Study of Ascetic Fluid Lactoferrin and Calprotectin as Diagnostic Markers in Cirrhotic Patients with Spontaneous Bacterial Peritonitis

Original Article

Mohamed Gamal, Omar Aly Hassan, Khalid Abd-Elhamid Sayed and Ahmed Samir Abd-Elsadik

Department of Internal Medicine Gastroenterology and Hepatology, Faculty of Medicine, Ain Shams University, Egypt.

ABSTRACT

Background: Liver cirrhosis and ascites patients are at higher risk for spontaneous bacterial peritonitis (SBP), requiring early diagnosis using laboratory procedures. The study investigated the effectiveness of ascetic calprotectin and lactoferrin in diagnosing SBP in liver cirrhosis patients.

Subjects and Methods: This case-control study divided 50 patients with cirrhosis and ascites into two distinct groups: Group A, which included 25 patients without spontaneous bacterial peritonitis (SBP), and Group B, which included 25 patients with SBP. We conducted detailed medical histories, physical exams, lab tests (such as complete blood counts, renal function tests, CRP, HBA1c, and virological markers), echocardiograms (for patients aged 60 and up), pelviabdominal ultrasounds, and ascitic fluid analysis to gather information. We analyzed lactoferrin and calprotectin in ascitic fluid.

Results: The levels of calprotectin and lactoferrin are linked in a good way to white blood cells, ESR, CRP, and ascetic fluid PNL. Conversely, there is a negative correlation between the levels of calprotectin and albumin. Ascetic fluid calprotectin had the greatest AUC = 0.980 with a sensitivity of 92% and a specificity of 96% (*p-value* <0.001), and its cut-off point was >221. Ascetic fluid lactoferrin had the greatest AUC = 0.980 with a sensitivity of 92% and a specificity of 80% (*p-value* <0.001), and its cut-off point was >64.5.

Conclusion: Cirrhotic patients with SBP had higher levels of lactoferrin and ascetic fluid calprotectin than cirrhotic individuals without SBP. People with liver cirrhosis can use ascetic fluid lactoferrin and calprotectin as good diagnostic markers to check and diagnose SBP.

Key Words: High-sensitivity C-reactive protein, spontaneous bacterial peritonitis, polymorphonuclear cells.

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Corresponding Author: Mohamed Gamal, Internal Medicine Gastroenterology and Hepatology, Faculty of Medicine, Ain Shams University, Egypt, **Tel.:** + 201003620310, **E-mail**: m.gamal.yousef@med.asu.edu.eg

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INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is described as an ascetic fluid infection that does not have an obvious intra-abdominal surgically curable source; it is most common in individuals with advanced cirrhosis[1]. Patients with ascites due to severe cirrhosis who exhibit symptoms such as fever, abdominal pain/tenderness, and altered mental status should be suspected of having SBP. Additional symptoms of SBP include peripheral leukocytosis, metabolic acidosis, azotemia, hypothermia, paralytic ileus, diarrhea, and hypotension[2]. Due to the limited window of opportunity to treat and guarantee a good outcome, early detection of spontaneous bacterial peritonitis is crucial. Missing out on this window of opportunity causes shock, which in turn causes organ failure in multiple systems[3]. Current diagnostic criteria for SBP involve laboratory tests such as a positive bacterial culture of ascitic fluid and a high absolute polymorphonuclear leukocyte (PMN) count in the ascitic fluid (>250 cells/mm³), while excluding other causes of bacterial peritonitis. However, these methods have limitations: bacterial cultures often yield false negatives due to low bacterial load or previous antibiotic use, and PMN count determination can be time-consuming and subject to inter-observer variability[4- 6].

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Calprotectin, a calcium- and zinc-binding protein found in nearly all neutrophils, correlates with neutrophil influx and can be measured rapidly using bedside testing instruments. Elevated levels of ascitic calprotectin can reliably predict a PMN count (>250 cells/mm³), aiding in the prompt diagnosis of SBP[7].

Lactoferrin is an iron-binding protein found mostly in external secretions and PMNs, and it is produced during degranulation. During times of inflammation or infection, polymorphonuclear cells (PMNs) express and secrete lactoferrin, which causes its levels to increase throughout the body. As a biomarker for gastrointestinal diseases, it shows promise and could be reliable. Measuring lactoferrin levels in ascitic fluid could provide a rapid and reliable method for detecting PMNs and diagnosing SBP in cirrhotic patients[8]. Despite these promising biomarkers, there remains a gap in the clinical application and validation of ascitic calprotectin and lactoferrin as routine diagnostic tools for SBP. Our study aims to evaluate the efficacy of ascitic calprotectin and lactoferrin as diagnostic markers for SBP in cirrhotic patients, addressing this gap and potentially offering more reliable and quicker diagnostic options.

SUBJECTS AND METHODS

Diagnostic Research IV used a case-control approach to evaluate 50 randomly selected cases. We gave a unique identifier to patients who met the admission criteria to the unit and used a random number generator to select 25 patients for Group A (those with cirrhosis and ascites but without spontaneous bacterial peritonitis) and 25 patients for Group B (those with cirrhosis and spontaneous bacterial peritonitis). We will establish the SBP diagnosis based on the presence of a PMN count >250 cells/mm3 in the ascites without secondary peritonitis, regardless of the ascetic fluid culture results.

We recruited all the studied cases from the Hepatology and Gastroenterology Department at Ain Shams University's Nasser Institute for Research and Treatment Hospital in Egypt. The Ain Shams University Ethical Committee regulations governed all procedures in this study, and we obtained patient consent from all subjects. We obtained informed written consent from all patients after explaining the aim of the study. The Ethics Committee promptly clarified any unexpected risks to the patients. The participants' privacy was safeguarded as follows: Every patient had a code number, which served as a symbol for their name and address, stored in a special file. We exclusively used the research results for scientific purposes. We kept the names of the patients confidential during the research process.

Inclusion Criteria: Participants were adults hospitalized in our department with a diagnosis of cirrhosis and ascites during the designated research period.

Exclusion Criteria: Patients who have previously received antibiotic treatment, those who have a history of diabetes, high blood pressure, heart disease, inflammatory bowel disease, cholangiocarcinoma, hepatocellular carcinoma (HCC), colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, ulcerative colitis, HIV infection, and patients who have undergone abdominal surgery in the three months prior to admission were excluded.

Tool of data collections:

History: The patient's medical history was meticulously documented, including their demographic details, current and former medical conditions, symptoms, analysis of each symptom, etiology of cirrhosis, other gastrointestinal tract complaints, medications, and surgeries.

General examination: Along with taking vital signs (heart rate, temperature, and blood pressure), a thorough physical examination was performed, which included a visual inspection of the chest, abdomen, and heart, as well as a local abdominal examination (inspection, palpation, percussion, and auscultation).

Laboratory investigations: Complete blood count (CBC), renal function tests (creatinine and urea), coagulation profile, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), random blood sugar, hemoglobin A1C (HBA1c), and virological markers (HCVAb, HBsAg, HIV Ab) were estimated for all cases.

Echocardiogram: It was ordered for patients above 60 years old.

Pelviabdominal ultrasound: It was ordered for all cases.

Ascetic fluid analysis: Aseptically performed diagnostic paracentesis. As part of the ascetic fluid analysis, the person was physically checked, their protein, albumin, lactoferrin, and calprotectin levels were measured chemically, their total white blood cell and platelet counts were checked, and bacteria were tested. Commercially accessible quantitative sandwich enzyme-linked immunosorbent tests for both ascetic Calprotectin and Lactoferrin were evaluated using Abcam's Human Calprotectin ELISA kit has catalog number ab267628 and Abcam's Human Lactoferrin ELISA Kit has catalog number ab200015.

Predicting scores: At admission, every patient had their Child Turcotte Pugh (CTP) score checked to determine the extent of liver illness.

Peritoneal fluid examination technique: The patient was anesthetized locally before a sterile diagnostic paracentesis was performed at the bedside using a 23-G needle connected to a 30-cc syringe. Next, within two hours after aspiration, the ascetic fluid that had been collected into three tubes was evaluated. The first tube is for culture and sensitivity testing, the second for electrophoresis-based determination of calprotectin and lactoferrin levels, and the third for the same purpose.

Blood sample tests: Two tubes were utilized to collect peripheral venous blood from patients: one tube without anticoagulant and another tube with EDTA anticoagulant. The hemoglobin and platelet count were measured automatically using Sysmex, a device from Japan. Non-fasting blood glucose, aspartate aminotransferase, alanine aminotransferase, total bilirubin, albumin, and creatinine were quantified using the Erba Mannheim XL-180, Germany, automated system. Prothrombin activity, indicated as INR (International Normalized Ratio), was assessed in all patients during their initial visit using normal laboratory methods.

Ascetic fluid sample: Every patient had a diagnostic abdomen puncture done within 6 hours of admission and before any antimicrobial treatment began. Each patient had 10 milliliters of ascetic fluid drawn into sterile tubes during abdominal paracentesis. The tubes were then spun at 2000–3000 RPM for 20 minutes, and the sediment-free supernatant was collected. We stored the liquid remainder in 2-milliliter cryotubes at a temperature of -80 degrees Celsius.

Analysis of Ascetic Calprotectin: After gathering Ascetic samples and centrifuging them to obtain serum, we measured the quantity of calprotectin. We analyzed sera using an enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions.

Analysis of Ascetic Lactoferrin: Centrifuging ascetic samples for serum extraction allowed us to assess the lactoferrin content. We analyzed sera using an enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions.

Bacterial examination:

Sample Collection: We collected ascitic fluid samples using aseptic techniques. Each patient had 20 mL of ascitic fluid extracted using a sterile syringe. We conducted the collection under both aerobic and anaerobic conditions to ensure a thorough bacterial examination.

Culture Methods: The ascitic fluid samples were taken and then placed on different types of culture media to detect any potential bacterial infections. The media utilized encompassed various forms:

MacConkey Agar is a type of growth media that selectively promotes the growth of Gram-negative bacteria and aids in distinguishing them based on their ability to ferment lactose.

Agar, sometimes known as Nutrient Agar, is a versatile medium that supports the development of a broad range of organisms that do not require specific nutritional requirements.

Blood Agar is a nutrient-rich substance that promotes the growth of various organisms, including bacteria that are difficult to cultivate, and aids in identifying hemolytic activity.

Bacterial identification:

MacConkey Agar is mostly utilized for the purpose of isolating Gram-negative bacteria, specifically Escherichia coli and Klebsiella pneumoniae.

Blood agar is utilized to identify microorganisms such as Streptococcus species and Staphylococcus species.

Nutrient Agar is employed to cultivate and distinguish diverse bacterial species.

Statistical analysis: Conducting statistical analyses utilizing the SPSS (Statistical Package for the Social Sciences) software (version 26; Inc., Chicago. IL). Mean μ, standard deviation (SD), and range were used to convey descriptive data, whereas numbers and percentages were used to express qualitative data. To compare the means and standard deviations of two sets of quantitative normally distributed data, a student t-test would be employed. To determine the association between the qualitative variables, we performed a chi-square test. Correlation coefficients for non-parametric and parametric values using Spearman and Pearson regression. To find the cutoff value with the best sensitivity and specificity, the Receiver Operating Characteristic (ROC) curve will be used. When the *p-value* is less than 0.05, it is deemed statistically significant.

RESULTS

(Table 1) illustrates the comparison of studied demographic data and biochemical parameters using the independent student T test, chi-square test, and Mann-Whitney U test, as well as the significant difference (*P-value*) between the two studied groups. The analysis showed that there is no statistically significant difference between patients with and without SBP regarding age, sex, child score, or classification. Among our studied population, bleeding, abdominal pain, and fever were statistically significantly more frequent in patients with SBP than those without SBP. And patients with SBP have a statistically significantly higher leucocyte count, ESR, and CRP than those without SBP. Additionally, patients with SBP have statistically significant higher levels of bilirubin, ALT, AST, and lower albumin levels compared to those without SBP. Finally, patients with SBP have statistically significantly higher levels of ascetic fluid PNL, calprotectin, and lactoferrin than those without SBP.

Table 1: Comparison of demographic data and biochemical parameters of the studied population.

		SBP	No SBP	Independent student T test/ chi-square test	
		N=25	N=25	t/X2	*p-value*
Age years	Range	42-62	45-63	-0.617	0.540
	Mean ± SD	51.48 ± 4.47	52.28 ± 4.70		
Sex	Male	13 (52%)	11 (44%)	0.321	0.571
	Female	12 (48%)	14 (56%)		
Classification	A	0 (0%)	1 (4%)		
	B	10 (40%)	10 (40%)	1.034	0.596
	C	15 (60%)	14 (56%)		
Bleeding	Yes	12 (48%)	5 (20%)	4.367	0.037
Abd. pain	Yes	17 (68%)	2 (8%)	19.100	<0.0001
Fever	Yes	8 (32%)	0 (0%)	9.524	0.002
Encephalopathy	Yes	3 (12%)	4 (16%)	0.166	0.684
Jaundice	Yes	21 (84%)	17 (68%)	1.754	0.185
Child score	Range	8 - 13	8 - 12	0.719	0.476
	Mean ± SD	10.28 ± 1.40	10.00 ± 1.35		
HB gm/dl	Mean ± SD	10.71±1.40	9.73±1.19	2.674	0.010
HCT	Mean ± SD	32.18±4.41	29.71±4.47	1.968	0.055
WBC thousand/mm3	Mean ± SD	9.58±4.47	8.08±2.76	1.433	0.160
Platelet thousand/ mm3	Mean ± SD	128.3±66.29	96.12±57.89	1.832	0.073
INR	Mean ± SD	1.54±0.34	1.48±0.24	0.716	0.477
ESR	Mean ± SD	43.40±15.36	11.64±2.87	10.165	<0.0001
CRP mg/dl	Mean ± SD	18.57±17.01	2.95±1.54	4.570	<0.0001
Albumin mg/dl	Mean ± SD	2.53±0.53	2.86±0.44	-2.357	0.023
Bilirubin mg/dl	Mean ± SD	2.68±1.74	2.10±1.38	1.303	0.199
ALT IU	Mean ± SD	107.6±94.42	37.88±14.47	3.653	0.001
AST IU	Mean ± SD	56.24±108.6	33.36±14.15	1.044	0.306
Creatinine mg/dl	Mean ± SD	0.92±0.22	0.80±0.22	1.846	0.071
Glucose mg/dl	Mean ± SD	93.83±6.39	94.64±6.73	-0.435	0.665

The results of ascetic fluid chemical analysis illustrated in (Table 2), and the results revealed that There is statistically significant higher ascetic fluid PNL, Calprotectin, Lactoferrin in patients with SBP than those without SBP.

Table 2: Comparison of ascetic fluid findings of the studied population.

		SBP	No SBP	Independent student T test	
		N=25	N=25	T	*p-value*
PNL	Range	255 - 325	100 - 195	16.928	<0.0001
	Mean ± SD	287.12 ± 21.33	148.88 ± 34.82		
Calprotectin	Range	221 - 384	167 - 225	7.529	<0.0001
	Mean ± SD	279.20 ± 45.15	205.00 ± 19.07		
Lactoferrin	Range	62 - 96	45 - 76	7.175	<0.0001
	Mean ± SD	76.72 ± 10.19	57.96	± 8.19	

(Table 3) showed The bacterial analysis findings of the 50 ascitic fluid samples revealed the following results:

Table 3: Results of Bacterial Analysis.

Positive Cultures	25 samples with SBP. (100%)
Escherichia coli	11 samples (44%)
Klebsiella pneumoniae	9 samples (36%)
Streptococcus viridans	3 samples (12%)
Streptococcus pneumoniae	2 samples (8%)
Negative Cultures	No bacterial growth was observed in 25 samples without SBP.

(Table 4) illustrated the results for the relation between liver disease severity and Calprotectin & Lactoferrin levels, and the results showed that the Ascetic fluid Calprotectin & Lactoferrin levels were significantly associated with liver disease severity.

Table 4: Relation between Calprotectin & Lactoferrin level and liver disease severity.

Classification		N	Mean	SD	Minimum	Maximum	F	*P-value*
Calprotectin	A	1	167.0	--	167	167		
	B	20	218.0	32.36	170	282	6.839	0.002
	C	29	261.6	52.6	199	384		
Lactoferrin	A	1	50.0	--	50	50		
	B	20	59.6	10.1	45	78	9.904	<0.0001
	C	29	73.28	12.0	53	96		

(Table 5) illustrates the Pearson correlation results between ascetic fluid calprotectin and lactoferrin with clinical and laboratory data, and the results showed that there is a statistically significant positive correlation between WBCs, ESR, CRP, and ascetic fluid PNL with both calprotectin and lactoferrin levels and a negative correlation of calprotectin with albumin.

Table 5: Correlation between ascetic fluid calprotectin and lactoferrin with clinical and laboratory data.

	Calprotectin		Lactoferrin	
	r	*P-value*	r	*P-value*
Age	-0.010	0.947	-0.041	0.779
Child score	-0.173	0.229	-0.139	0.334
HB	0.182	0.205	0.231	0.107
HCT	0.190	0.185	0.284	0.046*
WBC	0.460	0.001*	0.394	0.005*
Platelet	0.094	0.518	0.058	0.691
Albumin	-0.281	0.048*	-0.250	0.079
Bilirubin	0.216	0.131	0.176	0.221
ALT	0.504	<0.0001*	0.449	0.001*
AST	0.194	0.177	0.169	0.241
INR	0.071	0.623	0.173	0.230
creatinine	0.187	0.193	0.179	0.214
ESR	0.778	<0.0001*	0.709	<0.0001*
CRP	0.684	<0.0001*	0.617	<0.0001*
Glucose	-0.118	0.415	-0.185	0.198
HBA1c	0.141	0.330	0.109	0.452
PNL	0.755	<0.0001*	0.671	<0.0001*
Calprotectin			0.934	<0.0001*

Tables 6 and 7 show The ROC curve analysis was for ascetic fluid calprotectin and ascetic fluid lactoferrin for the prediction of SBP, as follows: Ascetic fluid calprotectin had the greatest AUC of 0.980, with a sensitivity of 92% and a specificity of 96% (*p-value* <0.001), and its cutoff point was >221. These results are illustrated in Figure 1.

Regarding ascetic fluid lactoferrin, its AUC was 0.980 with a sensitivity of 92% and a specificity of 80% (*p-value* <0.001), and its cut-off point was >64.5. These results are illustrated in figure 2.

Table 6: Sensitivity, specificity of ascetic fluid calprotectin for prediction of SBP.

Cutoff point	Area under curve	Std. Error	*p-value*	sensitivity%	specificity%	95% Confidence Interval	
						Lower Bound	Upper Bound
>221	0.980	0.015	<0.001	92%	96%	0.950	1.000

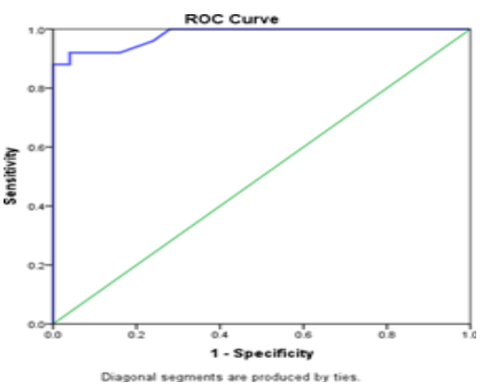

Fig. 1: ROC curve for ascetic fluid calprotectin for predicting SBP.

Table 7: Sensitivity, specificity of ascetic fluid lactoferrin for prediction of SBP.

Cutoff point	Area under curve	Std. Error	*p-value*	sensitivity%	specificity%	95% Confidence Interval	
						Bound Lower	Upper Bound
>64.5	0.938	0.031	<0.001	92%	80%	0.877	0.998

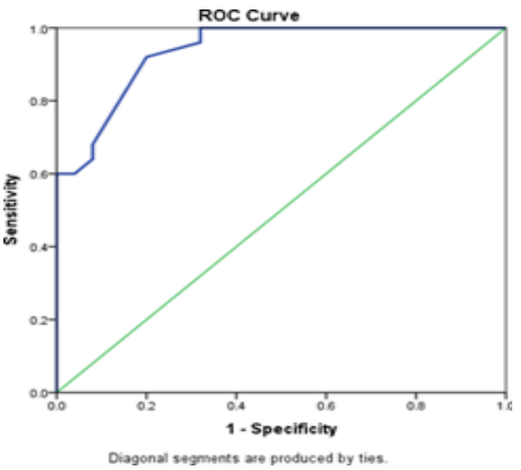

Fig. 2: ROC curve for ascetic fluid lactoferrin for predicting SBP.

DISCUSSION

Liver cirrhosis patients are at increased risk of developing spontaneous bacterial peritonitis (SBP), the most common and deadly infection in this condition, which necessitates quick diagnosis and treatment. If the abdomen is free of tumors or infections, a diagnosis of ascites with more than 250 polymorphonuclear cells (PMN)/mm3 is enough to diagnose the problem[9]. However, other factors, such as transient bacteremia after invasive procedures, can exacerbate the development of SBP in cases of nosocomial SBP. While BT (bacterial translocation) that stays inside the mesenteric lymph nodes (MLN) is perfectly normal, pathological BT occurs when the pace and severity of BT rises to dangerous levels that could affect the patient[10]. Only a few intestinal bacteria, such as E. coli, Klebsiella pneumoniae, and other Enterobacteriaceae, can translocate into MLN. Surprisingly, these species cause SBP more often than any other. DNA sequencing studies show that the bacteria in MLN and ascites in most patients can be identified by genotype[11].

In patients with liver cirrhosis, there are noticeable shifts in the stool microbial makeup, with potentially harmful bacteria such as Enterobacteriaceae being more common. In addition, patients with severe liver cirrhosis often have small intestinal bacterial overgrowth (SIBO), which is defined as more than 105 colony-forming units/ml of jejunal aspirate and/or colonic-type species. This condition has been associated with pathological BT, SBP, and endotoxinemia[12].

We designed a case-control study to evaluate the diagnostic accuracy of both ascetic calprotectin and lactoferrin in detecting SBP in cirrhotic patients. S100A8 and S100A9 are the names of the two proteins that comprise calprotectin. They are capable of binding divalent metal ions such as Zn2+, Mn2+, and Ni2+, in addition to two Ca2+ ions. The capacity of calprotectin to bind and sequester metal ions from microbes is believed to be the source of its antibacterial characteristics. Another way calprotectin helps with innate immune responses is by activating toll-like receptor 4, which it does as a damage-associated molecular pattern (DAMP)[13].

Human milk, tears, synovial fluid, serum, and other bodily fluids include lactoferrin, an iron-binding glycoprotein with a molecular weight of 76 kDa. Most mucosal membranes release it as an important part of polymorphonuclear neutrophil secondary granules. As part of their degranulation response to inflammation, polymorphonuclear neutrophils release secondary granules. This is because lactoferrin, an important component of innate defense, kills bacteria[14].

In our study comparing demographic data from the examined population, we discovered that there was no statistically significant difference in age, gender, child score, or classification between patients with and without SBP. In our study sample, bleeding, stomach pain, and fever were statistically significantly more common in patients with SBP than in those without SBP. To diagnose and predict the likelihood of spontaneous bacterial peritonitis

in patients with liver cirrhosis and ascites, ***Lutz et al.*** set out to examine the calprotectin-to-total protein ratio. There was no discernible gender or age gap between SBP and non-infected individuals ($p > 0.05$), as per the study. When comparing SBP and uninfected people, a statistically significant difference was found in Child-Pugh Stage B/C ($p<0.01$)[15].

In line with our research, ***Abudeif et al.*** aim to evaluate whether the mean platelet volume (MPV) and neutrophil-to-lymphocyte ratio (NLR) were reliable in cirrhotic individuals experiencing spontaneous bacterial peritonitis. In terms of temperature ($p = 0.008$) and abdominal pain ($p<0.001$), the study found a statistically significant difference between the groups that received SBP and those that did not. The two groups did not differ significantly with respect to hepatic encephalopathy. Furthermore, the incidence of jaundice was significantly different in the two groups ($p = 0.039$)[16].

Consistent with our findings, ***Abdel-Razik et al.*** set out to identify SBP predictors to develop a noninvasive approach for ruling out or confirming an episode of SBP. A child's score was not significantly different between the SBP and non-SBP groups, according to the study ($p > 0.05$)[17].

It was like what ***Tu et al.*** found when they used ascites from people with cirrhosis of the liver and positive microbiological cultures to make a multivariate predictive model for finding SBP early on in people who don't have any symptoms. The study found a statistically significant difference in child-Pugh score ($p = 0.004$) between the asymptomatic SBP and control groups[18]. ***Duah et al.'s*** study, which examined the prevalence and causes of spontaneous bacterial peritonitis in cirrhotic patients with ascites admitted to the medical block at Korle-Bu Teaching Hospital in Ghana, differed from ours. According to the results, there was no significant difference in abdominal pain between the SBP and non-SBP groups ($p>0.05$). When comparing encephalopathy, there was a statistically significant difference between the two groups ($p = 0.021$)[9].

We found that the leucocyte count, ESR, and CRP of patients with SBP were significantly greater than those of patients without SBP when comparing the biochemical parameters of the study group. Patients with SBP had significantly higher levels of bilirubin, ALT, and AST, as well as reduced albumin, compared to those without SBP.

In agreement with our study, ***Fernandes et al.*** found that the SBP group had significantly higher levels of C-reactive protein (CRP) than the control group did (7.3 vs. 2.1 mg/dl, $P<0.001$). The levels of white blood cells, neutrophils, bilirubin, albumin, creatinine, and prothrombin time were all the same[19].

Our findings are in line with those of ***Ali et al.,*** who attempted to determine the efficacy of AF-calprotectin as a diagnostic marker for SBP. Researchers have also studied the ratio of calprotectin to albumin in AF for its potential use in determining the severity of SBP. According to the results of the study, CRP was significantly higher in the SBP group compared to the non-SBP group ($P<0.001$)[20].

Additionally, ***Kalvandi et al.,*** who attempted to compare ascetic fluid biochemical markers across children with and without cirrhosis and spontaneous bacterial peritonitis, found results consistent with our own. Both CRP and ESR were shown to be significantly different between SBP and non-SBP ($p = 0.008$ and $p = 0.004$, respectively)[21]. In a study conducted by ***Naser Honar et al.,***[22] it was found that increased levels of ascitic calprotectin in cirrhotic patients can serve as a dependable diagnostic marker for detecting ascitic fluid infection (AFI). These elevated levels are also considered a substitute marker for polymorphonuclear leukocytes (PMN).

Like ***Abdel Hafez et al.'s*** investigation into the potential use of a serum-ascites 25-hydroxyvitamin D (25-OH vitamin D) gradient (SADG) for diagnosing SBP in cirrhotic ascites patients, they tested WBCs, AST, albumin, creatinine, and INR, finding no statistically significant differences between SBP and non-SBP ($p > 0.05$). However, the two groups showed no significant variation in ALT levels[23].

The research by ***Metwally et al.,*** which looked at the role of the granulocyte elastase enzyme in diagnosing spontaneous bacterial peritonitis, agreed with what we found. These results showed that there was no significant difference ($p > 0.05$) in the levels of hemoglobin, white blood cells, platelets, alanine aminotransferase activity, albumin, or creatinine between the SBP and non-SBP groups[24].

Our results conflicted with those of ***Luo et al.,*** who wanted to explore the role of ascetic prostaglandin E2 in the diagnosis of spontaneous bacterial peritonitis and the prediction of in-hospital mortality in patients with decompensated cirrhosis. The study found no significant difference in CRP and ALT levels between the SBP and non-SBP groups. ($p>0.05$)[25]. Similarly, our findings contradicted those of ***Elkafoury et al.,*** who aimed to investigate platelet indices as noninvasive predictors for

the diagnosis of SBP in cirrhotic patients. According to the results of the study, the SBP group had lower glucose levels than the ascites group ($p = 0.0001$)[26].

Patients with SBP had significantly higher levels of PNL, calprotectin, and lactoferrin in their ascetic fluid compared to those without SBP. Our results are like those of ***Ahmed et al.,*** whose goal was to find out how useful homocysteine and calprotectin were for diagnosing bacterial peritonitis in ascetic fluid from cirrhotic patients. We found significant differences in the ascetic PMNL count ($p = 0.002$) and the ascetic calprotectin ($p = 0.001$) between the SBP and non-SBP groups[27].

A study by ***El-Baz et al.*** also investigated the roles of hepcidin, calprotectin, and lactoferrin in ascetic fluid for early diagnosis and follow-up of spontaneous bacterial peritonitis, they found the same results. We found significant differences in calprotectin ($p = 0.002$) and lactoferrin ($p = 0.001$) between the SBP and non-SBP groups[28].

The study by ***Nasereslami et al.,*** Diagnostic and predictive role of ascetic fluid calprotectin level: six-month outcome findings in cirrhotic patients, fits with our study. Calprotectin levels were found to be significantly different between the SBP and non-SBP groups ($p < 0.01$)[29].

Our findings were in line with those of ***Josifovikj et al.,*** who wanted to determine whether calprotectin might be used as a diagnostic tool for patients with liver cirrhosis and ascites who had developed spontaneous bacterial peritonitis. Calprotectin levels in ascites were shown to be significantly different between SBP and non-SBP patients prior to treatment ($p<0.05$)[30].

We identified a substantial correlation between calprotectin and lactoferrin levels in ascetic fluid and the severity of liver disease. In our study correlating ascetic fluid calprotectin and lactoferrin with clinical and laboratory data, we found a positive correlation between white blood cell count, erythrocyte sedimentation rate, C-reactive protein, and ascetic fluid PNL with both calprotectin and lactoferrin levels, and a negative correlation between calprotectin and albumin.

Makhlouf et al. wanted to find out how to tell if someone has spontaneous bacterial peritonitis by looking at lactoferrin, calprotectin, and the calprotectin-albumin ratio in ascetic fluid. The study revealed a strong positive link between ascetic fluid calprotectin and both ascetic fluid WBC and PMN count (r = 0.777; p 0.001). Ascetic fluid lactoferrin was found to be negatively related to both the WBC count and the PMN count (r = 0.621; $p < 0.001$), and these relationships were found to be the same for both counts. Furthermore, we found a strong link between ascetic fluid lactoferrin and serum CRP (r = 0.302; $p = 0.024$)[4] These findings are like ours.

This study looked at how sensitive and specific ascetic fluid calprotectin was for predicting SBP. At a cutoff point of >221, ascetic fluid calprotectin was 92% sensitive and 96% specific for predicting SBP.

Rizk et al. tried to find that ascetic fluid calprotectin and serum C-reactive protein could be used to diagnose spontaneous bacterial peritonitis. Their results agreed with ours, which included a ROC curve for calprotectin's sensitivity and specificity. Ascetic fluid calprotectin had 86 percent specificity and 97.5 percent sensitivity for detecting SBP at a cutoff value of 270 mg/dl [Area under the receiver operating characteristics curve (AUC) = 0.924, with 96% and 69% negative and positive predictive values (NPV, PPV) for ascetic calprotectin, respectively][31].

Our findings were consistent with those of ***Abdel Rahman et al.,*** who sought to investigate ascetic calprotectin as a useful marker in the diagnosis of spontaneous bacterial peritonitis in adults. According to their findings, ascetic calprotectin >2 ng/mL exhibited 90% sensitivity, 92.5% specificity, 92.3% positive predictive value, and 90.2% negative predictive value (AUC 0.963, 95% C.I. 0.895–0.992, $P = 0.001$)[32]. For example, our results were the same as those of ***Gad et al.,*** who said that ROC curve analysis showed that calprotectin could diagnose SBP 90% of the time at a cutoff value of 2.89 ng/ml, but only 62.5 percent of the time at other levels[33].

Selim et al. also aimed to investigate the potential use of calprotectin in ascetic fluid for diagnosing spontaneous bacterial peritonitis in individuals with cirrhosis. In the study, ascetic fluid calprotectin was able to diagnose SBP with a sensitivity of 90.91% and a specificity of 95.45%. It also had a positive predictive value of 95.2% and a negative predictive value of 91%. In terms of ascetic fluid lactoferrin's sensitivity and specificity for predicting SBP, we discovered that at a cutoff point of >64.5, ascetic fluid lactoferrin had a sensitivity of 92% and a specificity of 80% for predicting SBP[34]. A systematic review and meta-analysis conducted by ***Patel et al.*** reported that summary sensitivity, specificity, and LDOR for lactoferrin were 0.954 (95% CI, 0.930, 0.979), 0.890 (95% CI, 0.836, 0.945), and 4.630 (95% CI, 3.800, 5.452), respectively. The AUC for lactoferrin was 0.958[35].

A systematic review and meta-analysis conducted by ***Kishan P. Patel et al.*** found that ascitic calprotectin and lactoferrin have a significant overall performance in detecting spontaneous bacterial peritonitis (SBP). Calprotectin and lactoferrin possess the capacity to emerge as a swift screening method. The clinical importance of these methods stems from the fact that fast and dependable diagnostic tests for SBP can help reduce the time it takes to diagnose the condition and start antibiotic treatment[36].

A study conducted by ***Amal A Mohamed et al.*** identified Mannose-binding lectin (MBL) as a potential predictive and prognostic marker in patients with cirrhosis and spontaneous bacterial peritonitis. The study found that Ascitic fluid MBL showed promise as an effective marker in assessing the prognosis and predicting outcomes in these patients[37].

Abu Rahma, M. Z et al.[38] conducted a study to determine the early diagnostic value of serum procalcitonin (PCT) levels in decompensated cirrhotic patients (DCPs) with spontaneous bacterial peritonitis (SBP). Their results indicate that serum procalcitonin is a reliable diagnostic biomarker for SBP, with a sensitivity of 93.1% and specificity of 73.2%. The area under the receiver operating characteristic curve (AUC) was 0.91 with a 95% confidence interval (CI) ranging from 0.83 to 0.99.

The limitation, our study has some limitations. The small sample size may limit our ability to apply our findings to a larger population and reduce our statistical strength. A single hospital conducted the study, potentially limiting its applicability to diverse environments or individuals. The absence of time-dependent data limits our comprehension of the biomarkers' long-term predictive efficacy. We did not confirm the results for a separate group of subjects. It's possible that we didn't fully consider other factors that could influence the results. Our recommendations for future clinical research are to conduct studies with a larger sample size and involve many hospitals to validate the findings and enhance the generalizability of the results. Longitudinal studies that include follow-up data are necessary to evaluate the long-term predictive significance of calprotectin and lactoferrin. It is necessary to create clinical recommendations that include these biomarkers as standard diagnostic tools for SBP. Scientists are currently studying the feasibility of combining calprotectin and lactoferrin with other biomarkers or clinical signs to provide a comprehensive diagnostic approach. Engaging in educational programs can help healthcare practitioners better understand and apply biomarkers in clinical practice. Additionally, the implementation of preventative measures, such as regular screening and prophylactic therapies, might be beneficial for high-risk patients.

CONCLUSION

In conclusion, Calprotectin and lactoferrin levels in ascetic fluid were higher in cirrhotic patients with SBP than in cirrhotic individuals without SBP. Lactoferrin and calprotectin in ascetic fluid may be useful diagnostic tests for screening and detecting SBP in individuals with liver cirrhosis.

DECLARATIONS

Ethics approval and consent to participate: the research protocol was approved consistent with the Research Ethics Committee, at Faculty of Medicine. Ain Shams University. (FWA00017585). Date of approval 18/10/2022.

AUTHORS' CONTRIBUTIONS

M.G. and O.H. were responsible for the conception and design of the study, collecting and entering data, literature search, methodology, laboratory investigations, statistical analysis of data and writing the results, the original draft, preparation, and editing of the final manuscript. M.G., K.S., and A.A. were responsible for the interpretation of the results. M.G., O.H., A.A., and K.S. revised the manuscript and contributed intellectual content. All authors revised and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no Conflict of interest.

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دراسة اللاكتوفيرين والكالبركتين في السائل البطني كعلامات تشخيصية في مرضى التليف الكبدي المصابين بالتهاب الصفاق الجرثومي العفوي

محمد جمال، عمر علي حسن، خالد عبد الحميد سيد و أحمد سمير عبد الصادق

قسم الجهاز الهضمي والكبد، كلية الطب، جامعة عين شمس، مصر

الخلفية: مرضى التليف الكبدي والاستسقاء معرضون بشكل أكبر للإصابة بالتهاب الصفاق الجرثومي العفوي (SBP)، مما يتطلب التشخيص المبكر باستخدام الإجراءات المعملية.

الموضوعات والأساليب: شملت الدراسة ٥٠ مريضًا تم تقسيمهم إلى مجموعتين، حيث أظهرت النتائج أن مستويات الكالبركتين واللاكتوفيرين مرتبطة بعدد خلايا الدم البيضاء، CRP، ESR، وعدد الخلايا متعددة النوى في السائل البطني.

النتائج: سجل الكالبركتين في السائل البطني أعلى قيمة AUC = ٠,٩٨٠ بحساسية ٩٢٪ ونوعية ٩٦٪، بينما سجل اللاكتوفيرين أعلى قيمة AUC = ٠,٩٨٠ بحساسية ٩٢٪ ونوعية ٨٠٪.