectin to total protein in ascites as an alternative diagnostic marker of SBP.

Patients and methods

This cross-sectional study was conducted at Gastroenterology and Hepatology Unit, Department of Internal Medicine, Ain Shams University Hospital and Ahmed Maher Educational Hospital. The study included a total number of 60 patients with liver cirrhosis and ascites were included in this study and were divided into two main groups:

1) Non-SBP Group: include 30 patients with liver cirrhosis and ascites without clinical or laboratory evidence of spontaneous bacterial peritonitis.

2) SBP Group: include 30 patients with liver cirrhosis and ascites and spontaneous bacterial peritonitis.

Diagnosis of SBP is based on a polymorphonuclear (PMN) cell count of $> 250/\mu\text{L}$ in the ascites in the absence of a surgically treatable intra-abdominal infection (**Arroyo et al., 2000**).

Inclusion Criteria: Ascitic patients with clinical, laboratory, ultrasonographic findings of liver cirrhosis were included.

Exclusion Criteria: Patients have ascites due to causes other than liver cirrhosis, recent abdominal surgery (< 3 months), abdominal malignancy (HCC, Colorectal carcinoma, Gastric carcinoma, Pancreatic carcinoma, Cholangio carcinoma), intra-abdominal infection e.g. abscess, appendicitis, cholecystitis, and pancreatitis.

Ethical Considerations: Patients were informed about the research, freedom of choice to participate in the research or not was given to patients.

Methods

All patients were subjected to the following: 1. Full history taking: 2. Clinical examination: - Signs of chronic liver disease - Signs of SBP 3. Laboratory investigations: - Complete blood count, Liver function tests: (Serum albumin, liver enzymes, Bilirubin and INR). - Kidney function tests (urea and creatinine). 4. Calculation of Child's Pugh score 5. Abdominal ultrasonography 6. Diagnostic abdominal paracentesis: It was done for: i-

Patients with cirrhosis and ascites at admission.
 ii- Patients who develop symptoms or signs of SBP during hospitalization .

Ascitic fluid analysis: 15 ml of the ascitic fluid were aspirated from each patient and divided as follow: (I) 5 ml for physical examination for: a) Color. b) Turbidity (II) 5 ml for biochemical tests including: a) Total protein content. b) Albumin. c) Calprotectin (ELISA). (III) 5 ml for WBCs (total and differential). **Calprotectin** was measured in 1 mL ascitic fluid by Enzyme-Linked Immuno Sorbent Assay (ELISA) to assay the level of Human Calprotectin in samples.

Statistical analysis

Data were collected, coded, revised and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The data were presented as number and percentages for the qualitative data, mean, standard deviations and ranges for the quantitative data with parametric distribution and median with inter quartile range (IQR) for the quantitative data with non parametric distribution. **Chi-square test** was

used in the comparison between two groups with qualitative data and **Fisher exact test** was used instead of the Chi-square test when the expected count in any cell found less than **Independent t-test** was used in the comparison between two groups with quantitative data and parametric distribution and **Mann-Whitney test** was used in the comparison between two groups with quantitative data and non parametric distribution. **Spearman correlation coefficients** were used to assess the significant relation between two quantitative parameters in the same group. **Receiver Operating Characteristic curve (ROC)** was used to assess the best cut off point between two groups with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the curve (AUC). The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: $P > 0.05$: Non significant, $P < 0.05$: Significant.

Results

Table (1): Comparison between SBP group & Non SBP group as regards sex

		SBP group (No.=30)		Non SBP group (No.=30)		CHI square test	
		No.	%	No.	%	X ²	P value
Sex	Female	13	43.3%	17	56.7%	1.067	0.302
	Male	17	56.7%	13	43.3%		

This table shows that there was no statistical significant difference between studied groups as regards sex .

Table (2): Comparison between SBP group & Non SBP group as regards age

		SBP group (No.=30)		Non SBP group (No.=30)		Independent t test	
		Mean	SD	Mean	SD	T	P value
Age	Mean	56.83	3.53	55.83	8.12	0.491	0.625
	Standard deviation	7.64	1.05	7.64	1.05		

This table shows that there was no statistical significant difference between studied groups as regards age .

Table (3): Comparison between SBP group & Non SBP group as regards laboratory investigations

	Normal value	SBP group (No.=30)		Non SBP group (No.=30)		Independent t test	
		Mean	SD	Mean	SD	T	P value
HB	(12-15)	8.81	1.05	9.51	1.23	-2.385	0.020
TLC	(4-11)	11.63	3.53	5.24	1.49	9.137	0.001
PLT	(150-400)	86.90	20.73	109.13	19.53	-4.276	0.001
ALT	(10-40)	72.70	14.08	65.43	15.28	0.977	0.271
AST	(10-40)	71.60	16.01	70.30	15.79	0.317	0.753
Total BIL	(0.3-1.2)	3.13	2.58	2.16	0.70	1.977	0.053
DIRECT BIL	(up to 0.2)	1.91	2.90	1.40	0.47	0.958	0.342

ALBUMIN	(3.5-5.3)	2.61	0.30	2.94	0.16	-5.408	0.001
INR	(1)	1.67	0.17	1.56	0.21	2.235	0.029
UREA	(20-40)	64.43	27.69	66.27	27.93	-0.255	0.799
CREATININ	(0.4-1.3)	1.84	0.53	1.44	0.32	3.495	0.001

This table shows that there was statistically significant increase in SBP group in comparison to non SBP group as regards TLC, INR and creatinin but decrease in SBP group in comparison to non SBP group with HB, PLT and albumin.

Table (4): Comparison between SBP group & Non SBP group as regards ascitic fluid analysis

	SBP group (No.=30)		Non SBP group (No.=30)		Independent t test	
	Mean	SD	Mean	SD	T	P value
Total protein (g/l)	9.2	1.6	17.4	2.1	-17.162	0.001
ALBUMIN(g/dl)	0.52	0.10	0.84	0.13	-10.999	0.001
SAAG	2.09	0.33	2.10	0.20	-0.085	0.932
TLC	955.33	222.30	303.33	186.59	12.304	0.001
PNLS	406.83	120.88	78.87	39.33	14.131	0.001

This table shows that there was statistically significant increase in SBP group in comparison to non SBP group as regards TLC and PNLs but decrease in SBP group in comparison to non SBP group with total protein and albumin.

Table (5): Comparison between SBP group & Non SBP group as regards calprotectin and calprotectin to total protein ratio

	SBP group (No.=30)		Non SBP group (No.=30)		Independent t test	
	Mean	SD	Mean	SD	t	P value
Calprotectin (ng/ml)	915.67	1232.20	102.23	119.97	3.599	0.001
Calprotectin to total protein ratio	116.98	145.94	5.88	8.10	4.164	0.001

This table shows that there was statistically significant increase in SBP group in comparison to non SBP group as regards calprotectin and ratio of calprotectin to total protein

Table (6): Comparison between SBP group & Non SBP group as regards ultrasound

		SBP group (No.=30)		Non SBP group (No.=30)		Chi square test	
		No.	%	No.	%	X ²	P value
LC	+ve	30	100.0%	30	100.0%	NA	NA
HFL	-ve	30	100.0%	30	100.0%	NA	NA
Ascites	moderate	14	46.7%	15	50.0%	0.067	0.796
	severe	16	53.3%	15	50.0%		

There is no patients with HFL to exclude any cause of ascites other than live cirrhosis.

Table (7): Comparison between SBP group & Non SBP group as regards child score

		SBP group (No.=30)		Non SBP group (No.=30)		CHI square test	
		No.	%	No.	%	X ²	P value
Child score	B	10	33.3 %	23	76.6 %	8.378	0.212
	C	20	66.7 %	7	23.4 %		

The majority of SBP patients were child –paugh class C (66.7%) but without significant difference in between SBP and non SBP groups.

Table (8): Correlation between calprotectin and calprotectin to total protein ration as regards all parameters in SBP group

	Calprotectin (ng/ml)		Calprotectin to total protein ratio	
	r	P value	R	P value

Age	0.043	0.820	-0.026	0.891
Laboratory investigations				
HB	0.246	0.190	0.200	0.290
TLC	0.178	0.346	0.067	0.725
PLT	-0.110	0.563	-0.144	0.449
ALT	-0.313	0.092	-0.243	0.196
AST	-0.196	0.299	-0.085	0.654
T.BIL	-0.227	0.228	-0.286	0.125
DIRECT BIL	-0.082	0.665	-0.129	0.496
ALBUMIN	0.051	0.789	0.083	0.664
INR	-0.118	0.535	-0.086	0.651
UREA	0.051	0.789	0.086	0.651
CREATININ	-0.007	0.969	0.012	0.949
Fluid analysis				
Total protein (g/l)	0.301	0.106	0.044	0.819
ALBUMIN	-0.401	0.028	-0.364	0.048
SAAG	0.089	0.640	0.110	0.563
TLC	0.439	0.015	0.462	0.010
PNLS	0.351	0.049	0.365	0.047

This table shows that calprotectin has negative correlation with albumin in ascitic fluid and positive correlation with TLC and PNLs in ascitic fluid but calprotectin to total protein ratio has negative correlation with albumin in ascitic fluid while has positive correlation with TLC and PNLs in ascitic fluid.

Table (9): Cutoff point, sensitivity and specificity of calprotectin between SBP group and non SBP group

Cutoff point	AUC	Sensitivity	Specificity	NPV	PPV
>96	0.877	86.67	76.67	85.2	78.8

This table shows that in calprotectin: The cutoff point of calprotectin >96, Its sensitivity is 86.67%, Its specificity is 76.67%, The positive predictive value is 85.2% and the negative predictive value is 78.8%

Table (10): Cutoff point, sensitivity and specificity of calprotectin to total protein ratio between SBP group and non SBP group

Cutoff point	AUC	Sensitivity	Specificity	NPV	PPV
>9.6	0.961	96.67	90.00	96.4	90.6

This table shows that in calprotectin to total protein ratio: The cutoff point of calprotectin to total protein ratio >9.6, Its sensitivity is 96.67%, Its specificity is 90%, The positive predictive value is 96.4%, The negative predictive value is 90.6%

Discussion

Ascites is one of the most common complications of patients with cirrhosis and its development carries a relatively poor prognosis but the overall course depends on the degree of reversibility of the underlying liver disease and the response to therapy ⁽¹⁴⁾.

Spontaneous bacterial peritonitis is defined as an infection of a previously sterile ascitic fluid in the absence of any evident intraabdominal surgical source of infection ⁽¹⁵⁾.

Calprotectin, a calcium and zinc-binding protein that belong to the S100 protein family. It is detected almost exclusively in neutrophils, and its presence in body fluids is proportional to the influx of neutrophils. Calprotectin is primarily expressed in neutrophils and macrophages, while it is not usually present in lymphocytes. Calprotectin constitutes up to 60% of soluble protein content in the cytosol of neutrophil granulocytes ⁽¹⁶⁾.

A high level of calprotectin reportedly exists in extracellular fluid during various inflammatory conditions, such as SBP ⁽¹⁷⁾.

The aim of this study is assessment of the role of ratio of calprotectin to total protein in ascites for diagnosis of SBP and to identify a cut - off level of this ratio.

In order to achieve this goal, we selected 60 patients with liver cirrhosis and ascites, 30 patients without SBP, and other 30 patients with SBP admitted in Ahmed Maher Educational Hospital during study period.

In this study, patients with SBP was 56.7 % males and 43.3 % females with no statically significant difference, mean age of patients with SBP is 56.83 ± 7.64 and mean age of patients without SBP is 55.83 ± 8.12 without statistically significant difference .

Regarding of the parameters of laboratory investigations in this study, SBP group showed statistically significant decrease in hemoglobin level when compared to non-SBP groups($p = 0.020$) .

Similar result was obtained by **Wojtacha A et al.**, ⁽¹⁸⁾ who reported decreased level of haemoglobin in SBP patients, this can be explained that patients with more severe liver disease have lesser HB and they are more likely to have SBP.

On the other hand, **Coşkun et al.**, ⁽¹⁹⁾ stated that no significant difference between SBP and non-SBP groups as regard hemoglobin level.

Regarding total leucocytic count, there was statistically significant increase in serum total leucocytic count when compared to non-SBP groups($p = 0.001$) .Similar result was obtained by **Rodríguez-Ramos et al.**, ⁽²⁰⁾ who

detected leucocytosis in their SBP cases with significant difference when compared to non SBP cases.

These results differ from some published studies as **Cholongitas et al.**, ⁽²¹⁾ who stated that there was leucocytosis in SBP patients but with no statistically significant difference between SBP and non SBP patients ($p= 0.11$).

In this study there was reduced platelets count in SBP and non SBP patients but it was much lower in SBP group (86.9 ± 20.73 versus $109.13 \pm 19.35 \times 1000$ cell/cmm). These results are consistent with those of **Lata et al.**, ⁽²²⁾ who reported statically significant lower platelet counts in a group of SBP patients and supposed that the reduction of the platelet count in patients with spontaneous bacterial peritonitis indicates the influence of portal hypertension in the aetiology of the disease.

As regard liver enzymes our result showed that there were no statistically significant differences between SBP and non SBP groups. This is in accordance with **Salama et al.**, ⁽²³⁾.

On the contrary, **Coral et al.**, ⁽²⁴⁾ stated that the level of serum ALT, AST was significantly higher in cirrhotic patients with SBP group than non-SBP group.

Regarding serum bilirubin, We noted higher serum bilirubin level in SBP compared to non SBP groups with no statistically significant differences in between, this match results of **Kim et al.**, ⁽²⁵⁾, **Thiele et al.**, ⁽²⁶⁾. These results differ from results of **Umgelting et al.**, ⁽²⁷⁾ who stated that the level of serum bilirubin was significantly higher in cirrhotic patients with SBP group than non-SBP group.

In the current study, serum albumin level in SBP groups was statistically significantly lower than in non -SBP group. This result goes in agreement with **Ruiz et al.**, ⁽²⁸⁾.

Regarding international normalization ratio (INR) it was it was significantly higher in SBP patients more than non SBP patients.

Similar result concluded by **Oladimeji et al.**, ⁽²⁹⁾ who reported that, INR was significantly higher in those patients with SBP compared with those without SBP.

In this study, the level of serum creatinine was significantly increased in patients with SBP when compared to non SBP patients and this result goes in agreement with **Angeloni et al.**, ⁽³⁰⁾, who concluded that the hospital mortality in SBP is high due to renal impairment which is common in SBP patients

either due to prerenal or hepatorenal causes and also reinforced by **Tsung et al.**,⁽³¹⁾ who stated that renal dysfunction occurs in patients with SBP and it is independent predictor of mortality.

On the other hand, **Zalam et al.**⁽³²⁾ stated that there was no statistical significant difference comparing SBP & non SBP patients as regards kidney function.

Chemical analysis of ascitic fluid showed that total protein concentration was significantly lower in SBP than non SBP group. These results were in line with **Paul K et al.**,⁽³³⁾ who reported that opsonic activity of ascitic fluid has been shown to correlate closely with the fluid's protein concentration; fluids with < 1.0 g/dl of protein have been reported to have diminished opsonic activity and therefore high susceptibility to SBP.

On the other hand this result disagree with **Abdel-Razik A et al.**⁽³⁴⁾ who found that patients with SBP has an increase in ascitic fluid total protein which has an important role in the inflammatory process in SBP.

The present study revealed that the mean value of SAAG was > 1.1 g/dl in both SBP and non-SBP groups but without significant difference, which confirms that etiology of ascites was portal hypertension in these patients. This finding is in concordance with **Agarwal et al.**⁽³⁵⁾ study which suggested that SAAG levels are > 1.1g/dl in all ascites due to portal hypertension irrespective of infection.

This can be explained by the results of current study that reported decreased level of both serum albumin and ascitic albumin in SBP patients .

Chemical analysis of ascitic fluid showed that, statistically significantly higher levels of TLC, PMN count in SBP group compared to non SBP group, these results

were in line with **Yildirim et al.**,⁽³⁶⁾ who reported higher ascitic TLC in SBP more than non SBP patients. Also **Jansen**⁽³⁷⁾ stated that, although ascitic TLC count increases in SBP cases, it suffers from low specificity because a large proportion of patients with sterile ascites

have increased white blood cell count. Also diuretic therapy has been shown to increase the TLC count but does not alter the PMN count.

In the present work, we found that the majority of SBP patients were child –paugh class C (66.7%) but without significant difference in between SBP and non SBP groups ($p = 0.212$). This result matched with that reported by **Cirera et al.**,⁽³⁸⁾ who conducted a study on 136 SBP patients and reported that about 70% of patients who developed SBP had Child class C.

In this study, that ascitic fluid calprotectin was detected in both groups. There was statistically significant increase in ascitic fluid calprotectin in SBP group when compared with non SBP group. This finding is in concordance with **Burri et al.**⁽¹⁰⁾.

Also **Abdel-Razik A et al.**⁽³⁴⁾ show that serum procalcitonin and ascitic calprotectin were significantly higher in SBP patients than in non-SBP patients.

This study revealed that ascitic fluid calprotectin to total protein ratio was statistically significant higher in SBP patients than non SBP patients (116.98 ± 145.94 & 5.88 ± 8.1) respectively ($p = 0.001$).

A result which is consistent with those demonstrated in the study of **Lutz et al.**⁽³⁹⁾.

Further analysis of results revealed statistically significant positive correlation between ascitic fluid calprotectin and ascitic fluid TLC and PNLs among SBP group. Similar result were obtained by **Soyfoo et al.**,⁽¹⁶⁾ who concluded that calprotectin is detected almost exclusively in neutrophils, and its presence in body fluids is proportional to the influx of neutrophils. And goes also in agreement with **Burri et al.**,⁽¹⁰⁾ who say that ascitic fluid calprotectin helpful in detection of neutrophil count .

While in current study there was a significant negative correlation between ascitic calprotectin and ascitic fluid albumin among SBP group ($p = 0.003$), this result goes in agreement with **Gupta et al.**⁽⁴⁰⁾ who explained that in cirrhotic patient there is a decrease in

synthetic function of the liver which aggravated by sepsis the net result is decrease serum albumin which by its role decrease the diffused albumin to ascitic fluid.

This study revealed statistically significant positive correlation between ascitic fluid calprotectin to total protein ratio and ascitic fluid TLC and PMNLs among SBP group and significant negative correlation between ascitic calprotectin to total protein ratio and ascitic fluid albumin among SBP group.

The present study demonstrated that, ascitic fluid calprotectin at a cutoff value of 96 ng/ml, had 86.67 % sensitivity and 76.67 % specificity with positive predictive value 78.8 % and negative predictive value 85.2 % in diagnosis of SBP, AUC was 0.877, while cut off value of calprotectin to total protein ratio was 9.6, had 96.67 % sensitivity and 90% specificity with positive predictive value 90.6 % and negative predictive value 96.4 %, AUC was 0.961.

Lutz et al. ⁽³⁹⁾ show that optimal cutoff value of calprotectin above 36 ng/ml, had 90% sensitivity and 51% specificity, AUC was 0.85, while cut off value of calprotectin to total protein ratio was 5.24, had 93 % sensitivity and 81 % specificity, AUC was 0.93.

The noticeable difference between cutoff value of calprotectin and the ratio of calprotectin to total protein between this study and **Lutz et al.** ⁽³⁹⁾ study may be due to low number of patients in both studies.

Many previous studies evaluated the diagnostic utility of measuring ascitic fluid calprotectin to diagnose SBP but this study added that the ratio of calprotectin to total protein can be used as an alternative diagnostic marker with better sensitivity and specificity than calprotectin alone.

Finally, larger studies are needed to evaluate this test in different clinical settings and to establish a reliable cut-off value for ascitic calprotectin to total protein ratio.

Conclusion

-The ratio of calprotectin to total protein had high sensitivity and specificity in diagnosis of SBP and better than calprotectin alone .

- The ratio of calprotectin to total protein could be a useful diagnostic test for SBP.

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