



The study of the C-reactive protein, calprotectin and calprotectin-ascetic albumin ratio in ascetic fluid of patient with spontaneous bacterial peritonitis

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Abstract: Background: Early indications for SB: According to the current guidelines, patients with symptoms of infection and ascites PMN counts <250/mm³ ought to be administered antibiotics while awaiting the findings of the culture, which is regarded as therapy for SB. Calprotectin is a heterodimer of two calcium-binding proteins, calprotectin is secreted and can be found in different body fluids and serum, as it is considered a useful clinical inflammatory indicator. CRP is 224 residue proteins released by liver and its level will rise if there is broad systematic inflammation. Hospital clinical laboratories employ the CRP test as one of the most important tests for diagnosis to detect and monitor many inflammatory disorders. **Methods:** Our study was conducted on Fifty (50) Cirrhotic patient has ascites can divide into two groups: Group 1 (the control group) comprised 25 patients with cirrhotic ascites who did not have SBP. Group 2 (the SBP group) comprised 25 patients with cirrhotic ascites and SBP. **Results:** The current results' statistical analysis revealed a significant positive correlation between AF CRP, AF Calprotectin of the studied group to their AF Calprotectin and Calprotectin - Albumin Ratio. Statistical analysis of current results showed that all variable results are significant indicator as denoted by the significantly large area under the curves (AUCs); with AF Calprotectin at cut-off value >73.5 being the most significant predictor followed by AF CRP at cut-off value >22.6 and also Calprotectin – Ascetic Albumin ratio at cut-off value >171.4. **CONCLUSION:** In patients with ascitic cirrhosis, AF CRP, AF Calprotectin, and the Calprotectin-Ascetic Albumin ratio were found to be sensitive, valuable, and useful diagnostic measures that yielded a satisfactory and accurate diagnosis of spontaneous bacterial peritonitis. When diagnosing cases of spontaneous bacterial peritonitis, AF calprotectin exhibits greater sensitivity and specificity compared to AF CRP and AF calprotectin albumin ratio. A significant positive association was observed between AF Calprotectin and AF CRP, as well as the AF Calprotectin albumin ratio, in patients suffering from spontaneous bacterial infections.

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Keywords: C-reactive protein; calprotectin; calprotectin-ascetic albumin ratio; ascetic fluid; patient; spontaneous; bacterial peritonitis

Introduction:

One of the most serious adverse effects of portal hypertension and liver cirrhosis is ascites. Ascites is related to a poor prognosis, with a 15% death rate at one year and a 46% death rate at five-year follow-ups (Biecker, 2011).

SBP is a commonly occurring and potentially fatal bacterial ascites infection that impacts those who have cirrhosis. Because it varies from secondary peritonitis, the diagnosis is made when there is no intra-abdominal source of infection or inflammatory process (Dever and Sheikh, 2015).

Early SB indications have yet to be identified. Reagent strips were tested for SB detection, but the findings are not satisfactory. According to the most recent guideline, those patients who exhibit indications of infection and PMN counts <250/mm³ ought to receive antibiotic treatment while we await the results of the culture. The “signs of infection” contain only a few clinical symptoms and no laboratory indicators, which can lead to antibiotic misuse or treatment delays (Zhu et al., 2016).

Calprotectin is a calcium-binding protein that exists as a heterodimer in neutrophil cytoplasm and on monocyte membranes. Calprotectin, a possibly helpful clinical

inflammatory indicator, is secreted with neutrophil activation or monocyte endothelial adherence. It can be found in bodily fluids or serum (**Stríz and Trebichavsk, 2004**).

In centralized hospital laboratories, the CRP test is one of the tests for diagnosis utilized to track various inflammatory disorders. When there is a broad systematic inflammation, its level rises. (**Vihari et al., 2015**).

Aim of the work

to assess the calprotectin and CRP levels in ascetic fluid's use in the diagnosis of SBP. When diagnosing SBP, consider the ascetic fluid's calprotectin-to-albumin ratio as well, and be aware of a clinically significant cut-off level that may be applied in the future to develop a significant clinical diagnosis of SBP that will save time.

Patients and Methods

Type of Study: Case control study

Study Setting:

The study was conducted at Ain-shams university and Theodor Bilharz Research Institute (TBRI)

Study Population: -

Our study was performed on Fifty (50) Cirrhotic patient with ascites split into two groups:

Group 1: (the control group) comprised 25 patients with cirrhotic ascites who did not have SBP.

Group 2: (the SBP group) comprised 25 patients with cirrhotic ascites and SBP.

-Inclusion Criteria:

- 1-Egyptian patient
- 2-Age above 18 years old, including males and females.
- 3-Accepting participation in our study
- 4-Patients has chronic decompensated liver disease

Exclusion Criteria:

- 1-Ascitic patients due to any cause rather than liver cirrhosis
- 2-Patient has evidence of active infection rather than Ascetic Fluid infection cause
- 3-Patients with pre hospitalization antibiotic administration
- 4-Patients with any other cause such as pancreatitis, peritoneal carcinomatosis, and haemorrhagic ascites
- 5-Patient with a history of abdominal surgery within 3 months of the study.

Sampling Method:

A random sample of patients of Ain-shams university and Theodor Bilharz Research Institute (TBRI) was subjected to measure the level of Calprotectin, CRP in Ascetic fluid.

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Sample Size:

Using PASS11 program for sample size calculation (selim F o et al ,2019) detected that ascetic fluid calprotectin has a high diagnostic accuracy in detection of spontaneous bacterial peritonitis (AUC=0.967)

based on these result a sample size of 50 patients (25/group) will be enough to achieve study objectives

Ethical Considerations:-

All cases were informed about the aim of the study then they signed a consent form.

All patients will undergo: -

- 1- Taking history and Clinical examination
- 2-Liver function tests:alanine, , serum bilirubin aspartate aminotransferase, aminotransferase, serum albumin.
- 3--Complete blood picture
- 4-Abdominal ultrasonography was performed to assess the size of the liver and spleen, the degree of ascites, the presence or absence of any localized lesions, and the hepatic, portal, and splenic vein diameters.
- 5- CRP was assessed quantitatively in Ascetic fluid by Immunoturbidimetric method.
- 6-AsceticFluid calprotectin was measured by ELISA.

Diagnostic abdominal paracentesis:

It was performed under complete aseptic conditions. Samples was withdrawn from each patient and divided as follows: samples for examining differential PMN count by Leishman stain and total leukocytic count (TLC) on automatic cell counter. serum-ascites albumin gradient will be estimated. A 5 ml of Ascetic Fluid will be collected then measure calprotectin, using ELISA.

Statistical analysis:

The Statistical Package for Social Sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA), was used to analyze the recorded data. Quantitative data has been presented as the mean \pm SD. Qualitative data has been presented as percentages and frequencies.

We performed the following tests:

- When comparing two means, the independent-samples t-test of significance was employed.
- The Mann-Whitney U test is used when comparing two groups of non-parametric data.

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- We compared the proportions between the qualitative factors using the Chi-square (χ^2) test of significance.

- The Fisher's exact test is thought to be more accurate compared with the chi-squared test when the predicted numbers are small. It was employed to investigate the link between two qualitative variables when the predicted count was less than 5 in more than 20% of the cells.

- If one or both of the sets of variables were skewed, the degree of relationship between them was determined using Spearman's rank correlation coefficient (rs).

Value of "r" ranges from -1 to 1

0 = no linear correlation

1=perfect positive correlation

-1=perfect negative correlation

- Positive = Increase in the independent variable which lead to increase in the dependent variable.
- Negative= Increase in the independent variable which lead to decrease in the dependent.
- Scatter plot: It's a graph in which two variables' values are displayed along two axes, and the pattern of the resultant points shows whether there is a correlation.
- Receiver operating characteristic (ROC curve) analysis has been utilized for determining the optimal cut-off value at which to detect both sensitivity and specificity.
- Sensitivity = (true +ve)/ [(true +ve) + (false -ve)].
- Specificity = (true -ve) / [(true -ve) + (false +ve)].
- PPV = (true +ve) / [(true +ve) + (false +ve)].
- NPV = (true -ve)/ [(true -ve) + (false -ve)].

$$\text{Accuracy} = (TP+TN)/[TP+FP+TN+FN]$$

- The margin of error accepted has been set at 5%, whereas the confidence interval has been set at 95%. So, the following p-value has been regarded as significant:
- Probability (P-value)
- P-value <0.05 is significant.
- P-value <0.001 is highly significant.
- P-value >0.05 is insignificant.

Results

This study has been done on group ranging age from 47 to 57 years, (mean age of 61.82±7.92 years). There was female predominance with female to male ratio about 1.08:1.

Table (1): Comparison between non-SBP and SBP groups based on age and sex demographics.

Demographic data	Non SBP Group (n=25)	SBP Group (n=25)	Test	p-value
Age (years) Mean±SD Range	60.20±8.86 47-75	63.04±6.86 52-74	T=-1.267	0.211
Sex Female Male	12 (48.0%) 13 (52.0%)	14 (56.0%) 11 (44.0%)	$\chi^2=0.080$	0.777

Using: *t*-Independent Sample *t*-test; χ^2 : Chi-square test *p*-value >0.05 NS

The two groups' mean ages were comparable, with 63.04±6.86 for the SBP group and 60.20±8.86 for the non-SBP group, respectively. The two groups do not differ statistically significantly (*p* = 0.211). This table also showed the sex were comparable in each of Non-SBP group were 12 patients (48%) and

13 patients (52%) were male and female, respectively in comparison with SBP Group were 14 patients (56%) and 11 patients (44%) were female and male respectively. There isn't a statistically significant difference in terms of sex between the two groups (*p* = 0.478).

Table (2): Comparison of the non-SBP group and the SBP group according to their CBC regarding Hb., TLC, and PLT.

CBC	Non SBP Group (n=25)	SBP Group (n=25)	Test	p-value
HB Mean±SD Range	9.93±1.60 7-13	9.92±2.13 6.6-14.2	T=0.015	0.988
TLC Mean±SD Range	6.56±3.43 3.1-17	8.59±4.35 1.6-24.26	Z=-2.387	0.017*
PLT Mean±SD Range	119.28±55.70 33-250	141.68±72.19 53-376	Z=-1.126	0.26

Using: *t*-Independent Sample *t*-test; *z*-Mann-Whitney test *p*-value >0.05 NS; **p*-value <0.05 S

TLC revealed a significantly significant difference (*p*<0.05) between the two groups. Comparing the SBP Group (8.59±4.35) to the non-SBP Group (6.56±3.43), the SBP Group had the highest value. However, according

to Hb and PLT, with a *p*-value (>0.05 NS), there's no statistically significant difference between the two groups.

Table (3): Comparison Non SBP Group and SBP Group according to their liver function regarding albumin, AST, ALT and bilirubin.

Liver-Function	Non SBP Group (n=25)	SBP Group (n=25)	Test	p-value
Albumin				
Mean±SD	2.70±0.61	2.57±0.48	T=0.864	0.392
Range	1.7-4.6	1.8-3.7		
AST				
Mean±SD	70.75±56.65	59.04±39.24	Z=-0.359	0.72
Range	19-270	9-180		
ALT				
Mean±SD	36.71±21.96	34.52±18.01	Z=-0.146	0.884
Range	9-76	12-89		
Bilirubin				
Mean±SD	2.35±2.06	5.38±5.00	Z=-2.213	0.027*
Range	0.4-10.3	0.4-16.8		

Using: *t*-Independent Sample *t*-test; *z*-Mann-Whitney test *p*-value >0.05 NS; **p*-value <0.05 S

There was a statistically significant difference between the two groups according to bilirubin ($p < 0.05$). In comparison to the non-SBP group (2.35±2.06), the SBP group had the highest value (5.38±5.00). However,

with a *p*-value (>0.05 NS), there's no statistically significant difference between the two groups in terms of albumin, AST, and ALT.

Table (4): Comparison Non SBP Group and SBP Group according to their ascetic fluid biochemical regarding AF Albumin, AF CRP, AF Calprotectin, Calprotectin - Albumin Ratio, AF TLC and Neutrophil%.

Ascetic Fluid Biochemical	Non SBP Group (n=25)	SBP Group (n=25)	Test	p-value
AF Albumin				
Mean±SD	0.83±0.58	0.67±0.46	Z=1.329	0.184
Range	0.15-2.32	0.21-2.17		
AF CRP				
Mean±SD	8.73±7.04	21.09±15.86	Z=2.562	0.010*
Range	1.4-30.4	1.3-67.2		
AF Calprotectin				
Mean±SD	119.30±111.31	196.72±135.00	Z=3.085	0.002*
Range	31.6-483.4	61.2-550		
Calprotectin - Albumin Ratio				
Mean±SD	187.83±186.07	379.60±354.71	Z=3.269	<0.001**
Range	41.15-792.46	81.32-1677.62		
AF TLC				
Mean±SD	196.00±105.99	756.04±513.50	Z=6.004	<0.001**
Range	100-500	400-3001		
Neutrophil%				
Mean±SD	54.07±20.35	63.77±14.83	Z=1.893	0.058
Range	19-93	42-91		

Using: *t*-Independent Sample *t*-test; *z*-Mann-Whitney test *p*-value >0.05 NS; **p*-value <0.05 S

The two groups' AF CRP levels ($p < 0.05$) differed statistically significantly. The highest value was detected in the SBP group (21.09±15.86) compared to the non-SBP group (8.73±7.04).

Also shown in this table is the statistically significant difference in AF Calprotectin ($p < 0.05$) between the two groups. The highest value was detected in the SBP group (196.72±135.00) compared to the non-SBP group (119.30±111.31).

The findings also indicate that the calprotectin-albumin ratio ($p < 0.001$) differed statistically significantly between the two groups. The highest value was found in SBP Group (379.60 ± 354.71) in comparison with Non SBP Group (187.83 ± 186.07).

The table indicates that there was a significant statistical difference in the AF TLC ($p < 0.001$) between

the two groups. The highest value was detected in SBP Group (756.04 ± 513.50) in comparison with Non SBP Group (196.00 ± 105.99).

While, there is no statistically significant difference between two groups in terms of AF Albumin and Neutrophil%, with p-value (> 0.05 NS).

Table (5): Correlation between AF CRP, AF Calprotectin and Calprotectin - Albumin Ratio with different parameters, using Pearson Correlation Coefficient, in the Non SBP Group.

Non SBP Group	AF CRP		AF Calprotectin		Calprotectin-Albumin Ratio	
	r	p-value	r	p-value	r	p-value
AF CRP	--	--	0.504	0.010*	0.493	0.042*
AF Calprotectin	0.504	0.010*	--	--	0.827	<0.001**
Calprotectin - Albumin Ratio	0.493	0.042*	0.827	<0.001**	--	--
Age (years)	-0.140	0.504	0.093	0.660	-0.061	0.772
HB	0.063	0.763	0.010	0.962	-0.103	0.624
TLC	-0.046	0.826	0.308	0.135	0.269	0.193
PLT	0.076	0.718	0.349	0.088	0.149	0.476
Albumin	0.308	0.135	0.154	0.462	-0.288	0.162
Ast	0.372	0.067	0.539	0.005*	0.503	0.010*
Alt	-0.017	0.936	0.063	0.764	0.007	0.974
Billirubin	0.024	0.909	0.053	0.802	0.089	0.672
AF Albumin	0.155	0.459	0.188	0.367	-0.348	0.089
AF TLC	0.348	0.088	0.627	<0.001**	0.326	0.112
Neutrophil%	0.060	0.774	-0.063	0.764	-0.004	0.984
Spleen measurment (cm)	0.102	0.627	-0.185	0.377	-0.253	0.222
PT	0.222	0.285	0.063	0.765	0.070	0.738
PC	-0.182	0.384	-0.028	0.896	-0.116	0.581
INR	0.310	0.132	0.198	0.344	0.198	0.343

**Highly statistical significant differences ($p < 0.001$). *A statistical significant difference ($p < 0.05$)
p-value > 0.05 NS r-Pearson Correlation Coefficient

There were significant statistically correlation between AF CRP of the studied group according to their AF Calprotectin and Calprotectin - Albumin Ratio. It reveals that there was significant positive correlation with AF Calprotectin and Calprotectin-Albumin Ratio, with (p -value <0.05).

It shows that, there were significant statistically correlation between AF Calprotectin of the studied group according to their Calprotectin - Albumin Ratio, AST and AF TLC. It reveals that there was significant positive correlation with Calprotectin - Albumin Ratio, AST and AF TLC, with (p -value <0.05). Also, there was statistically significant correlation between

Calprotectin - Albumin Ratio of the studied group according to their AST. It reveals that there was

significant positive correlation with AST, with (p-value<0.05).

Table (6): Correlation between AF CRP, AF clprotectin and Calprotectin - Albumin Ratio with different parameters, using Pearson Correlation Coefficient, in the SBP Group.

SBP Group	AF CRP		AF Calprotectin		Calprotectin - Albumin Ratio	
	r	p-value	r	p-value	r	p-value
AF CRP	--	--	0.439	0.014*	0.396	0.034*
AF Calprotectin	0.439	0.014*	--	--	0.566	0.003*
Calprotectin - Albumin Ratio	0.396	0.034*	0.566	0.003*	--	--
Age (years)	0.187	0.372	-0.199	0.341	-0.172	0.410
HB	0.060	0.777	0.212	0.309	0.034	0.871
TLC	0.219	0.292	0.278	0.179	-0.241	0.245
PLT	-0.459	0.021*	-0.156	0.458	-0.168	0.423
Albumin	-0.124	0.554	-0.294	0.153	-0.604	<0.001**
Ast	0.163	0.437	-0.111	0.599	-0.005	0.982
Alt	0.196	0.348	-0.259	0.212	-0.203	0.331
Billirubin	0.052	0.806	-0.330	0.107	-0.246	0.236
AF Albumin	0.043	0.838	0.365	0.073	-0.362	0.075
AF TLC	0.292	0.156	0.337	0.099	-0.057	0.786
Neutrophil%	-0.166	0.428	-0.133	0.525	-0.035	0.868
Spleen measurement (cm)	-0.099	0.639	-0.254	0.220	-0.137	0.514
PT	-0.050	0.811	-0.302	0.142	0.188	0.369
PC	-0.300	0.145	0.070	0.741	-0.089	0.672
INR	0.300	0.145	0.201	0.336	0.167	0.425

**Highly statistical significant differences ($p < 0.001$). *A statistical significant difference ($p < 0.05$)
p-value >0.05 NS r-Pearson Correlation Coefficient

There were statistically significant correlation between AF CRP of the studied group according to their AF Calprotectin, Calprotectin - Albumin Ratio and PLT. It reveals that there was significant positive correlation with AF Calprotectin, Calprotectin-Albumin Ratio, while PLT significant negative correlation, with (p-value<0.05).

It shows that, there were statistically significant correlation between AF Calprotectin of the studied

group according to their Calprotectin - Albumin Ratio. It reveals that there was significant positive correlation with Calprotectin-Albumin Ratio, with (p-value<0.05). This table shows that, there were significant statistically correlation between Calprotectin - Albumin Ratio of the studied group according to their Albumin. It reveals that there was significant negative correlation with Albumin, with (pvalue<0.05).

Variable	Cutoff	Sen.	Spe.	PPV	NPV	AUC [95% C.I.]	p. value
AF CRP	>22.6	88%	84%	84.6%	87.5%	0.914 [0.80-0.98]	<0.001
AF Calprotectin	>73.5	96%	89%	85.7%	95.5%	0.960 [0.86-0.99]	<0.001
Calprotectin-Ascetic Albumin ratio	>171.4	80%	86%	85.2%	82.8%	0.878 [0.76-0.95]	0.013

Receiver operator characteristics (ROC) curves were constructed for AF CRP, AF Calprotectin and Calprotectin -Ascetic Albumin ratio for spontaneous bacterial peritonitis. All variable indices consider

significant predictors as it showed by large area under the curves (AUCs); with AF Calprotectin is consider most valuable predictor with [AUC 0.960 and p-value <0.001 highly significant], AF CRP with [AUC 0.914

and p-value <0.001 highly significant], and also Calprotectin –Ascetic Albumin ratio with [AUC 0.878 and p-value <0.05 significant]. This indicates that the AF CRP, AF Calprotectin and Calprotectin –Ascetic Albumin ratio the most diagnosis of SBP.

Discussion

SBP is considered a fatal infection that affects cirrhotic patients with different symptoms. The diagnosis is made by distinguishing it from secondary peritonitis (Song et al., 2019).

Theodor Bilharz Research and Ain Shams University Hospital conducted this case-control study in order to assess the utility of ascetic fluid calprotectin, CRP, and calprotectin-to-albumin ratio in the development of a significant clinical diagnosis of SBP in the future. A total of fifty cirrhotic patients who were diagnosed with ascites were split into two groups: group 1 (the non-SBP control group) consisted of 25 patients who had ascites without SBP, and group 2 (the SBP group) consisted of 25 patients who had ascites with SBP.

The present study's statistical analysis revealed that, with respect to mean age, which was 60.20 ± 8.86 in comparison to 63.04 ± 6.86 ($p = 0.211$), there wasn't a statistically significant difference between the two groups. Moreover, with regard to sex, 12 patients (48%) and 13 patients (52%) were female and male, respectively, in comparison with 14 patients (56%) and 11 patients (44%) in the SBP Group ($p = 0.478$). This agrees with the study of Rahman et al. (2020) who assessed ascitic calprotectin for SBP diagnosis. This study involved 80 patients out of 300 patients. Patients have been split into 2 equal groups; with and without SBP. Nasereslami et al. (2020) who, assessed the value of calprotectin in early diagnosis of SBP in cirrhotic patients and investigated the prognosis value in determination of the 6-month outcome. As well, Ali and Mohamed (2019) evaluated AF calprotectin as a diagnostic marker of SBP. 72 patients with ascites had cirrhosis in this research. They have been split into two groups: 50 patients with SBP and 22 ascitic patients with cirrhosis without evidence of SBP.

Regarding CBC parameters, statistical analysis of current findings indicated that there was a difference between two groups in terms of TLC ($p < 0.05$). The highest value has been detected in SBP Group (8.59 ± 4.35) in comparison with Non SBP Group (6.56 ± 3.43).

Rahman et al. (2020) agreed with current study and stated that both groups had comparable blood hemoglobin and patients with SBP had statistically significant higher WBCs (9800 vs. 4150 μL)

Ali and Mohamed (2019) agreed with current study and stated that, serum total leukocytic count was statistically significant.

Regarding liver function tests, statistical analysis of current results showed that there was a valuable difference between the two groups in terms of bilirubin ($p < 0.05$). The highest value was detected in SBP Group (5.38 ± 5.00) in comparison with Non SBP Group (2.35 ± 2.06). A statistically significant difference was not shown between the two groups according to albumin, AST, or ALT, with a p-value > 0.05.

Rahman et al. (2020) agreed with current study and stated that both groups had comparable serum AST and ALT. Patients had SBP has higher median total bilirubin (4.2 vs. 2.3 mg/dL).

Nasereslami et al. (2020) agreed with current study and stated that SBP positive patients had markedly higher levels of total bilirubin compared to SBP negative group ($p\text{-value} < .01$).

Ali and Mohamed (2019) agreed with this study and showed that the comparison of total bilirubin of the SBP group and non-SBP group was significant statistically. The comparison between both groups for alanine aminotransferase, total serum protein, and serum albumin was not significant statistically. But they disagreed with current study and stated that aspartate aminotransferase was significantly higher in patient with spontaneous bacterial peritonitis.

Regarding ascetic fluid biochemicals, the current findings indicate that there was a statistically significant difference ($p < 0.05$) in AF CRP, AF Calprotectin, Calprotectin-Albumin Ratio, and AF TLC between the two groups. The highest value has been detected in SBP Group (21.09 ± 15.86), (196.72 ± 135.00), (379.60 ± 354.71) and (756.04 ± 513.50) compared to Non SBP Group (8.73 ± 7.04), (119.30 ± 111.31), (187.83 ± 186.07) and (196.00 ± 105.99) respectively. However, AF albumin and neutrophil% did not show a statistically significant difference between the two groups ($p\text{-value} > 0.05$ NS).

Rahman et al. (2020) agreed with the current study and stated that SBP patients exhibited higher CRP (49 vs. 12 mg/dL) and ascetic calprotectin (7.57 vs. 1.1 ng/mL). Nasereslami et al., 2020 also agreed with this study and stated that patient with SBP positive, calprotectin was markedly higher than un-infected patients. The relation between calprotectin to the total protein in patient with ascites is higher in the SBP group.

As well, did the study of Ali and Mohamed (2019). It stated that there was a significant statistical difference between the two groups based on TLC, CRP, and AF calprotectin (ng/ml), as they were elevated in SBP more than non-SBP group ($P < 0.001$ for each). Also, AF albumin didn't show statistically significant different. However, it disagreed with current study and stated that neutrophils were higher in SBP cases. Fouda et al (2018) which assessed the role of ascetic

fluid calprotectin in diagnosis of SBP also agreed with current study and stated that ascetic calprotectin has been elevated in SBP patients if it compare with non-SBP patients.

The current results illustrate that there was significant statistically positive correlation between AF CRP, AF Calprotectin of the studied group to their Calprotectin - Albumin Ratio, AST and AF TLC.

Hadjivasilis et al. (2021) assessed the accuracy of ascetic calprotectin fluid in confirm of SBP in ascetic patient. There are 10 studies in this study. Ascetic samples have been split into patient with a SBP and patient with a non-SBP to compare the two samples., 406 samples with SBP and 635 from patients without. They agreed with current study and stated that all previous studies had relation between the calprotectin in ascetic patient and PMN count.

Nasereslami et al. (2020) stated that calprotectin in ascites is positively related with total leukocyte counts in ascetic fluid ($r=.53$, $p\text{-value}<.001$) and PMN counts ($r=.54$, $p\text{-value}<.001$) but they was against current study and stated that there is no correlation was seen between calprotectin and albumin in ascetic fluid ($p\text{value}= .69$).

Weil et al. (2019) evaluated value of the level of calprotectin in ascetic fluid (AF), to state the (SBP). They collected 236 AF samples from 119 patients with cirrhosis hospitalized including 36 patient with SBP (15.2% in 23 patients) and 200 non-SBP (84.8%). They agreed with current study and showed that calprotectin levels were positively correlated with TLC in AF ($r=0.57$, $P<0.001$ and $r=0.33$, $P<0.001$), C-reactive protein (CRP; $r=0.43$, $P<0.001$ and $r=0.19$, $P = 0.036$). However, calprotectin levels were positively correlated with total albumin concentration in AF which disagreed with current study.

Ali and Mohamed (2019) agreed with current study and stated that there was a strong relation between calprotectin ascitic fluid and main parameters [AF TLC, AF PMN, AF calprotectin–albumin ratio, AF calprotectin–protein ratio, serum and AF CRP]. Also, the study stated that, there was a no relation between AF calprotectin and prothrombin concentration and serum albumin.

Fouda et al. (2018) agreed with current study and stated that ascetic fluid calprotectin positively correlated well with the TLCs count in ascetic fluid and reliably diagnose SBP.

Statistical analysis of current results showed that all indices were significant, as stated by the large AUCs; with AF Calprotectin at cut-off value $>73.5\text{ng}$ with sensitivity 96%, specificity 89%, PPV 85.7% and NPV 95.5% being the most significant predictor followed by AF CRP at cut-off value >22.6 also AF Calprotectin – Albumin ratio at cut-off value >171.4 with

specificity 86%, sensitivity 80%, PPV 85.2% and NPV 82.8%.

Hadjivasilis et al. (2021) agreed with current study and stated that ascetic calprotectin consider to be an valuable alternative to PMN leucocyte count to confirm SBP with more faste time to confirm diagnosis. While specificity was 89% (95% CI 80–95%), the sensitivity of a "positive" ascetic fluid calprotectin in SBP was 93% [95% CI 90–95%].

Rahman et al. (2020) agreed with current study but with different values and stated that the independent predictors of SBP were serum CRP (odds = 1.1), and ascitic calprotectin (odds = 7.4). Ascitic calprotectin >2 ng/mL had 92.5% specificity 90% sensitivity, 92.5%, 92.3% PPV and 90.2% NPV (AUC 0.963, 95% C.I 0.895–0.992, $P = 0.001$). On multivariate analysis, only ascitic calprotectin can consider (odds = 13.1) the independent predictor of SBP.

Dibas et al. (2020) showed a meta-analysis systematic review to study asceti meta-analysis c calprotectin for the diagnosis of spontaneous bacterial peritonitis. They included 10 studies that confirm that calprotectin as a diagnostic marker for SBP in patients with cirrhosis.

Nasereslami et al. (2020) agreed with current study but with anther different values and stated that calprotectin in ascetic fluid at value of 168 ng/ml

77.4% for specificity and 85.7 for sensitivity (AUC = 0.815, $p\text{-value}<.001$). Additionally, according to ROC analysis, an AUC of 0.840 ($p<.001$) and a sensitivity of 88% and a specificity of 83.9% were obtained with a calprotectin/protein ratio of 101. Unfortunately, they cannot confirm the diagnostic value of calprotectin/protein ratio to calprotectin alone.

Weil et al (2019) is with the study and stated that patients has ascitic SBP had higher median levels of calprotectin (1.81 $\mu\text{g/mL}$) than patients with no SBP (0.25 $\mu\text{g/mL}$; $P<0.001$). Using percentiles of calprotectin range, the best threshold value optimizing the Youden Index (sensitivity + specificity-1) to diagnose SBP was 1.51 $\mu\text{g/mL}$.

Ali and Mohamed (2019) agreed with current study but with different values and stated that From the ROC curve, the optimum calprotectin level cutoff point for the SBP diagnosis was 372 ng/ml with a, PPV, NPV, and accuracy of 100% for each value overall, with an AUC of 1.000 ($P<0.001$). Moreover, ROC curve analysis asse that the calprotectin-to-albumin ratio cutoff points for SBP in cirrhotic patient was 535.25 ng/ml, with a specificity, sensitivity, PPV, NPV, and accuracy of 78, 77.3, 88.64, 60.71, and 77.78%, respectively, with an AUC of 0.814 ($P<0.001$). The ROC analysis for AF CRP stated that the cutoff value was 6.05 mg/dl, with a specificity, sensitivity, NPV, PPV, and all accuracy of 96, 100, 100, 91.67, and

97.22%, respectively, with an AUC of 0.999 ($P < 0.001$).

There are a large variation in cut off values of different studies this different is might be due to small sample size or might be needed to be done in larger scale.

The value of the study was result to every effort to collect all data of follow-up. All clinical data, paracentesis sonographic measurement and results of study were done by the same team.

Summary

Cirrhosis represents a common variety of chronic liver diseases. Cirrhosis is described as diffuse fibrosis that converts normal liver architecture into a structurally aberrant nodule. Some cirrhosis patients have no symptoms at all and lead normal lives (**Friedman et al., 2008**).

Ascites is one of the major complications of portal hypertension and liver cirrhosis. The ascites is associated with a poor prognosis, (**Biecker, 2011**).

SBP is a common, deadly bacterial infection of the ascites that occurs in cirrhotic people (**Dever and Sheikh, 2015**).

Signs and symptoms of spontaneous bacterial peritonitis (SBP) include abdominal pain and chills, and nausea fever, vomiting, tenderness, abdominal pain altered mental status, and worsening ascites (**Lata et al., 2009**).

No early indicators for SB were identified. Reagent strips were tested, but the findings are inaccurate for detecting SB. According to a recent guideline, patients who have ascites and PMN counts $< 250/\text{mm}^3$ should treat any infection-related symptoms with antibiotics while they wait for culture findings, which may be used to treat SB. Nevertheless, the "signs of infection" contain just a few clinical symptoms and no objective laboratory indicators, which leads to antibiotic abuse or treatment delays (**Zhuet et al., 2016**).

Monocyte membranes and the cytoplasm of neutrophils both contain calprotectin. Calprotectin is secreted and can be found in serum or bodily fluids, serving as a helpful clinical inflammatory marker.

(**Stríz and Trebichavský, 2004**).

The liver produces the protein CRP, which is elevated in cases of widespread, systemic inflammation. The CRP test is one of several tests for diagnosis utilized by hospitals and labs to keep an eye on inflammatory conditions (**Vihari et al., 2015**).

The liver produces serum albumin. The oncotic pressure required for the distribution of bodily fluids between blood vessels and tissues in the body is maintained by albumin; in the absence of albumin, the high blood vessel pressure would force more fluids into the tissues. It also functions as a transport protein for fatty acids and hemin, as well as a plasma carrier by non-specifically attaching a number of hydrophobic steroid hormones (**Hawkins and Dugaiczky, 1982**).

It was found that calprotectin to albumin ratio was more higher in SBP patients who died in the hospital than the patients with SBP who survived. The calprotectin-to albumin ratio in ascites is valuable in the SBP diagnosis (**Makhlouf et al., 2018**).

At Ain Shams University Hospital and Theodorbilharz Research Institute, a case-control study was conducted to evaluate ascetic fluid calprotectin, CRP, and the calprotectin-to-albumin ratio for the SBP diagnosis. Additionally, a clinically useful cut-off level was identified to potentially serve as a cost-effective method for SBP diagnosis.

A total of fifty cirrhotic patients who were diagnosed with ascites were split into two groups: group 1 (the non-SBP control group) consisted of 25 patients who had ascites without SBP, and group 2 (the SBP group) consisted of 25 patients who had ascites with SBP.

In regard to demographic data, statistical analysis of the present findings revealed that there existed no statistically significant difference between the two groups with respect to mean age, which was 60.20 ± 8.86 compared with 63.04 ± 6.86 ($p = 0.211$). In terms of sex, 12 patients (48%) and 13 patients (52%) were female and male, respectively, in comparison to the SBP Group, which had 14 patients (56%) and 11 patients (44%), who were of the same sex ($p = 0.478$). In terms of CBC parameters, the statistical analysis of the present outcomes indicated a statistically significant difference ($p < 0.05$) in TLC between the two groups. In comparison to the non-SBP group (6.56 ± 3.43), the SBP group had the highest value (8.59 ± 4.35). However, the p-value (> 0.05 NS) indicated that there wasn't a statistically significant difference between the two groups in terms of Hb and PLT.

Regarding liver function tests, statistical analysis of current results showed that there was a significant statistical difference between two groups in terms of bilirubin ($p < 0.05$). In comparison to the non-SBP group (2.35 ± 2.06), the SBP group had the highest value (5.38 ± 5.00). However, with a p-value (> 0.05 NS), there was no statistically significant difference between the two groups in terms of albumin, AST, or ALT.

In regard to ascetic fluid, the statistical analysis of the present findings revealed a statistically significant difference in AF CRP, AF Calprotectin, Calprotectin-Albumin Ratio, and AF TLC ($p < 0.05$) between the two groups. In comparison to the non-SBP group (8.73 ± 7.04), (119.30 ± 111.31), (187.83 ± 186.07), and (196.72 ± 135.00), the SBP group had the highest values (21.09 ± 15.86), (196.72 ± 135.00), (379.60 ± 354.71), and (756.04 ± 513.50), respectively. However, the p-value (> 0.05 NS) indicated that there wasn't a statistically significant difference between the two groups in terms of AF albumin and neutrophil%.

Analysis of this results statistically stated that there were valuable significant statistically positive correlation between AF CRP, AF Calprotectin of the studied group to their AF Calprotectin and Calprotectin - Albumin Ratio. Statistical analysis of these results showed that all variable indices were considered significant variables, as shown by the significantly large AUCs, with AF Calprotectin at a cut-off value >73.5 being the most significant predictor, followed by AF CRP at a cut-off value >22.6 and also the Calprotectin-Ascetic Albumin ratio at a cut-off value >171.4 .

This indicates that the AF CRP, AF Calprotectin and Calprotectin – Ascetic Albumin ratio is considering the most significant diagnosis of spontaneous bacterial peritonitis.

The importance of this study stemmed from the fact that every effort was made to ensure that all follow-up data were correct and that only full information had been included in the analysis of the data. The same team performed all clinical evaluations, sonographic measurements, and study evaluations via paracentesis. The COVID-19 pandemic, the comparatively small sample size, and the researcher's blinding were the reasons behind the study's limitations.

Conclusion:

The diagnosis of SBP in cirrhotic ascitic patients appears to be satisfactorily accurate when based on significant and sensitive diagnostic criteria such as AF Calprotectin, AF CRP, and the Calprotectin-Ascetic Albumin ratio.

When diagnosing patients with SBP, AF calprotectin exhibits greater sensitivity and specificity compared to AF CRP and AF calprotectin albumin ratio.

Among patients who have SBP, there was a significant positive association between AF Calprotectin and AF CRP, as well as the AF Calprotectin albumin ratio.

Measurements of AF CRP, AF Calprotectin, and Calprotectin-Ascetic Albumin ratio may be utilized, if there are no contraindications, to diagnose SBP with a high degree of diagnostic accuracy.

Larger studies are needed to evaluate AF calprotectin and AF CRP and AF calprotectin to albumin ratio before and after treatment in order to validate their use as a marker of recovery.

Due to relatively small sample size in the current study, so it needed more studies with more data and larger sample size for management

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