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Ascitic Fluid Calprotectin Level as a Diagnostic Marker of Spontaneous Bacterial Peritonitis in Cirrhotic Patients

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ABSTRACT

Background: Spontaneous bacterial peritonitis (SBP) is the infection of pre-existing ascitic fluid without evidence of a secondary infection. **Objective:** To evaluate ascitic fluid calprotectin as a diagnostic marker of spontaneous bacterial peritonitis in cirrhotic patients.

Patients and Methods: This study was conducted on 50 cirrhotic patients with ascites. Patients were divided into 2 groups: Group (I): Included 40 cirrhotic patients with SBP on the basis of polymorphonuclear leukocytes (PMN) count in the ascitic fluid ≥250 cells/μL in the absence of secondary peritonitis, irrespective of ascitic fluid culture results, Group (II): Included 10 cirrhotic patients with ascites but without SBP (control group).

Results: There was a statistically significant difference between the two studied groups regarding hemoglobin (Hb) (P=0.006), white blood cells (WBCs) (P=0.015), platelet count (P>0.001), C-reactive protein (CRP) (P=0.001) and bilirubin (P=0.013). There was also significant difference between the two studied groups regarding ascitic fluid analysis parameters; as ascitic lactate dehydrogenase (LDH) and PMN count were significantly higher in SBP group (P<0.001, for both). Ascitic fluid calprotectin was significantly higher in SBP group compared to non-SBP group (26.3 ng/ml (6.5 – 75) vs. 15 ng/ml (6.5-33); P=0.013). Ascitic calprotectin was significant at a cutoff level of 18 ng/ml with a sensitivity of 90% and 70% specificity for diagnosing SBP with an area under the curve (AUC) = 0.835).

Conclusion: Ascitic fluid calprotectin could be used to serve as a convenient reliable diagnostic marker for SBP in cirrhotic patients with ascites.

Key words: Ascites, Ascitic fluid calprotectin; Cirrhosis; Spontaneous Bacterial Peritonitis.

INTRODUCTION

Liver cirrhosis (LC) is the last stage of chronic liver disease and is caused by increasing fibrosis. Cirrhosis can cause hepatic dysfunction and/or portal hypertension. Ascites, varices, hepatic encephalopathy, hepatocellular cancer, hepatopulmonary syndrome, and coagulation abnormalities can all result from one of these drugs alone or in combination. Cirrhosis and its consequences have a negative impact on quality of life as well as survival [(1)].

In the absence of a gastrointestinal perforation and intra-abdominal inflammatory diseases such as abscess, cholecystitis, or acute pancreatitis, spontaneous bacterial peritonitis (SBP) is a bacterial infection of previously sterile ascitic fluid. It is a well-known and common consequence in cirrhotic individuals with ascites, with 10–25 percent of these patients experiencing it [(2)].

Bacterial translocation is the major cause of SBP; therefore, no intra-abdominal source of infection can be found. Ascites culture is the gold standard for SBP diagnosis, and a high ascites PMN count is accepted as an early indicator of SBP. An ascites PMN count ≥250/mm3 is considered to indicate empirical antibiotic therapy based on the current guidelines [(3)].

Calprotectin is a calcium and zinc-binding protein and detected mainly in neutrophils. Its presence in body fluids is directly proportional to the rate of influx of neutrophils [(4)]. Calprotectin is present mainly in neutrophils, macrophages, and very rarely appears in lymphocytes. Calprotectin accounts for about 60% of cytosolic proteins of neutrophils [(5)].

Ascitic fluid calprotectin may be helpful in detection of neutrophil count greater than or equals to 250 cells/mm^3, so it may have an important role in diagnosis of SBP and this will be a rapid bedside test in quick management of SBP [(6)].

The aim of this work is to evaluate ascitic fluid calprotectin as a diagnostic marker of spontaneous bacterial peritonitis in cirrhotic patients.

PATIENTS AND METHODS

This study was conducted on 50 Ascitic cirrhotic patients who were admitted at internal medicine Department, Benha University Hospital. Patients with established liver cirrhosis and ascites based upon clinical, laboratory and ultrasonographic findings, aged >18 years were included in this study.

They were divided into 2 groups: Group (I): Included 40 cirrhotic patients with SBP on the basis of PMN count in the ascitic fluid ≥250 cells/μL with or without positive ascitic fluid culture, in absence of an intra-abdominal source of infection or malignancy [(7)], and **Group (II):** Included 10 cirrhotic patients with ascites and without SBP (control group).

Patients with ascites due to other causes (malignancy, cardiac diseases, renal diseases, Budd-

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Chiari syndrome, tuberculosis or hypothyroidism), patients with history of antibiotics 2 weeks prior to paracentesis, patients with evidence of secondary bacterial infection, and patients with hepatocellular carcinoma (HCC) were excluded from the study.

All patients were subjected to detailed history taking and complete clinical examination for stigmata of LC including ascites and features of SBP like fever, abdominal tenderness, altered mental status, diarrhea, worsening ascites and new onset renal failure; biochemical investigations including; complete blood count (CBC), CRP, markers of liver injury: alanine amino transferase (ALT), aspartate amino transferase (AST), liver function tests: serum bilirubin (total, direct), serum albumin, prothrombin time and international normalized ratio (INR) and renal function tests: serum creatinine and blood urea.

Diagnostic abdominal paracentesis and examination of ascitic fluid for: PMN count, glucose, albumin, serum ascites albumin gradient (SAAG) and LDH. Ascitic fluid calprotectin level was measured using enzyme-linked immunosorbent assay (ELISA).

Pelvi-abdominal ultrasonography was done to detect radiological features of LC, spleen length, PV diameter, ascites and to exclude HCC. An assessment of disease severity using Child Pugh Score[8] and a model for end-stage liver disease (MELD score)[9] was performed.

Method and sampling:

Blood sample: Five milliliters of fresh venous blood were collected under complete aseptic conditions and was divided to: 1 milliliter of whole blood was collected into EDTA containing vacutainer and mixed well for CBC, which was performed by automated hematology system (Sysmex XE 5000; Sysmex America, Inc, Mundelein, USA). 1.6 milliliters were collected into sodium citrate containing vacutainer and mixed well for coagulation profile using Coatron A4 automated coagulometer. The rest of blood was collected into empty tube and allowed to clot for 20 minutes then centrifuged for 5 min at 5000 rpm for serum preparation. The serum was separated in Eppendorf tubes for measurement of liver and kidney function tests using Biosystem A15 autoanalyzer (Biosystem S.A, Barcelona, Spain).

Ascitic fluid sample: 20 mL were obtained through paracentesis performed using a 20-gauge sterile needle under local anesthesia with lidocaine under complete aseptic conditions in the right or left lower quadrant with the patient in the supine position, part of the specimen was directly sent to the laboratory for examination of differential leukocyte counts (PMNLs). The other part (about three mL) of the ascitic fluid was centrifuged for 15 min, the supernatant was transferred to three sterile Eppendorf tubes and stored at –20ºC until analysis by ELISA technique for measuring the levels of Glucose, total protein, LDH using Biosystem A15 autoanalyzer (Biosystem S.A, Barcelona, Spain).

By appropriate chemical principles calprotectin level was measured by ELISA technique using calprotectin human ELISA kit (Demeditech Diagnostic, GMbH, Keil Wellsee, Germany) according to the manufacturer's instructions.

Ethical clearance:

An informed consent was obtained from all subjects after taking approval of Institutional Review Board, Faculty of Medicine, Benha University. The work had been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Statistical Analysis

All data were collected, tabulated and statistically analyzed using SPSS 22 for windows (SPSS Inc., Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Wilk test. Quantitative data were expressed as mean ± SD (Standard deviation) for parametric and median and range for non-parametric data. Independent T test and Mann Whitney test were used to calculate difference between quantitative variables in two groups for parametric and non-parametric variables respectively. Receiver operating characteristic (ROC) curve was constructed to permit selection of threshold values for test results and comparison of different testing strategies. Areas under ROC curves and their standard errors were determined using the method of Cantor, and compared using the normal distribution, with correction for correlation of observations derived from the same cases. The optimal cutoff point was established at point of maximum accuracy. All statistical comparisons were two tailed with significance level of P-value $\leq$ 0.05 indicates significant and p <0.001 indicates highly significant difference.

RESULTS

Regarding the parameters of laboratory investigations in this study, WBCs, ALT, AST, and median total and direct bilirubin and CRP were significantly higher in group I than group II. Platelets, hemoglobin were significantly lower in group I than group II. There were no significant differences regarding albumin, INR, urea, and creatinine **(Table 1).**

Table (1): Laboratory findings in both groups

		Group I (n = 40)	Group II (n = 10)	P-value
Hemoglobin (g/dl)	Mean ±SD	9 ±1	10 ±0.9	0.006
WBCs (10^3 cell/cmm)	Median ±SD	9.7 ±2	5.6 ±1.2	0.015
Platelets (10^3 cell/cmm)	Mean ±SD	77 ±3	126 ±5	<0.001
ALT (IU/l)	Mean ±SD	53 ±11	40 ±9	0.039
AST (IU/l)	Mean ±SD	53 ±7	41 ±5	0.049
Total bilirubin (mg/dl)	Mean ±SD	3.2 ±0.7	1.8 ±0.31	0.013
Direct bilirubin (mg/dl)	Mean ±SD	1.6 ±0.32	0.65 ±0.11	0.006
Albumin (g/dl)	Mean ±SD	2.52 ±0.31	2.68 ±0.2	0.128
INR	Mean ±SD	2.08 ±0.4	1.69 ±0.6	0.09
Urea (mg/dl)	Mean ±SD	71 ± 15.62	52.5 ± 11.81	0.765
Creatinine (mg/dl)	Mean ±SD	1.4 ± 0.25	1.55 ± 0.271	0.296
CRP (mg/L)	Mean ±SD	42.5 ± 9. 73	7 ± 1.31	0.001

Comparison of ascitic fluid parameters between the two studied groups: TLC, PMNLs, LDH and total protein were significantly higher in group I than group II. However, there was no significant difference between the two studied groups regarding SAAG, glucose, and albumin **(Table 2).**

Table (2): Ascitic fluid analysis in both groups

		Group I (n = 40)	Group II (n = 10)	P-value
TLC (cell/ul)	Mean ±SD	4100 ± 89.81	280 ± 6.81	<0.001
PMNLs	Mean ±SD	388.12 ±9.13	71.6 ±6.82	<0.001
SAAG	Mean ±SD	1.80 ±0.33	1.94 ±0.25	0.166
LDH (U/L)	Mean ±SD	283.82 ±48.51	214.8 ±32.03	<0.001
Glucose (mg/dL)	Mean ±SD	92.57 ±13.82	97.4 ±14.30	0.346
Total protein (g/dl)	Mean ±SD	1.82±0.26	1.52±0.23	<0.001
Albumin (g/dl)	Mean ±SD	0.776 ±0.2	0.712 ±0.1	0.545

PMNLs: polymorphonuclear leukocytes, SAAG: serum ascites albumin gradient, LDH: Lactate dehydrogenase.

The ascitic fluid calprotectin was significantly higher in group I than group II **(Table 3).**

Table (3): Ascitic fluid calprotectin levels in both groups

		Group I (n = 40)	Group II (n = 10)	P-value
Calprotectin (ng/ml)	Mean ±SD	26.3 ±5.61	15 ± 3.81	0.013

Mann Whitney U test was used

Ascitic fluid calprotectin was significant at a cutoff level of 18 ng/ml for diagnosing SBP with an area under the curve (AUC) = 0.835) **(Table 4, Figure 1).**

Table (4): ROC analysis for ascitic fluid calprotectin in diagnosing SBP

ROC characteristics	
AUC (95% CI)	0.835 (0.703 – 0.925)
Best cutoff	≥ 18
Sensitivity	90%
Specificity	70%
PPV	92.3%
NPV	63.6%
P-value	<0.001

AUC; Area Under Curve. 95% CI: 95% confidence interval.
PPV; Positive predictive value. NPV; Negative predictive value.

ROC curve of ascitic calprotectin as a diagnostic marker for SBP in cirrhotic patients (Figure 1).

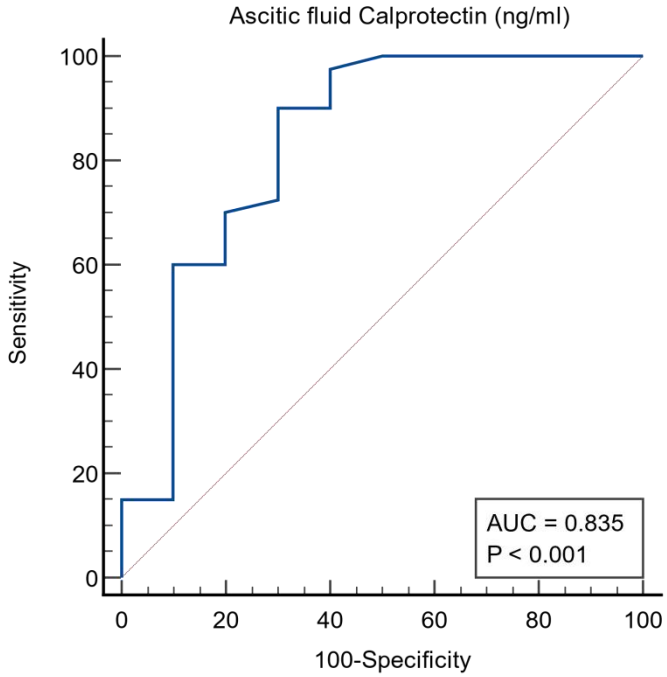

Figure (1): ROC analysis for ascitic fluid calprotectin in diagnosing SBP

In group I, ascitic fluid calprotectin was positively correlated with ascitic fluid PMNLs, while negatively correlated with ascitic fluid albumin **(Table 5).**

Table (5): Correlation between ascitic fluid calprotectin and ascitic fluid and laboratory parameters in group I (SBP)

Group I	Ascitic fluid calprotectin	
	r	P-value
TLC (cells/µl)	-0.286	0.074
PMNLs	0.495	0.001
SAAG	0.283	0.077
LDH (U/L)	0.161	0.321
Glucose (mg/dL)	0.223	0.166
Total protein (g/dl)	0.449	0.036
Albumin (g/dl)	-0.442	0.004
Hb (g/dl)	-0.003	0.987
WBCs (10^3 cell/cmm)	-0.088	0.588
Platelets (10^3 cell/cmm)	.359*	0.023
ALT (IU/l)	-0.191	0.237
AST (IU/l)	0.034	0.833
Total bilirubin (mdl/g)	-0.237	0.141
Direct bilirubin (mg/dl)	-0.028	0.865
Albumin (g/dl)	0.105	0.521
INR	-0.268	0.094
Urea (mg/dl)	0.007	0.965
Creatinine (mg/dl)	-0.118	0.467

r: correlation coefficient

DISCUSSION

SBP is a unique and a widespread complication in cirrhotic patients. The prevalence ranges from 10 to 30% in cirrhotic ascitic patients at the time of hospital admission and about 50% develop during hospitalization, with a mortality rate about 20–30% depending on several factors. In ascitic fluid, local and systemic immune dysfunction with bacterial translocation and reduced opsonic activity are the cornerstone mechanisms in the pathogenesis of SBP [(10)].

The diagnosis of SBP is still based upon diagnostic paracentesis [(7)]. There was a need for novel reliable and rapid diagnostic methods for SBP in patients with hepatic cirrhosis. Calprotectin is a calcium and zinc-binding protein and detected mainly in neutrophils. Its presence in body fluids is directly proportional to the rate of influx of neutrophils [(4)], so ascitic fluid calprotectin may be helpful in detection of neutrophil count greater than or equals to 250 cells/mm^3, and then may have an important role in diagnosis of SBP. The main aim of this study was to evaluate ascitic fluid calprotectin as a diagnostic marker of spontaneous bacterial peritonitis in cirrhotic patients.

The results of our study showed that regarding the parameters of laboratory investigations, WBCs were significantly higher in group I (9.7) than group II (5.6). Similar result was obtained by **Cholongitas *et al.*** [(11)] who reported that leukocytosis was higher in SBP patients as a part of reaction of the body against infection.

As regard platelet count of studied patients, there was reduced platelet count in SBP and non-SBP, but it was much lower in SBP group with highly significant difference between both groups. These results are consistent with those of **Lata *et al.*** [(12)] who reported significant lower platelets count in group of SBP patients when compared to non-SBP group and supposed that the decrease of platelets count in SBP reflects the increase of portal pressure. Portal hypertension could probably contribute to the amount of protein in ascites, which is an important factor influencing the incidence of SBP even by increasing bacterial translocation. Also, **Kuckleburg *et al.*** [(13)] suggested that platelets have numerous immunologic functions as their role in activating neutrophil granulocytes in bacterial infections. Thus, a low platelets count might result in insufficient activation of neutrophils and risk of infections in cirrhotic patients.

In this study, hemoglobin level was lower in group I (9 g/dl) than group II (10 g/dl). This disagreed with **Coskun *et al.*** [(14)] who stated that patients with SBP have normal hemoglobin, which does not affected by ascitic fluid infection, but hemoglobin level may be related to severity of liver disease which reported by **Paul *et al.*** [(15)]. Also we found that, the level of serum ALT and AST was significantly higher in SBP group than non-SBP group. These results were in line with **Coral *et al.*** [(16)] who strongly suggested that liver functions may be worsened by bacterial infection.

As regard bilirubin level of studied patients, we noted that median total and direct bilirubin were significantly higher in group I (3.2 mg/dl and 1.6 mg/dl, respectively) compared to group II (1.8 mg/dl and 0.65 mg/dl, respectively). Also, these results were similar to data recorded by **Abdel-Rahman *et al.*** [(17)] who found that patients with SBP had statistically significant higher median total bilirubin compared to control group (4.2 vs. 2.3 mg/dL) respectively.

As regard serum albumin and INR in both groups, we noted that there was slight decrease in serum albumin in group I compared to group II and slight increase in INR in group I than group II, but with no significant differences between both groups regarding serum albumin and INR. These results were in agreement with those reported by **Runyon *et al.*** [(18)] who stated that, hypoalbuminemia and prolonged prothrombin time are not related to SBP per se, but rather to the underlying liver disease. Regarding the kidney functions tests, there were no statistical significant difference between the two groups and this is in agreement with **Zalam *et al.*** [(19)] who reported no statistical significant difference comparing SBP and non-SBP patients as regards kidney function. This is in disagreement with results of the study conducted by **Ajitpal *et al.*** [(20)] in which the levels of serum creatinine were significantly higher in patients with SBP compared to those without (2.44 ± 0.84 vs. 1.8 ± 1.35, $P<0.05$).

Chemical analysis of ascitic fluid showed that, statistically significantly higher levels of TLC, PMN count in SBP group compared to non-SBP group, these results were in line with **Yildirim *et al.*** [(21)] who reported higher ascitic TLC in SBP more than non-SBP patients. Ascitic fluid total protein in our study was statistically significant high in SBP patients than in non-SBP patients. This result was consistent with **Abdel-Razik *et al.*** [(22)] who found that, patients with SBP had an obvious increase in ascitic fluid total protein, which has an important role in the inflammatory process in SBP, so it can be measured as an inflammatory marker in the early phase of the illness. On the other hand, this result disagrees with **Paul *et al.*** [(15)] who noted that patients with poor synthetic function have diminished level of protein in ascitic fluid that correlate with low level of opsonization and this play a role in SBP susceptibility and noted also ascitic fluid total protein < 1 g/dl as important predictor for SBP.

Also ascitic fluid LDH in this study was significantly higher in SBP patients than non-SBP patients. Similar result was obtained by **Krastev *et al.*** [(23)] who found that patients with SBP frequently have ascitic fluid LDH greater than that of non-SBP group. However, ascitic fluid glucose level had no significant difference between two groups in our study. This result goes in agreement with **Lin *et al.*** [(24)] who found that

there was no significant difference between SBP and non-SBP patients regarding ascitic fluid glucose level, therefor it has low diagnostic sensitivity and specificity in differentiation SBP from non-SBP patients. So the application of ascitic glucose analysis is limited in routine practice. On the other hand, this result disagrees with **Tsung *et al.*** [25] who reported that, SBP patients show lower level of ascitic fluid glucose due to consumption by bacteria and white blood cells.

Our study revealed that the mean value of SAAG was > 1.1 g/dl in both SBP and non-SBP groups but without significant difference, which confirms that the etiology of ascites was portal hypertension in these patients. This finding is in concordance with **Agarwal *et al.*** [26] study, which suggested that SAAG levels are > 1.1 g/dl in all ascites due to portal hypertension irrespective of infection. SBP patients in this study had lower mean SAAG value (1.80±0.33 g/dl) as compared to non-SBP patients (1.94±0.25 g/dl), but without significant difference. Similar results were reported by **Thiele *et al.*** [27] as mean value of SAAG in SBP group was (1.3 g/dl) and in non SBP group was (1.7 g/dl) and this can be explained by **Tarn and Lapworth** [28] who stated that SBP is advanced liver disease associated with low serum albumin concentration and so on lower SAAG than cirrhotic patients without SBP.

Regarding ascitic fluid calprotectin level in this study, the median level was significantly higher in patients with evidence of spontaneous bacterial peritonitis (SBP) (26.3 ng/ml) compared to control group (15 ng/ml). In agreement with our results, **Weil *et al.*** [29] reported that, among 236 ascitic fluid samples with available calprotectin levels, patients with SBP had significantly higher median levels of calprotectin (1.81 μg/mL) than patients without SBP (0.25 μg/mL; P<0.001). Also, **Lutz *et al.*** [30] showed that, calprotectin levels in SBP (median 928 ng/mL, range 21–110,480 ng/mL) were significantly increased in comparison to uninfected samples (median 34 ng/mL, range 5–795 ng/mL; p<0.001). The current study showed that ascitic fluid calprotectin at a cutoff level ≥ 18 ng/ml, it had a sensitivity of 90 % and 70% specificity for diagnosing SBP with an area under the curve (AUC) = 0.835. Our results were supported by study of **Abdel-Razik *et al.*** [22] as they reported that ascitic calprotectin at a cutoff value of 445 ng/ml had 95.4% sensitivity and 85.2% specificity for detecting SBP (AUC=0.921). On correlating ascitic calprotectin with other parameters in patients with SBP, there was a significant positive correlation between calprotectin and PMNLs (r = 0.495) and significant negative correlation between calprotectin and albumin (r = -0.442). This was in agreement with the study of **Burri *et al.*** [6] who reported that ascitic calprotectin levels correlated well with PMN count. Samples with PMN ≥250/mm^3 also had higher ascitic calprotectin levels than the samples with PMN <250/mm^3.

Limitation of the study: The number of patients was small.

CONCLUSION

Ascitic fluid calprotectin could be used as a reliable diagnostic marker for SBP in cirrhotic patients with ascites.

Conflict of interest: The authors declare no conflict of interest.

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Author contribution: Authors contributed equally in the study.

REFERENCES

1. **Nusrat S, Khan M, Fazili J *et al.* (2014):** Cirrhosis and its complications: evidence based treatment. World J Gastroenterol., 20: 5442-48.
2. **Marciano S, Diaz J, Dirchwolf M *et al.* (2019):** Spontaneous bacterial peritonitis in patients with cirrhosis: incidence, outcomes, and treatment strategies. Hepatic Med Evid Res., 11: 13-17.
3. **Wiest R, Garcia-Taso G (2005):** Bacterial translocation (BT) in cirrhosis. Hepatology, 411: 422-27.
4. **Brophy M, Nolan E (2015):** Manganese and microbial pathogenesis: sequestration by the mammalian immune system and utilization by microorganisms. ACS Chem Biol., 10:641–651.
5. **Soyfoo M, Roth J, Vogl T *et al.* (2009):** Phagocyte-specific S100A8/A9 (calprotectin) protein levels during disease exacerbations and infections in systemic lupus erythematosus. J Rheumatol., 36:2190–2194.
6. **Burri E, Schutle F, Muser J *et al.* (2013):** Measurement of calprotectin in ascitic fluid to identify elevated polymorphonuclear cell count. World J Gastroentrol., 19(13): 2028-2036.
7. **Rimola A, Gracia-Tsao G, Navasa M *et al.* (2000):** Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. International Ascites Club. J Hepatol., 32: 142-153.
8. **Wayne J, Lauwers G, Ikai I *et al.* (2002):** Preoperative predictors of survival after resection of small hepatocellular carcinomas. Annals of Surgery, 235(5)722-26.
9. **Jung G, Encke J, Schmidt J *et al.* (2008):** Model for end-stage liver disease. New basis of allocation for liver transplantation. Chirurg., 79(2):157-63.
10. **Wiest R, Lawson M, Geuking M (2014):** Pathological bacterial translocation in liver cirrhosis. J Hepatol., 60:197–209.
11. **Cholongitas E, Theodoridis G, Manesis E *et al.* (2006):** Spontaneous bacterial peritonitis in cirrhotic patients: Is prophylactic propranolol therapy beneficial? J Gastroenterol and Hepatology, 21:581-587.
12. **Lata J, Fejfar T, Krechler T *et al.* (2003):** Spontaneous bacterial peritonitis in the Czech

Republic: prevalence and etiology. Eur J Gastroenterol Hepatol., 15(7): 739-43.

13. **Kuckleburg C, Tiwari R, Czuprynski C (2008):** Endothelial cell apoptosis induced by bacteria-activated platelets requires caspase-8 and -9 and generation of reactive oxygen species. Thromb Haemost., 99:363-72.
14. **Coskun U, Ozenirler S, Sancak B *et al.* (2001):** Serum and ascitic fluid hemoglobin levels in patients with cirrhosis. Clin Chin Acta., 306(1-2):127-32.
15. **Paul K, Kaur J, Kazal H (2015):** Predictive factors and clinical outcome of spontaneous bacterial peritonitis in patients of cirrhosis with ascites. Journal of Clinical & Diagnostic Research, 15: 09–12.
16. **Coral G, Mattos A, Valiatti F *et al.* (2002):** Bacterial infections in cirrhotic patients. World J Gastroenterol., 20(39): 14079-86.
17. **Abdel-Rahman E, Attia F, Alsebaey A *et al.* (2020):** Ascitic calprotectin as a useful marker in the diagnosis of spontaneous bacterial peritonitis in adults. Egypt Liver Journal, 10: 14-18.
18. **Runyon B, Akriviadis E, Sattler R *et al.* (1991):** Ascitic fluid and serum cefotaxime and desacetyl cefotaxime levels in patients treated for bacterial peritonitis. Dig Disc Sci., 12:1782-1786.
19. **Zalam S, El Taher S, Al Taher S *et al.* (2010):** The values of serum and ascetic fluid tumor necrosis factor-Alpha (TNF- alpha), TNF-receptor 1 and C-reactive protein (CRP) in patients with cirrhotic ascites. Scientific Medical Journal, 41:491-501.
20. **Ajitpal S, Amandeep S, Prithpal S *et al.* (2012):** Spontaneous bacterial peritonitis in alcoholic cirrhosis: An Indian perspective. Euroascian Journal of Gastreoenterology, 1: 14-19.
21. **Yildirim B, Sari R, Isci N *et al.* (2005):** Patients with spontaneous bacterial peritonitis, and malignant and cirrhotic ascites. J Natl Med Assoc., 97: 276-80.
22. **Abdel-Razik A, Mousa N, Elhammady D *et al.* (2016):** Ascitic fluid calprotectin and serum procalcitonin as accurate diagnostic markers for spontaneous bacterial peritonitis. Gut Liver, 10(4):624-631.
23. **Krastev N, Djurkov V, Murdjeva M *et al.* (2013):** Diagnosis of spontaneous and secondary bacterial peritonitis in patients with hepatic cirrhosis and ascites. Khirurgiia (Sofiia), 3: 20-25.
24. **Lin-Lin H, Harry Hua X, Sen-Lin Z (2014):** Ascitic fluid analysis in the differential diagnosis of ascites; focus on cirrhotic ascites. J Clin Transl Hepatol., 2 (1): 58-64.
25. **Tsung P, Ryu S, Cha I *et al.* (2013):** Predictive factors that influence the survival rates in liver cirrhotic patients with spontaneous bacterial peritonitis. J Clinical and Molecular Hepatology, 19:131-139.
26. **Agarwal M, Choudhury B, Banerjee B *et al.* (2008):** Ascitic fluid examination for diagnosis of spontaneous bacterial peritonitis in cirrhotic ascites. Indian Academy of Clinical Medicine, 9 (1):29-32.
27. **Thiele G, Silva O, Fayad L *et al.* (2014):** Clinical and laboratorial features of spontaneous bacterial peritonitis in southern Brazil. Sao Paulo Med J., 132 (4): 205-210.
28. **Tarn A, Lapworth R (2010):** Biochemical analysis of ascitic (peritoneal) fluid: what should we measure? Annals of Clinical Biochemistry, 47:397-407.
29. **Weil D, Heurgue-Berlot A, Monnet E *et al.* (2019):** Accuracy of calprotectin using the Quantum Blue Reader for the diagnosis of spontaneous bacterial peritonitis in liver cirrhosis. Hepatol Res., 49 (1):72–81.
30. **Lutz P, Pfarr K, Nischalke H *et al.* (2015):** The ratio of calprotectin to total protein as a diagnostic and prognostic marker for spontaneous bacterial peritonitis in patients with liver cirrhosis and ascites. Clinical Chemistry and Laboratory Medicine, 53(12): 2031-2039.