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Elevated Ascitic Fluid Lactoferrin Levels as a Diagnostic Marker for Spontaneous Bacterial Peritonitis in Cirrhotic Patients

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ABSTRACT

Background: Spontaneous bacterial peritonitis (SBP) is a severe infection in cirrhotic patients with ascites, often leading to decompensation and poor outcomes. Current diagnostic methods rely on ascitic fluid (AF) polymorphonuclear cell (PMN) counts and culture results, which are time-consuming and have limitations. Lactoferrin, an iron-binding glycoprotein released from neutrophils during degranulation, has emerged as a potential biomarker for SBP diagnosis.

Objective: This study aimed to assess the diagnostic accuracy of AF lactoferrin as a biomarker for the rapid detection of SBP in cirrhotic patients with ascites.

Patients and Methods: This prospective study included 60 patients with decompensated liver cirrhosis and ascites, 30 with SBP (PMN ≥ 250/mm³ or culture-positive neutrocytic ascites) and 30 without SBP. Patients underwent clinical, laboratory, and AF analyses. AF lactoferrin levels were measured using ELISA, and diagnostic accuracy was assessed using receiver operating characteristic (ROC) curve analysis.

Results: The median AF lactoferrin level was considerably higher in the SBP group compared to controls (217.5 ng/mL vs. 50.0 ng/mL, $p < 0.001$). At a cut-off of ≥75 ng/mL, lactoferrin demonstrated a sensitivity of 85% and specificity of 100% (AUC = 0.896, $p < 0.001$). AF PMN and total leukocyte counts also correlated strongly with lactoferrin levels ($r = 0.581$, $p < 0.001$ and $r = 0.634$, $p < 0.001$, respectively).

Conclusion: AF lactoferrin is a reliable, rapid biomarker for SBP diagnosis with excellent sensitivity and specificity. Its clinical utility may enhance timely detection and management of SBP in cirrhotic patients, potentially improving outcomes.

Keywords: Lactoferrin, Spontaneous bacterial peritonitis, Cirrhosis, Ascitic fluid, biomarker.

INTRODUCTION

Patients with liver cirrhosis have impaired immune defences, resulting in reduced bacterial clearance. This immune deficiency facilitates bacterial translocation by increasing intestinal permeability and promoting the proliferation of gut microbiota. As a result, over 30% of cirrhotic patients develop bacterial infections, either upon admission or during hospitalization, with spontaneous bacterial peritonitis (SBP) being the most common manifestation [1].

SBP represents a frequent and severe complication in cirrhotic patients with ascites. The ascitic fluid (AF), predominantly transudative with low opsonic activity, provides an ideal environment for bacterial growth [2]. Diagnosis of SBP is based on a positive AF culture and/or a polymorphonuclear cell (PMN) count of ≥ 250 cells/mm³. Unlike secondary peritonitis, SBP lacks an identifiable intra-abdominal source of infection or other causes of elevated ascitic PMN counts, such as pancreatitis, hemorrhage, carcinomatosis, or peritoneal tuberculosis [3].

Among bacterial infections in cirrhotic patients, SBP ranks as the most prevalent, followed by urinary tract infections, skin and soft tissue infections, pneumonia, and spontaneous bacteremia. SBP frequently leads to clinical decompensation, manifesting as worsening ascites, hepatic encephalopathy, gastrointestinal bleeding, or systemic complications such as renal failure [4].

Lactoferrin, an iron-binding glycoprotein released during neutrophil degranulation, functions as a biomarker of inflammation. The presence of neutrophils in AF suggests its potential as a valuable diagnostic tool for spontaneous bacterial peritonitis [5, 6]. This study aimed to evaluate AF lactoferrin as a diagnostic marker for SBP, potentially facilitating the development of a rapid, cost-effective bedside test to improve clinical outcomes in cirrhotic patients.

PATIENTS AND METHODS

This study involved 60 patients diagnosed with decompensated chronic liver disease (CLD) and ascites, with or without SBP. The patients were recruited from The Gastroenterology and Hepatology Inpatient and Outpatient Clinics at Ain Shams University Hospitals through the period from April 2023 to September 2023. Of the participants, 31 (51%) were male and 29 (49%) were female, with an age ranged from 40 to 79 years.

Patient Selection: Participants were selected based on predefined inclusion and exclusion criteria.

Inclusion criteria: Egyptian nationals, 18 years or older, with a confirmed diagnosis of decompensated CLD (Child-Pugh class B or C) accompanied by ascites. Both male and female participants were included.

Exclusion criteria: Cases with ascites caused by conditions other than liver cirrhosis, those with active infections unrelated to AF, or cases who had received antibiotics prior to admission. Additionally, patients with neutrocytic ascites due to other causes such as pancreatitis, appendicitis, tuberculosis, peritoneal carcinomatosis, and hemorrhagic ascites. Cases diagnosed with hepatocellular carcinoma (HCC) and those who had undergone abdominal surgery within three months preceding the study.

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Patient classification: The research participants were categorized into two categories. The first cohort, designated as the SBP group, included 30 patients with cirrhotic ascites with SBP. The controls consisted of 30 patients with cirrhotic ascites who had no signs of SBP.

Diagnosis of SBP: The diagnosis of SBP was based on recognised clinical criteria. Patients were designated as having SBP if the polymorphonuclear cell (PMN) count in AF over 250 cells/mm³ and the fluid culture yielded positive results. Moreover, culture-negative neutrocytic ascites (CNNA) and non-neutrocytic bacterascites (positive AF culture with polymorphonuclear cell count <250 cells/mm³) were included into the diagnostic [3]. The diagnosis of SBP explicitly excludes instances exhibiting characteristics indicative of subsequent peritonitis or other intra-abdominal infections.

Patient assessment: All patients underwent a comprehensive evaluation consisting of clinical history, physical examination, laboratory investigations, imaging, and specific diagnostic procedures.

Clinical history: A thorough history was taken, focusing on signs of liver cell failure such as jaundice, lower limb edema, altered consciousness, and bleeding tendencies. Symptoms suggestive of SBP, including fever and abdominal pain, were noted. Additionally, the history of previous SBP episodes and complications, such as hepatic encephalopathy and variceal bleeding, was carefully documented.

Physical examination: General and local examinations were performed to identify signs of CLD, including palmar erythema, spider nevi, changes in liver and spleen size, ascites, jaundice, and encephalopathy. Specific signs of SBP, such as fever, hypotension, tachycardia, and abdominal tenderness, were also evaluated.

Laboratory investigations: Laboratory assessments included a complete blood count (CBC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Renal function tests (serum creatinine, blood urea, sodium, and potassium levels). Liver function tests [serum alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), total and direct bilirubin, albumin, and total protein levels]. Additional tests included prothrombin time and concentration, arterial blood gases (pH, serum bicarbonate, $PaCO_2$, and serum lactate), and serological tests for viral markers (HBsAg and HCV Ab using ELISA). Serum alpha-fetoprotein levels were measured as a tumor marker.

Abdominal ultrasonography: Ultrasound evaluation was performed to assess liver size and echotexture (evidence of cirrhosis or fibrosis, and focal lesions), portal vein diameter and patency, spleen size and echotexture, and the presence and extent of ascites (minimal, moderate, or marked). Signs of complications such as loculated ascites or intra-abdominal adhesions were also recorded.

Blood sample collection: Venous blood (10 mL) was collected under aseptic conditions and distributed for various analyses:

1. **2 mL** in an EDTA tube for CBC.
2. **6 mL** in a plain test tube for routine biochemical tests. After clotting at room temperature for 30 minutes, the samples were centrifuged at 1500 rpm for 15 minutes, and serum was separated for further analysis.
3. **1.8 mL** in a sodium citrate tube for prothrombin time testing.

Diagnostic abdominal paracentesis: Abdominal paracentesis was performed under aseptic precautions using a wide-bore needle. The procedure was explained to the patient beforehand, and the "Z Tracking" technique was employed to prevent fluid leakage. Ten milliliters of AF were aspirated for analysis:

1. **Physical examination**: Assessment of color and appearance.
2. **Biochemical tests:** Measurement of total protein content, glucose, chloride, and lactate dehydrogenase (LDH) levels.
3. **Cellular analysis:** Total and differential white blood cell counts. SBP was diagnosed when the PMN count in AF exceeded 250 cells/mm³ with a positive AF culture or in cases of CNNA or non-neutrocytic bacterascites (positive AF culture with PMN < 250 cells/mm³).

Principle of the assay: The detection of lactoferrin in AF was conducted using an Enzyme-Linked Immunosorbent Assay (ELISA) kit. The assay involved pre-coating a plate with human lactoferrin (LF) antibodies. LF in the sample binds to these antibodies, followed by the addition of biotinylated LF antibody and Streptavidin-HRP. After washing away unbound Streptavidin-HRP, a substrate solution was added, causing a color change proportional to the LF concentration. The reaction was terminated with an acidic stop solution, and absorbance was measured at 450 nm. The assay sensitivity was 2.52 µg/mL, with a standard curve range of 5–1000 µg/mL. The reagents were stored at 2–8°C.

Ethical considerations: Ethical permission for the research was secured from The Gastroenterology and Hepatology Inpatient Department and Outpatient Clinics at Ain Shams University Hospitals, Faculty of Medicine. The research complied with the regulations of the Institutional Committee for the Protection of Human Subjects and aligned with the ethical standards established in the Declaration of Helsinki (18th World Medical Assembly). Informed agreement was secured from

each participant before enrolment, guaranteeing their anonymity and comprehension of the research.

Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics version 23 (IBM Corp., Armonk, NY) and MedCalc® version 18.2.1 (MedCalc Software bvba, Ostend, Belgium). For continuous variables with a normal distribution, data were expressed as means and standard deviations (SD) and analyzed using the unpaired t-test. Skewed data were summarized as medians with interquartile ranges (IQR) and assessed using the Mann-Whitney U-test. Categorical variables were reported as counts and percentages, with group comparisons conducted using either the Pearson Chi-squared test or Fisher's exact test, as appropriate. Ordinal variables were evaluated using the Chi-squared test for trend. Relationships between variables were examined through Spearman rank correlation analysis, and the diagnostic accuracy of biochemical and hematological markers for SBP was evaluated using receiver-operating characteristic (ROC) curve analysis. A p-value ≤ 0.05 (two-tailed) was considered statistically significant.

RESULTS

Females were more frequently affected by SBP than males. SBP also demonstrated a higher incidence among elderly patients compared to the controls. However, the difference between the studied groups was not statistically significant ($p > 0.05$). The severity of liver disease in both groups was evaluated using the Child-Pugh classification. In the non-SBP group, 10% of patients were classified as Child-Pugh B and 20% as Child-Pugh C. In the SBP group, 16% were classified as Child-Pugh B and 84% as Child-Pugh C. There was no notable variation between the groups regarding Child-Pugh classification ($p = 0.553$) (Table 1).

Table (1): Demographic and clinical characteristics of patients with SBP and control group

Variable		SBP (n=30)		Control (n=30)		χ2	P value*
		n	%	n	%		
Gender	F	18	60.00%	11	36.67%	3.2703	0.071
	M	12	40.00%	19	63.33%		
Age category	40-49 yr	7	23.33%	7	23.33%	0.1176	0.99
	50-59 yr	8	26.67%	9	30.00%		
	60-69 yr	9	30.00%	8	26.67%		
	70-79 yr	6	20.00%	6	20.00%		
Risk factors	Smoking	9	30.00%	7	23.33%	-	0.771#
	Hypertension	14	46.67%	12	40.00%	-	0.794#
	DM	16	53.33%	14	46.67%	-	0.796#
Child class	Child B	5	16.67%	6	20.00%	0.113	0.739
	Child C	25	83.33%	24	80.00%		

Data were presented as number (n) and percentage (%). *Pearson chi-squares test unless otherwise indicated. #Fisher's exact test, SBP: Spontaneous Bacterial Peritonitis, DM: Diabetes Mellitus, χ^2: Chi-squared, P: Probability Value.

HCV Ab positivity was observed in 66.67% of SBP patients compared to 70.00% in the controls ($p = 0.736$), while HBsAg positivity was noted in 20.00% of SBP patients versus 13.33% of controls ($p = 1.000$). In contrast, there was a significant difference in the inflammatory marker CRP status between groups. CRP positivity was markedly higher in the SBP group (90.00%) compared to the controls (53.33%; $p = 0.013$) (Table 2).

Table (2): Serological and inflammatory marker status in patients with SBP and control group

Variable		SBP (n=30)		Control (n=30)		χ2(df,1)	P-value*
		n	%	n	%		
HCV Ab	Negative	10	36.67%	9	30.00%	0.114	0.736
	Positive	20	66.67%	21	70.00%		
HBsAg	Negative	24	80.00%	26	86.67%	-	1.000#
	Positive	6	20.00%	4	13.33%		
CRP	Negative	3	10.00%	14	46.67%	6.144	**0.013**
	Positive	27	90.00%	16	53.33%		

Data were presented as number (n) and percentage (%). Pearson Chi-squares test unless otherwise indicated. #Fisher's exact test. **HBsAg:** Hepatitis B Surface Antigen, HCV Ab: Hepatitis C Virus Antibody, **CRP:** C - reactive protein, χ^2: Chi-squared, P: Probability Value, **df:** Degrees of Freedom.

Among the ultrasonographic findings, cirrhosis was observed in 76.67% of patients with SBP and 80.00% of the controls, with no notable variation (p = 1.000). Parenchymal liver disease was noted in 23.33% of SBP patients compared to 20.00% of controls (p = 1.000). Splenomegaly was more common in the SBP group (66.67%) compared to the controls (46.67%), but this difference was not significant (p = 0.204). Calcified gallbladders were found in 20.00% of SBP patients versus 40.00% in controls (p = 0.168). Ascites severity varied, with mild ascites in 30.00% of SBP patients and 10.00% of controls, moderate ascites in 40.00% of SBP patients and 56.67% of controls, and marked ascites in 30.00% of SBP patients and 33.33% of controls, with no significant difference (p = 0.274). Nephropathy was absent in 73.33% of SBP patients and 86.67% of controls (p = 0.836) (Table 3).

Table (3): Ultrasonographic findings in patients with SBP and control group

Variable		SBP (n=30)		Control (n=30)		χ2/(df,1)	P value*
		n	%	n	%		
US findings	Cirrhosis	23	76.67%	24	80.00%	-	1.000#
	Parenchymatous liver disease	7	23.33%	6	20.00%	-	1.000#
	Splenomegaly	20	66.67%	14	46.67%	-	0.204#
	Calcular GB	6	20.00%	12	40.00%	-	0.168#
Ascites by US	No ascites	0	0.00%	0	0.00%	1.196	0.274§
	Mild ascites	9	30.00%	3	10.00%		
	Moderate ascites	12	40.00%	17	56.67%		
	Marked ascites	9	30.00%	10	33.33%		
Nephropathy by US	No nephropathy	22	73.33%	26	86.67%	0.043	0.836§
	Grade 1 nephropathy	6	20.00%	1	3.33%		
	Grade 2 nephropathy	0	0.00%	2	6.67%		
	Grade 3 nephropathy	2	6.67%	1	3.33%		

Data are number (n) and percentage (%), SBP: Spontaneous Bacterial Peritonitis, US: Ultrasonography, GB: Gallbladder, χ^2: Chi-squared, P: Probability Value, df: Degrees of Freedom.

In biochemical analysis, ALT was significantly higher in the SBP group (69.7 ± 48.4 IU/L) than in controls (40.7 ± 29.6 IU/L; p = 0.028), and ALP was also elevated in SBP patients (121.0 ± 47.5 IU/L) compared to controls (90.0 ± 33.9 IU/L; p = 0.023). Other parameters, such as AST, serum albumin, total serum protein, total bilirubin, and direct bilirubin, showed no statistically notable variations between groups, although direct bilirubin approached significance (p = 0.053). Hematological parameters revealed no significant differences in blood urea, serum creatinine, serum uric acid, sodium (Na), potassium (K), pH, bicarbonate (HCO_3^-), and $PaCO_2$ between groups. The MELD score was slightly higher in the SBP group (19.9 ± 6.1) than in controls (18.3 ± 5.5), but this was not statistically significant (p = 0.381) (Table 4).

Table (4): Biochemical and hematological parameters in patients with SBP and control group

Variable	SBP (n=30)	Control (n=30)	95% CI	P-value*
AST (IU/l)	58.0±6.7	50.4±8.0	-25.1 - 10.0	0.389
ALT (IU/l)	69.7±8.4	40.7±9.6	-54.6 - -3.3	0.028
ALP (IU/l)	121.0±7.5	90.0±3.9	-57.4 - -4.6	0.023
Serum albumin (g/dl)	2.9±0.5	3.0±0.1	-0.4 - 0.6	0.596
Total serum protein (g/dl)	6.6±0.8	6.1±1.1	-1.1 - 0.1	0.101
Total bilirubin (mg/dl)	2.5±0.2	1.9±0.1	-1.4 - 0.1	0.079
Direct bilirubin (mg/dl)	1.3±0.08	0.8±0.06	-0.9 - 0.01	0.053
Blood urea (mg/dl)	55.5±4.0	49.4±4.0	-29.9 - 17.6	0.603
Serum creatinine (mg/dl)	1.4±0.09	1.7±0.07	-0.3 - 0.7	0.346
Serum uric acid (mg/dl)	8.6±2.0	8.4±1.8	-1.4 - 1.03	0.771
Serum Na± (mmol/l)	133.5±8.0	132.5±9.4	-6.6 - 4.6	0.720
Serum K± (mmol/l)	3.9±0.9	4.3±1.0	-0.2 - 0.9	0.234
pH	7.4±0.1	7.4±0.1	-0.1 - 0.03	0.370
Serum HCO3- (mmol/l)	18.1±4.4	18.9±3.1	-1.7 - 3.2	0.540
PaCO2 (mmHg)	31.2±5.9	36.2±6.3	0.03 - 9.9	0.059
Serum lactate (mmol/l)	2.0±0.5	1.7±0.09	-0.8 - 0.3	0.317
MELD score	19.9±4.1	18.3±3.5	-5.3 - 2.1	0.381

Data are mean and standard deviation (SD). 95% CI = 95% confidence interval. *Unpaired t-test, SBP: Spontaneous Bacterial Peritonitis, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, Na: Sodium, K: Potassium, HCO_3^-: Bicarbonate, $PaCO_2$: Partial Pressure of Carbon Dioxide, MELD: Model for End-Stage Liver Disease, CI: Confidence Interval, P: Probability Value.

The analysis of hematological parameters showed that the INR was significantly higher in patients with SBP (1.9 ± 0.4) compared to the controls (1.7 ± 0.3; $p = 0.050$). MCV was significantly lower in the SBP group (76.1 ± 6.5 fl) compared to controls (83.6 ± 11.5 fl; $p = 0.016$). TLC was markedly elevated in SBP patients (12.9 ± 3.8 k/mm³) compared to the controls (8.8 ± 3.5 k/mm³; $p = 0.001$). Platelets counts were significantly reduced in SBP patients (103.7 ± 26.2 k/mm³) compared to controls (128.3 ± 33.0 k/mm³; $p = 0.013$). No significant differences were observed in hemoglobin levels (9.4 ± 1.5 g/dL in SBP vs. 9.6 ± 1.5 g/dL in controls; $p = 0.643$) or MCH (28.7 ± 3.6 pg in SBP vs. 29.9 ± 4.2 pg in controls; $p = 0.323$) (Table 5).

Table (5): Hematological parameters in patients with SBP and control group

Variable	SBP (n=30)	Control (n=30)	95% CI	P-value*
	Mean ±SD	Mean ±SD		
INR	1.9±0.4	1.7±0.3	-0.4 - 0.0002	0.050
Hemoglobin (g/dl)	9.4±1.5	9.6±1.5	-0.7 - 1.2	0.643
MCV (fl)	76.1±6.5	83.6±11.5	1.5 - 13.4	**0.016**
MCH (pg)	28.7±3.6	29.9±4.2	-1.3 - 3.8	0.323
TLC (k/mm3)	12.9±3.8	8.8±1.5	-6.5 - -1.8	**0.001**
Platelets (k/mm3)	103.7±26.2	128.3±33.0	5.6 - 43.7	**0.013**

Data are mean and standard deviation (SD), *Unpaired t-test, SBP: Spontaneous Bacterial Peritonitis, INR: International Normalized Ratio, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, TLC: Total Leukocyte Count, CI: Confidence Interval, P: Probability Value.

The analysis of AF parameters revealed several significant differences between patients with SBP and the controls. Median AF protein levels were higher in SBP patients (2.0 g/dL; IQR 1.9–2.5) compared to controls (1.9 g/dL; IQR 1.2–2.0; $p = 0.043$). TLC in AF was significantly elevated in SBP patients (median 1040 cells/mm³; IQR 755–2600) compared to controls (median 19 cells/mm³; IQR 0.0–385.0; $p < 0.001$). Similarly, PMN count in AF was markedly higher in the SBP group (median 525.5 cells/mm³; IQR 320–1300) compared to controls (median 30 cells/mm³; IQR 0.0–115.0; $p < 0.001$). AF lactoferrin levels were also significantly elevated in SBP patients (median 217.5 µg/mL; IQR 52.5–385.0) compared to controls (median 50.0 µg/mL; IQR 30.0–60.0; $p < 0.001$). No substantial changes were observed in AF chloride (Cl^-) levels (93.5 mmol/L in SBP vs. 100 mmol/L in controls; $p = 0.256$), AF glucose (173.5 mg/dL in SBP vs. 151.5 mg/dL in controls; $p = 0.441$), or AF LDH levels (202 IU/L in SBP vs. 189.5 IU/L in controls; $p = 0.279$) (Table 6).

Table (6): AF analysis in patients with SBP and control group

Variable	SBP (n=30)		Control (n=30)		P-value*
	Median	IQR	Median	IQR	
AF Cl- (mmol/l)	93.5	89.5 - 101.0	100	89.5 - 105.5	0.256
AF glucose (mg/dl)	173.5	120.0 - 207.5	151.5	119.0 - 175.5	0.441
AF LDH (IU/l)	202	145 - 275	189.5	122.0 - 217.5	0.279
AF protein (g/dl)	**2**	**1.9 - 2.5**	**1.9**	**1.2 - 2.0**	**0.043**
AF TLC (cell/mm3)	**1040**	**755 - 2600**	**19**	**0.0 - 385.0**	**<0.001**
AF PMN (cell/mm3)	**525.5**	**320 - 1300**	**30**	**0.0 - 115.0**	**<0.001**
AF lactoferrin (µg/ml)	**217.5**	**52.5 - 385**	**50**	**30.00 - 60.0**	**<0.001**

Data were presented as median and interquartile range (IQR), Av. Rank = average rank, SBP: Spontaneous Bacterial Peritonitis, AF: Ascitic Fluid, Cl^-: Chloride, LDH: Lactate Dehydrogenase, TLC: Total Leukocyte Count, PMN: Polymorphonuclear Cells, P: Probability Value, *Mann-Whitney U test.

The area under the ROC curve (AUC) for the use of AF lactoferrin in diagnosis of SBP in the 60 patients with ascites caused by cirrhosis was 0.896 (95 % CI, 0.639–0.987, $p < 0.001$). The sensitivity and specificity for different cut-off levels of AF lactoferrin for the diagnosis of SBP in this patient group are shown in table (12). At the cut-off level of ≥ 75 ng/mL, the sensitivity and specificity of the test were 85 % and 100 %, respectively. While AUC for the use of AF TLC in diagnosis of SBP caused by cirrhosis was 0.863 (95 % CI, 0.717–0.951, $p < 0.001$). At the cut-off level of ≥ 430 cell/mm^3, the sensitivity and specificity of the test were 100 % and 80 %, respectively. AUC for the use of AF proteins in diagnosis of SBP caused by cirrhosis was 0.686 (95 % CI, 0.520–0.823, p=0.029). At the cut-off level of ≥ 1.9 g/dl, the sensitivity and specificity of the test were 65 % and 65 %, respectively (Table 7).

Table (7): Receiver-operating characteristic (ROC) curve analysis of biochemical and hematological markers for discrimination between SBP and control groups

ROC metric	Marker							
	ALT	ALP	MCV	TLC	Platelets	AF proteins	AF TLC	AF lactoferrin
AUC	0.715	0.699	0.676	0.791	0.735	0.686	0.863	0.896
95% CI	0.550-0.846	0.533-0.833	0.510 -0.815	0.634-0.903	0.572-0.862	0.520-0.823	0.717-0.951	0.639-0.987
P-value	0.01	0.024	0.046	0	0.004	0.029	<0.001	0
Cu-ff criterion	≥34	≥94	≤74	≥11.2	≤122	≥1.9	≥430	≥75
Sensitivity	80	80	55	75	75	65	100	85
95% CI	56.3 - 94.3	56.3 - 94.3	31.5 - 76.9	50.9 - 91.3	50.9-91.3	40.8 -84.6	83.2-100.	60.8 - 94.6
Specificity	55	60	85	80	70	65	80	100
95% CI	31.5-76.9	36.1-80.9	62.1-96.8	56.3-94.3	45.7-88.1	40.8-84.6	56.3-94.3	83.2-100
±LR	1.8	2	3.7	3.8	2.5	1.9	5	-
95% CI	1.0-3	1.1-3.6	1.2-11.2	1.5-9.3	1.2 - 5.1	0.9 - 3.7	2.1-12	-
-LR	0.4	0.3	0.5	0.3	0.4	0.5	0	0.4
95% CI	0.1-1	0.1-0.9	0.3-0.9	0.1-0.7	0.2 - 0.8	0.3 - 1.1	-	0.2 - 0.6
±PV	64	66.7	78.6	78.9	71.4	65	83.3	100
95% CI	51.1-75.2	52.8 -78.1	54.6-91.8	60.1-90.3	55.0-83.6	48.5-78.5	67.5-92.3	-
-PV	73.3	75	65.4	76.2	73.7	65	100	74.1
95% CI	51.2-87.8	53.8-88.5	52.9-76	59.2-87.6	55.4-86.3	48.5-78.5	-	61.1 - 83.8

ROC: Receiver-Operating Characteristic, **AUC:** Area under curve, **ALT:** Alanine Transaminase, **ALP:** Alkaline Phosphatase, **MCV:** Mean Corpuscular Volume, **TLC:** Total Leukocyte Count, **AF:** Ascitic Fluid, **CI:** Confidence Interval, ± LR: Positive Likelihood Ratio, **-LR:** Negative Likelihood Ratio, **±PV:** Positive Predictive Value, **-PV:** Negative Predictive Value, **P**: Probability Value.

The analysis revealed a significant positive correlation between AF lactoferrin levels and CRP ($r = 0.485$, $p < 0.001$) and TLC ($r = 0.34$, $p = 0.042$), indicating an association between higher AF lactoferrin levels and markers of inflammation. Conversely, a significant negative correlation was observed with platelet count ($r = -0.392$, $p = 0.018$). Other variables, including age, liver enzymes (AST, ALT), hemoglobin, serum albumin, total serum protein, INR, and MELD score, did not show significant correlations with AF lactoferrin levels (Table 8).

Table 8: Correlations between AF Lactoferrin Levels and Laboratory Parameters

Variable	AF lactoferrin (µg/ml)	
	Spearman rho	P-value
Age	0.048	0.84
AST	-0.078	0.744
ALT	-0.131	0.582
CRP	0.485	<0.001
Serum albumin	-0.15	0.529
Total serum protein	0.002	0.994
INR	-0.276	0.238
Hemoglobin	-0.017	0.942
MCV	0.092	0.701
MCH	0.021	0.931
TLC	0.34	0.042
Platelets	-0.392	0.018
BUN	-0.26	0.269
Serum creatinine	-0.244	0.3
Serum uric acid	-0.107	0.652
MELD score	-0.305	0.19

AF: Ascitic Fluid, **AST:** Aspartate Aminotransferase, **ALT:** Alanine Aminotransferase, **CRP:** C - reactive protein**, INR:** International Normalized Ratio, **MCV:** Mean Corpuscular Volume, **MCH:** Mean Corpuscular Hemoglobin, **TLC:** Total Leukocyte Count**, BUN:** Blood Urea Nitrogen, **MELD:** Model for End-Stage Liver Disease**, P:** Probability Value.

DISCUSSION

SBP is a serious complication in cirrhotic patients with ascites, with a prevalence of 1.5–3.5% in outpatients and 10–30% in hospitalized patients. Diagnosed by AF PMN ≥ 250 cells/mm³ or positive culture, SBP is distinct from secondary peritonitis [7]. Lactoferrin, a protein elevated during infections, shows potential as a diagnostic marker [8]. This study was conducted at Ain Shams University Hospitals to evaluate AF lactoferrin in 60 patients with cirrhotic ascites (40–75 years, 55% male), divided into SBP (n=30) and non-SBP (n=30) groups.

Our findings revealed that females were more commonly affected by SBP than males, with 18 cases (60%) compared to 12 cases (40%), resulting in a female-to-male ratio of 1.5:1. No notable changes were noted between SBP and non-SBP groups in terms of mean age or gender distribution. The severity of liver disease, assessed using the Child-Pugh classification, showed that 10% of the non-SBP group and 5% of the SBP group were classified as Child-Pugh B, while the majority, 90% and 95% respectively, were classified as Child-Pugh C. The variation between the groups was not statistically substantial (p = 0.553). Notably, most SBP cases were classified as Child-Pugh C (68.2%), consistent with **Cirera *et al.*** [9], who reported a similar prevalence of 70% in SBP patients.

Our study demonstrated that 90% of cases in the SBP group had a positive CRP response compared to 55% in the controls, with a statistically significant difference. Consistent with our findings, CRP has been widely recognized as an inflammatory marker in bacterial infections, including liver diseases. Similarly, **Hamed *et al.*** [10] reported that CRP showed excellent sensitivity, specificity, and AUC-ROC in differentiating cirrhotic cases with SBP from those without SBP. Previous studies have also associated elevated CRP levels with AF infections [11, 12]. Beyond its diagnostic utility, CRP has been linked to predicting prognosis, mortality [13], and antibiotic response in SBP cases [14].

Additionally, liver enzymes (AST and ALT) were considerably higher in SBP cases compared to non-SBP cases. However, no notable changes were observed between the groups in other biochemical parameters. Serum TLC was significantly elevated in the SBP group compared to the controls. While the INR was higher in SBP cases than in non-SBP patients, the variation was not statistically notable. These findings align with those of **Oladimeji *et al.*** [15], who reported considerably elevated INR levels in SBP cases, indicative of poor prognosis.

The current study revealed that AF in SBP cases had notably higher levels of total protein, TLC, and PMN compared to non-SBP cases. PMN count in AF remains the gold standard for SBP diagnosis. Total protein in AF was notably elevated in SBP patients, suggesting its role in the inflammatory process and potential as an early marker of SBP. The median AF lactoferrin level was significantly higher in SBP cases compared to non-SBP cases (217.5 ng/mL vs. 50.0 ng/mL, p < 0.001), corroborating findings by **Parsi *et al.*** (2008). The elevated lactoferrin levels can be attributed to its release from polymorphonuclear leukocytes in response to inflammation, bacterial infection, and cytokine activation [16]. Conversely, no notable variations were observed between the groups regarding glucose, AF chloride, and LDH levels.

The area under the ROC curve (AUC) for AF lactoferrin in diagnosing SBP among 60 cirrhotic cases with ascites was 0.896 (95% CI: 0.639–0.987, p < 0.001). At a cut-off level of ≥75 ng/mL, lactoferrin demonstrated a sensitivity of 85% and specificity of 100%. Lactoferrin, a protein released from PMNs during infection or inflammation, showed strong positive correlations with inflammatory markers in this study, including serum CRP (r = 0.485, p < 0.001), TLC (r = 0.340, p = 0.042), AF TLC (r = 0.634, p < 0.001), and AF PMN count (r = 0.581, p < 0.001).

Platelet counts were considerably lower in SBP patients compared to non-SBP cases (p = 0.000), with AF lactoferrin levels showing a negative correlation with platelet count (r = -0.392, p = 0.018). These findings align with **Lata *et al.*** [17], who observed notably reduced platelet counts in SBP cases. The decrease in platelets may reflect increased portal pressure and portal hypertension, potentially contributing to higher protein levels in AF and facilitating bacterial translocation, a key factor in SBP development.

LIMITATIONS OF THE STUDY

This study has notable limitations that should be addressed. First, the relatively small sample size that may limit the broader applicability of the findings, highlighting the necessity for larger studies to validate and refine cut-off values for ascitic lactoferrin in SBP diagnosis. Second, the absence of follow-up post-discharge prevents the assessment of ascitic lactoferrin as a potential prognostic biomarker in the outpatient setting. Moreover, the manual evaluation of AF parameters, including PMN counts, introduces variability due to operator dependency, potentially affecting consistency and reliability. The development of standardized, commercially available kits for AF lactoferrin measurement could offer a practical solution for bedside testing. Given lactoferrin's exceptional stability and resistance to degradation at room temperature, such a test is not only feasible but could also significantly enhance its utility as a diagnostic tool for SBP.

CONCLUSION

AF lactoferrin is a reliable, rapid biomarker for SBP diagnosis with excellent sensitivity and specificity. Its clinical utility may enhance timely detection and management of SBP in cirrhotic patients, potentially improving outcomes.

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