Original Article

Medical Journal of Viral Hepatitis (MJVH)

Culture negative neutrocytic ascites versus culture positive spontaneous bacterial peritonitis; Is there a Difference; A Multi-Centric Study

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Abstract

Background: There are two variants of spontaneous bacterial peritonitis (SBP) include, culture negative neutrocytic ascites (CNNA) and culture positive SBP. Some suggested that, the clinical presentation of two variants is nearly similar, however, other reported that, patients with culture positive have a more severe course and higher mortality than CNNA. The aim of this study is to determine the clinical characteristics and predictors of CNNA in comparison to culture positive SBP.

Materials and methods: This study included 300 consecutive patients with HCV related cirrhotic ascites. All patients underwent abdominal paracentesis and the ascitic fluid was processed for cell count and culture. Clinical and laboratory parameters of these patients were recorded at index admission.. **Results:** Out of 300 patients included in the study, 150 patients had SBP. Among the 150 patients with SBP, 100 patients were culture positive SBP (culture positive SBP with ascitic fluid PMNL ≥250 cells/m3) and 50 patients were CNNA (culture negative SBP with ascitic fluid PMNL ≥250 cells/m3). Compared to patients with culture positive SBP, patients with CNNA showed, a significant decrease as regards, fever, prevalence of DM, hepatic encephalopathy, platelets, blood PMNL and ascetic PMNL. Logistic regression analysis demonstrated that, decreased platelets count, blood PMNL and ascetic PMNL were independent predictor factors for CNNA.

Conclusion: Patients with CNNA have a lower incidence of fever, prevalence of diabetes mellitus, hepatic encephalopathy, blood PMNL and ascetic PMNL versus culture positive SBP. Independent predictors of culture negative SBP are decreased platelets, blood PMNL and ascetic PMNL.

Keywords: Ascitic fluid infections, spontaneous bacterial peritonitis, culture negative neutrocytic ascites, culture positive spontaneous bacterial..

Received: 1-5-2021; Accepted: 18-6-2021

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Introduction

In cirrhotic patients, bacterial infections of ascites increase the morbidity and mortality [1,2]. Bacterial infections occur in 25% to 30% in cirrhotic patients and are responsible for 30% to 50% of the mortality in patients with chronic liver disease [3,4]. In abscence of any evidence of external or intra-abdominal source of infection, two variants of spontaneous bacterial peritonitis (SBP) have been described, the first is spontaneous bacterial peritonitis diagnosed by positive ascitic fluid culture associated with ascitic PMNL count >250/mm^3 and the second is patients have culture-negative SBP with an ascitic fluid neutrophil count ≥250 cells/mm3 named culture negative neutrocytic ascites (CNNA). Due to the high rate of culture negativity, reportedly up to 60%, SBP is diagnosed based on the ascitic fluid polymorphonuclear (PMN) count. Culture negative neutrocytic ascites, which is considered to be a variant of SBP, was first defined in 1984 [5-7].

The outpatient prevalence of SBP is 1.5–3.5% and exceeds 10% in hospitalized patients [8,9]. When first described, its mortality exceeded 90%, but it has been reduced to approximately 20% with early diagnosis and treatment[10].

Several studies have reported that, microorganisms are present in only 40–73% of SBP cases by using blood culture bottles [11,12]. The most prevalent types of microorganism isolated from patient ascetic fluid with SBP are *Escherichia coli* (~70%) and *Klebsiella* species [13]. While culturing bacteria needs time, delays in antibacterial treatment can be fatal and result in the death of the patient from overwhelming infection. However, ascites culture is essential to guide antibiotic therapy[14]. Up to the present time, only a scarce research, including a small number of patients has compared the clinical course and prognosis of CNNA and SBP [7,15-17]. Runyon and Hoefs[7] first described the similarities between CNNA and SBP in terms of clinical signs, symptoms and mortality, In contrast, some observed lower mortality in patients with CNNA than those with SBP[15] and concluded that CNNA was a less severe variant of SBP [15,17].

Although, some suggested that, the clinical presentation of two types AFI is nearly similar and should be treated in a similar manner [6,15], Many papers reported that, patients with SBP have a more severe course and higher mortality than CNNA [16, 17].

The aim of this study is to evaluate the characterization and predictors for culture negative neutrocytic ascites versus culture positive spontaneous bacterial peritonitis.

Materials and methods

In this prospective multi-centric study, we recruited 300 patients with HCV related cirrhotic ascites referred to the Tropical and Internal Medicine Departments, Mansoura University and Gastroenterology and Hepatology Department, Damietta Cardiology and Gastroenterology center, Egypt for paracentesis from October 2018 to September 2019. Complete history taking and physical examination include, abdominal ultrasonography and triphasic CT when indicated were done for all patients. The exclusion criteria were non-cirrhosis ascites (e.g. cardiac, renal, malignant and TB ascites), immunocompromised patients, and patients who had taken antibiotics prior to hospital admission or on prophylactic antibiotics for SBP. Furthermore, secondary bacterial peritonitis due to any surgical causes and patients with infection that may influence the levels of blood WBC or CRP, e.g. skin and lung infection, were also excluded.

Methodology

1- At the bedside, fifteen ml of ascitic fluid was aspirated under complete aseptic condition. Ten ml was immediately inoculated in bedside aerobic and anaerobic blood culture bottles and the residual amount was sent for biochemical and cytological examination in tubes containing EDTA and analyzed within 3 hours of aspiration [18]. Ziehl-Neelsen staining of the ascitic fluid was done when needed. Inoculated blood culture bottles were incubated for 3 successive days at 37°C with daily subculture on blood, MacConkey and chocolate agars. Antimicrobial susceptibility tests and bacterial identification were done using standard procedures. In the absenteeism of hemorrhagic ascites and secondary peritonitis AFI diagnosis was built on the existence of ≥250 PMNL cells/cmm ascitic fluid, with positive culture of ascitic fluid (culture positive spontaneous bacterial peritonitis). Patients with an ascitic fluid neutrophil count ≥250 cells/mm3 and negative culture have culture-negative SBP[19]. Patients with ascitic fluid PMNL cell count < 250 cells/mm3 and a culture-negative ascitic fluid was assigned as the control group.

2. At the time of paracentesis, five ml of venous blood was withdrawn; one ml in polystyrene EDTA tube for CBC and four ml delivered in another tube and allowed to clot. Non-hemolyzed sera were separated by centrifugation and used for determination of liver functions (ALT, AST, albumin, bilirubin and prothrombin time) blood sugar, creatinine, urea, alpha-fetoprotein and carcinoembryonic antigen. This study was performed with the approval of the Mansoura Faculty of Medicine Institutional Review Board "MFM-IRB". All patients provided written informed consent prior to participation in any protocol-specific procedure.

Statistical analysis

Data were analyzed with SPSS version 21. The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described using number and percentage. Association between categorical variables was tested using Chi-square. Continuous variables were presented as mean ± SD (standard deviation) for parametric data but Median and IQR were used for non-parametric data. The two groups were compared with Student *t* test (parametric data). Clinical variables were considered statistically significant when ($p<0.05$).

Results

(Table 1) shows the clinical, laboratory and demographic details of the studied groups. The two groups were matched for age and sex. There was a significant increase in SBP group versus non non-SBP group as regard, fever, abdominal pain, hepatic encephalopathy, bilirubin, AST, prothrombin time (PT), CRP, blood WBC and PMNL, ascetic WBC and ascetic PMNL, though a significant decrease in platelets, blood and ascetic lymphocyte. No significant differences were found as regards, DM, rupture esophageal varies, serum albumin, ALT, Child–Pugh score and hemoglobin between both groups.

Among the 150 patients with AFI, one hundred were culture positive SBP and fifty patients were CNNA(culture negative SBP). **(Table 2)** shows that, compared to patients with culture positive SBP, patients with CNNA (culture negative SBP) showed, a significant decrease as regards, fever, prevalence of DM, hepatic encephalopathy, platelets, blood PMNL and ascetic PMNL, however a significant increase in ascetic lymphocyte. No significant differences were found between both variants as regards, age, sex, abdominal pain, rupture esophageal varies, serum albumin, bilirubin, ALT, AST, serum creatinine, PT,CRP, Child–Pugh score, blood WBC, blood lymphocyte and ascitic WBC between both groups.

To determine predictive parameters for the diagnosis of CNNA (culture negative SBP), variables with significant associations on univariate analysis were subjected to a logistic regression analysis. (Table 3) shows multiple logistic regression analysis of predictors of CNNA. Decreased platelets count, blood PMNL and ascetic PMNL were independent predictor factors for CNNA. To determine predictive parameters for the diagnosis of SBP, variables with significant associations of AFI on univariate analysis were subjected to a logistic regression analysis. Increased serum creatinine, serum bilirubin, CRP, WBC, blood PMNL, ascitic PMNL and decreased platelets and

ascitic lymphocytes were independent predictors of SBP development (Table 4).

Isolation of cultured organisms.

Out of 150 patients with ascetic fluid infection,, microorganisms in ascitic fluid were isolated in 100 patients (66.6%). Among the 100 patients, 24 patients (24%) had two or more species of microorganisms. Gram negative organisms were found in 67 patients (67%), Gram positive organisms were found in 23 patients (23%) and 10 patients (10%) had both Gram positive and negative organisms in their ascitic fluid. E. coli was the most frequently isolated organism (53 of 100 cases, 53%), followed by Klebsiella species (10 cases, 10%), streptococcus species (12 cases, 12%), pneumococcus (7 cases, 7%), enterococcus species (6 cases, 6%) staphylococcus (5 cases, 5%) and Enterobacteriaceae (4 cases, 4%).

Table 1. Clinical and biochemical characteristic of studied patients with SBP vs. non-spontaneous bacterial peritonitis patients.

	Case (N=150)	Control (N=150)	P value
Age	55.38±8.91	54.34±4.16	0.19
Sex (M/F)	94/56	86/64	0.40
Abdominal pain (N/%)	79 (52.7%)	5 (3.3%)	<0.001
Fever (N/%)	80 (53.3%)	2 (1.3)	<0.001
DM (N/%)	49 (32.7%)	42 (28%)	0.45
HE (N/%)	118 (78.7%)	88 (58.6%)	0.003
REV (N/%)	35 (23.3%)	33 (22.0%)	0.89
Albumin (g/dL)	2.36±61169	2.4±0.6	0.41
Bilirubin (µmol/L)	291.7 (159.1-556.9)	79.5 (61.8-362.4)	<0.001
ALT (U/L)	30 (21.9-42)	36 (20-56)	0.118
AST (U/L)	61 (37-107)	31 (22-95)	<0.001
Creatinine (µmol/L)	128.1±11.49	11.49±8.8	<0.001
PT (seconds)	1.6±0.5	1.4±0.4	0.022
CRP (mg/dL)	48.63±34.03	4.19±3.44	<0.0001
CPS (N): A/B/C	0/12/138	0/6/144	0.14
Platelets (10^3/cmm)	76.0±13	93.0±31	0.002
Blood WBC (10^3/cmm)	8410 (5452.5-12700)	4400 (3400.0-5600.0)	<0.001
Blood PMNL (10^3/cmm)	69.58±13.587	55.16±14.724	<0.0001
Blood lymphocyte (10^3/cmm)	17.77 ±11.573	26.34± 5.916	<0.001
Hemoglobin (g/dL)	10.4±2.04021	10.05 ±2.1	0.51
Ascetic WBC (10^3/cmm)	1750 (900-4800)	180 (120-200)	<0.001
Ascetic PMNL (10^3/cmm)	68.50±10.87	64.90±12.54	0.008
Ascetic lymphocyte (10^3/cmm)	32.08±2.16	38.06±8.77	<0.001

HE, *Hepatic encephalopathy*; **REV**, *Ruptue esophageal varices*; **CPS**, *Child–Pugh score*; **ALT**, *Alanine aminotransferase*; **AST**, *aspartate aminotransferase*; **PT**, *prothrombin time*; **WBC**, *White blood cells*, **PMNL**: *Polymorphonuclear leukocytes*.

Table 2. Clinical and biochemical characteristic of patients with culture positive SBP versus CNNA.

	Culture Positive SBP N = 100	Culture Negative SBP N = 50	P value
Age	54.77±8.40	56.60±9.81	0.54
Sex. M/F	30/20	64/36	0.76
Abdominal pain (N/%)	56(56%)	23(46%)	0.18
Fever (N/%)	61 (61%)	19(38%)	0.012
DM (N/%)	41(41%)	8(16%)	0.003
Hepatic encephalopathy (N/%)	87 (87%)	31 (62%)	0.009
Rupture esophageal varices (N/%)	21(21.0%)	14 (28%)	0.45
Albumin (g/dL)	2.40±0.66	2.34±.585	0.13
Bilirubin (µmol/L)	274 (150.2-437.5)	291.7 (159.1-627.6)	0.31
ALT (U/L)	32 (20-37)	29.50 (22-46.0)	0.33
AST(U/L)	68 (29-96)	60.5 (37.-112)	0.91
Creatinine (µmol/L)	123.7 (79.5-256.3)	114.9 (70.7-185.6)	0.09
PT time	1.75±0.55	1.54±0.49	0.08
CRP(mg/dL)	45.65±31.18	53.15±37.85	0.11
Child–Pugh score (N): A/B/C	0/8/92	0/4/46	0.053
Platelets (10^3/cmm)	87.0±31	72.0±23	0.003
Blood WBC(10^3/cmm)	8600 (5652-13780)	8290 (5280-12400)	0.69
Blood PMNL(10^3/cmm)	74.00± 8.769	67.22±15.074	0.001
Blood lymphocytes(10^3/cmm)	14.20 (9.75-19.70)	16.90(7.50-30.0)	0.25
Ascetic WBC(10^3/cmm)	1800 (1050-8075)	1500.0 (900.0-3725.0)	0.12
Ascetic PMNL (10^3/cmm)	71.64±11.90	66.87±9.97	0.005
Ascetic lymphocyte(10^3/cmm)	30 (19.5-37.0)	35 (25-42)	0.012

CNNA, *culture negative neutrocytic ascites*

Table 3. Logistic regression analysis of pediatrics of culture positive SBP

Parameters	Exp (B)	95.0% C.I for EXP (B)		P value
		Lower	Upper	
Platelets	1.000	1.000	1.000	0.011
Blood PMNL	1.046	1.013	1.081	0.007
Ascetic PMNL	1.161	1.035	1.302	0.011
Ascetic lymphocyte	1.106	.998	1.226	0.055

Table 4. Logistic regression analysis of predictors of AFI versus control.

Parameters	Exp (B)	95.0% C.I for EXP (B)		P value
		Lower	Upper	
Creatine	10.420	0.000	30.5.	0.019
Bilirubin	0.133	0.000	7.72	0.025
AST	0.826	0.000	1.6	0.919
CRP	0.761	0.000	1.03	0.009
Platelets	1.000	1.000	1.000	0.024
Blood WBC	1.000	0.610	1.637	0.008
Blood PMNL	1.439	0.000	1.861	0.007
Blood lymph	2.910	0.000	9.504	0.99
Ascetic WBC	0.943	.054	16.41	0.096
Ascetic PMNL	0.666	0.000	8.83	0.011
Ascetic lymphocyte	0.556	0.000	3.06	0.023

Discussion

The widespread antibiotic use in patients with cirrhosis has changed the spectrum of bacteria responsible for SBP. Consequently, the choice of antibiotics for SBP has become a topic of discussion rising the importance of bacterial culture. As culturing bacteria need time, resulting in delay in antibacterial treatment and fatal outcome. It is thus important to understand the predictive factors for SBP specially culture negative SBP [20, 21].

In this study, we tried to demonstrate the characterization and predictive factors of culture negative SBP (CNNA) versus culture positive SBP. In this study, incomparsion to CNNA, patients with culture positive SBP patients showed, a significant increase as regards, prevalence of fever, diabetes mellitus and incidence of hepatic encephalopathy, without significant change as regards abdominal pain. On the other side, Kamani et al studied the two groups of ascitic fluid infection, i.e., SBP and CNNA and found that, the patients with SBP (culture positive SBP) had a statistically significant incidence of hepatic encephalopathy with no significant differences as regards abdominal pain and fever as compared to CNNA group[22]. Although, previous studies found that, majority of hematological and biochemical laboratory tests, including hemoglobin, white blood cells counts, and liver function tests were similar in the two groups of SBP [23,24]. Our results demonstrated a significant increase as regards, serum creatinine, prothrombin time and blood PMNL, similar to Kamani et al[22], we found no significant differences between SBP and CNNA as regards, ascetic TLC and gastrointestinal bleeding.

Using logistic regression analysis for predictors of culture positive SBP demonstrated that, blood polymorphonuclear and ascitic polymorphonuclear cells are independent predictor factors for culture positive SBP. Many studies declared that, the total lecocytic count (TLC) is independently predictor of SBP [24,25] . However Kamani et al in his study compared TLC and asctic PMN in culture positive SBP versus CNNA and found no significant difference between two studied groups as regards total lecocytic and ascitic polymorphonuclear [22]. Also, Terg et al found none of the values of TLC and ascitic PMN were significantly different in culture positive SPB from those of CNNA[23]. In contrast, Na et al found that, patients with the SBP group had a higher ascites neutrophil count, and positive blood culture rate in comparison to CNNA patients[26].

Several laboratory tests have been presented as predictive for SBP, including C-reactive protein level [25,27], platelet count [28], impaired prothrombin time and serum creatinine level [29].

In this study, we found that, using logistic regression, increased serum creatinine, serum bilirubin, CRP, WBC, blood PMN, ascitic PMN and decreased ascitic lymphocytes were independent predictors of SBP development.

Tsung et al found that, higher serum bilirubin and increased renal dysfunction, are associated with higher mortality rates in patients with SBP [30]. In addition, Tu B et al demonstrated that, blood neutrophil percentage, blood PMN and increased serum creatinine were predictors of SBP development, moreover, blood neutrophils and PMNL in ascitic fluid might be effective factors for early SBP screening in liver cirrhosis patients [31]. In his study Mousa et al found that, CRP values were significantly higher in SBP and could be used as simple, low-cost, non-invasive test for SBP diagnosis [25].

Though, in the last few decades there is an increasing prevalence of gram-positive, quinolone-resistant, and multidrug-resistant bacteria in SBP, our results show that, Gram negative organisms were the most predominant organisms found versus Gram positive organisms (67% versus 23%) while, 10% of patients had both Gram positive and negative organisms in their ascitic fluid. These results are in accordance with previous results demonstrated that, SBP in patients with cirrhosis is typically caused by gram-negative bacteria that are part of the intestinal microbial flora [32,33].

This study represents an advance in biomedical science because it shows that, decreased platelets, blood PMNL and ascetic PMNL are independent predictors of CNNA (culture negative SBP). Patients with culture positive SBP are expected to have a more morbid course of the disease with more complications (e.g. hepatic encephalopathy) versus CNNA. So, we recommend that, patients with culture positive SBP are promptly admitted to the hospital and not treated on an outpatient basis as in hospital, they will be more closely monitored by well qualified personnel for the development of any complications.

Conclusion

Patients with CNNA have a lower incidence of fever, prevalence of diabetes mellitus, hepatic encephalopathy, blood PMNL and ascetic PMNL versus culture positive SBP. Independent predictors of culture negative SBP are decreased platelets, blood PMNL, ascetic PMNL.

References

1. Arvaniti, V., D'Amico, G., Fede, G., et al. (2010). Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology*, *139*: 1246–1256.
2. El Shabrawi, A., Abdelaziz, M., Mousa, N., et al. (2019). Infections in cirrhotic patients. *Med J Viral Hepatitis*, *4* (1) - pp. 5-14.
3. Pleguezuelo, M., Benitez, J. M., Jurado, J., et al. (2013). Diagnosis and management of bacterial infections in decompensated cirrhosis. *World journal of hepatology*, *5*(1), 16.
4. Runyon, B. A. (2009). Management of adult patients with ascites due to cirrhosis: an update. *Hepatology*, *49*(6), 2087-2107.
5. Such, J., and Runyon, B. A. (1998). Spontaneous bacterial peritonitis. *Clinical infectious diseases*, *669*-674.
6. Piano, S., Singh, V., Caraceni, P., et al. (2018). Epidemiology, predictors and outcomes of multi drug resistant (MDR) bacterial infections in patients with cirrhosis across the world. Final results of the" Global study".
7. Runyon, B. A., and Hoefs, J., C. (1984). Culture-negative neutrocytic ascites: a variant of spontaneous bacterialperitonitis. *Hepatology*, *4*(6), 1209-1211.
8. Shizuma, T. (2018). Spontaneous bacterial and fungal peritonitis in patients with liver cirrhosis: A literature review. *World journal of hepatology*, *10*(2), 254.
9. Evans, L. T., Kim, W. R., Poterucha, J. J., et al. (2003). Spontaneous bacterial peritonitis in asymptomatic outpatients with cirrhotic ascites. *Hepatology*, *37*(4), 897-901.
10. Nousbaum, J. B., Cadranel, J. F., Nahon, P., et al. (2007). Diagnostic accuracy of the Multistix 8 SG reagent strip in diagnosis of spontaneous bacterial peritonitis. *Hepatology*, *45*(5), 1275-1281.
11. Wiest, R., Krag, A., and Gerbes, A. (2012). Spontaneous bacterial peritonitis: recent guidelines and beyond. *Gut*, *61*(2), 297-310.
12. Koulaouzidis, A., Bhat, S., Karagiannidis, A., Tan, W. C., & Linaker, B. D. (2007). Spontaneous bacterial peritonitis. *Postgraduate Medical Journal*, *83*(980), 379-383.
13. Paul, K., Kaur, J., and Kazal, H. L. (2015). To study the incidence, predictive factors and clinical outcome of spontaneous bacterial peritonitis in patients of cirrhosis with ascites. *Journal of clinical and diagnostic research: JCDR*, *9*(7), OC09.
14. Tandon, P., Kumar, D., Seo, Y. S., et al. (2013). The 22/11 risk prediction model: a validated model for predicting 30-day mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *Official journal of the American College of Gastroenterology| ACG*, *108*(9), 1473-1479.
15. Pelletier, G., Salmon, D., Ink, O., et al. (1990). Culture-negative neutrocytic ascites: a less severe variant of spontaneous bacterial peritonitis. *Journal of hepatology*, *10*(3), 327-331.
16. European Association for The Study of the Liver. (2018). EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *Journal of hepatology*, *69*(2), 406-460.
17. Al Amri SM, Allam AR, Al Mofleh IA. Spontaneous bacterial peritonitis and culture negative neutrocytic ascites in patients with non-alcoholic liver cirrhosis. J. Gastroenterol. Hepatol. 1994; 9: 433–6
18. Sahar, Z., Nasser, M., Mohamed, A. E., et al. (2018). The predictive factors for recurrence of spontaneous bacterial peritonitis. *Medical Journal of Viral Hepatitis*, *2*(2), 25-29.
19. European Association for The Study of the Liver. (2018). EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *Journal of hepatology*, *69*(2), 406-460.
20. Reuken, P. A., Pletz, M. W., Baier, M., et al. (2012). Emergence of spontaneous bacterial peritonitis due to enterococci–risk factors and outcome in a 12-year retrospective study. *Alimentary pharmacology & therapeutics*, *35*(10), 1199-1208.
21. Alexopoulou, A., Papadopoulos, N., Eliopoulos, D. G., et al. (2013). Increasing frequency of gram-positive cocci and gram-negative multidrug-resistant

bacteria in spontaneous bacterial peritonitis. *Liver International*, *33*(7), 975-981.

22. Kamani, L., Mumtaz, K., Ahmed, U. S., et al. (2008). Outcomes in culture positive and culture negative ascitic fluid infection in patients with viral cirrhosis: cohort study. *BMC gastroenterology*, *8*(1), 1-6.

23. Terg, R., Levi, D., Lopez, P., et al. (1992). Analysis of clinical course and prognosis of culture-positive spontaneous bacterial peritonitis and neutrocytic ascites. *Digestive diseases and sciences*, *37*(10), 1499-1504.

24. Metwally, K., Fouad, T., Assem, M., et al. (2018). Predictors of spontaneous bacterial peritonitis in patients with cirrhotic ascites. *Journal of clinical and translational hepatology*, *6*(4), 372.

25. Mousa, N., Besheer, T., Abdel-Razik, A., et al. (2018). Can combined blood neutrophil to lymphocyte ratio and C-reactive protein be used for diagnosis of spontaneous bacterial peritonitis?. *British journal of biomedical science*, *75*(2), 71-75.

26. Na, S. H., Kim, E. J., Nam, E. Y., et al. (2017). Comparison of clinical characteristics and outcomes of spontaneous bacterial peritonitis and culture negative neutrocytic ascites. *Scandinavian journal of gastroenterology*, *52*(2), 199-203.

27. Wehmeyer, M. H., Krohm, S., Kastein, F., et al. (2014). Prediction of spontaneous bacterial peritonitis in cirrhotic ascites by a simple scoring system. *Scandinavian journal of gastroenterology*, *49*(5), 595-603.

28. Lata, J., Fejfar, T., Krechler, T., et al. (2003). Spontaneous bacterial peritonitis in the Czech Republic: prevalence and aetiology. *European journal of gastroenterology & hepatology*, *15*(7), 739-743.

29. Shi, K. Q., Fan, Y. C., Ying, L., et al. (2012). Risk stratification of spontaneous bacterial peritonitis in cirrhosis with ascites based on classification and regression tree analysis. *Molecular biology reports*, *39*(5), 6161-6169.

30. Tsung, P. C., Ryu, S. H., Cha, I. H., et al. (2013). Predictive factors that influence the survival rates in liver cirrhosis patients with spontaneous bacterial peritonitis. *Clinical and molecular hepatology*, *19*(2), 131.

31. Tu, B., Zhang, Y. N., Bi, J. F., et al. (2020). Multivariate predictive model for asymptomatic spontaneous bacterial peritonitis in patients with liver cirrhosis. *World Journal of Gastroenterology*, *26*(29), 4316.

32. Piano, S., Brocca, A., Mareso, S., et al. (2018). Infections complicating cirrhosis. *Liver International*, *38*, 126-133.

33. Marciano, S., Dirchwolf, M., Diaz, J. M., et al. (2019). Spontaneous bacterial peritonitis recurrence in patients with cirrhosis receiving secondary prophylaxis with norfloxacin. *European journal of gastroenterology & hepatology*, *31*(4), 540-546.