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Original Article

Spontaneous ascitic fluid infection of patients with liver cirrhosis: culture-negative neutrocytic ascites vs. spontaneous bacterial peritonitis in Huai'an, China

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Abstract: *Aim*: Spontaneous ascite infections (SAI) include culture-negative neutrocytic ascites (CNNA) and spontaneous bacterial peritonitis (SBP). The present study compared the clinical characteristics, laboratory results and prognosis of CNNA and SBP in cirrhotic patients. *Methods*: 156 consecutive patients in Huai'an Hospital of Xuzhou Medical College due to the first episode of SAI between January 2010 and December 2015 through statistical analyses were analyzed. *Results*: 108 patients (69.2%) had CNNA, and 48 patients (30.8%) had SBP; and 19 patients (12.2%) died during hospitalization. Patients with SBP had higher in-hospital mortality than that in CNNA ($P<0.05$). Child-Pugh score (odds ratio [OR]: 0.328, 95% confidence interval [CI]: 0.136-0.791) was the only independent predictor of in-hospital mortality. In-hospital mortality was not significantly different between cirrhotic patients with different reasons ($P>0.05$). Gram-positive bacterium infections have an upward tendency in spontaneous ascite infections. *Conclusions*: Patients with SBP had higher in-hospital mortality than CNNA, but CNNA can turn to be SBP. In-hospital mortality was only related to a patient's liver function without the causes of cirrhosis or other reasons. Therapy to spontaneous ascite infections needs to be considered for Gram-positive bacteria infection.

Keywords: Culture-negative neutrocytic ascites, spontaneous bacterial peritonitis, in-hospital mortality, prognosis

Introduction

It has been demonstrated that 30-50% of cirrhotic patients with spontaneous ascitic fluid infection (SAI) had one type of infectious complication when admitted to the hospital. Furthermore, 15-35% of patients developed infections during hospitalization [1-3]. Thus, ascitic fluid infection has become one of the most important clinical problems in patients with decompensated cirrhosis.

Spontaneous bacterial peritonitis (SBP) is defined as the infection of ascitic fluid without a contiguous source of intra-abdominal infection and in the absence of evident intra-abdominal surgical treatment [4]. The diagnosis of SBP could be established by a positive ascitic fluid bacterial culture and an elevated ascitic fluid absolute polymorphonuclear leukocyte (PMN) count (≥250 cells/mm^3) [5, 6]. If the sample of ascites contains blood, SBP diagnosis is made by finding more than one neutrophilic granulocyte per 250 erythrocytes. Culture-negative neutrocytic ascites (CNNA), which is considered to be a variant of SBP, was first described by Runyon BA and Hoefs JC [7]. The diagnosis of CNNA was made when a patient has an elevated ascitic fluid and PMN count (≥250 cells/mm^3), a negative ascitic fluid culture, and no evident intra-abdominal surgically treatable source of infection.

To date, few investigations have compared the clinical course and prognosis of CNNA and SBP [7-11]. In these studies, the clinical characteristics and therapeutic approach of CNNA and SBP in patients with hepatitis B virus-related liver cirrhosis were similar. In the year 2000, the consensus conference of the International Ascites Club also recommended that there was no difference between these two entities [11]. In order to continue these studies, we compared the clinical characteristics, laboratory

Table 1. Description of Child-Pugh classification score

	Points		
Parameter	1	2	3
Hepatic encephalopathy	None	Stage 1-2	Stage 3-4
Ascites	Nil	Mild-moderate	Moderate-severe
Total bilirubin (μmol/L)	<34	34-51	>51
Prolonged prothrombin time	<4	4-6	>6
Albumin (g/dL)	>35	28-35	<28

results and in-hospital mortality of CNNA and SBP in patients.

Patients and methods

Patients and symptom

A total of 156 consecutive patients with liver cirrhosis-complicated SAI by the first episode, who were admitted to the Huai'an Hospital of Xuzhou Medical College between January 2010 and December 2015, were included into this study. Patients without ascitic fluid examination, which was required for the diagnosis of CNNA or SBP, and patients with secondary bacterial peritonitis were excluded. Data on age, gender, previous hepatic decompensation episodes such as hepatorenal syndrome and hepatic encephalopathy (HEP), and comorbid disorders and other clinical features at the time of admission were collected from medical records. In addition, laboratory parameters were collected for all patients at the time of admission.

Definitions

Cirrhosis in all patients was confirmed through clinical, hematological and biochemical laboratory findings and ultrasound. Liver cirrhosis was defined as: (1) a history of overt complication(s) of cirrhosis such as ascites, variceal bleeding, and hepatic encephalopathy; (2) platelet count $<1\times10^5/mm^3$ and ultrasonographic findings suggestive of cirrhosis such as blunted or nodular liver edge, accompanied by splenomegaly (≥12 cm); (3) the presence of esophageal or gastric varices. The Child-Pugh classification score was designed by Child CG III and Turcotte JG [12, 13]. They assigned a numeric score from 1-3 to signify the clinical severity of each predictor (**Table 1**). The five clinical variables were calculated including hepatic encephalopathy, ascites, serum bilirubin level, serum albumin level, and prolonged prothrombin time.

Paracentesis and culture techniques

Diagnostic paracentesis with ascitic fluid culture was performed in all patients with ascites, who developed local symptoms or signs suggestive of peritoneal infection, systemic signs of infection such as fever or leukocytosis, or clinical deterioration without any obvious precipitating factor. When an ascitic PMN count was ≥250 cells/mm^3, a blood culture was performed. Ascitic fluid and peripheral blood were inoculated into aerobic and anaerobic blood-culture bottles (BACTEC NR 600; Becton Dickinson, Sparks, MD, USA) at bedside [13]. At least 10 ml of ascitic fluid was inoculated into each bottle for aerobic and anaerobic cultures. All isolated organisms in the culture were tested for antimicrobial susceptibility, according to the standard method [14].

Treatment

All patients were treated with antibiotics, which was based on the culture and sensitivity results of the initial ascitic fluid testing. Treatment failure was established when the condition of patients rapidly deteriorated within the first hours of antibiotic therapy or no significant decrease in ascitic PMN count was observed in the follow-up paracentesis. Recovery from SAI was assessed clinically by the disappearance of fever, abdominal pain, improvement in encephalopathy, or reduction in ascitic fluid PMN count to <250 cells/mm^3 and negative cultures. After recovery from the first episode of AFI, norfloxacin was given for prophylaxis.

Statistical analyses

Statistical analyses were performed using SPSS 17.0. Patient characteristics were reported as means ± standard deviation or medians (range), as appropriate. Continuous variables were compared using independent *t*-test, and categorical variables were compared using Fisher's exact test. Variables associated with in-hospital mortality were assessed by binary logistic regression analysis.

Table 2. Baseline characteristics at the first episode of SAI

	Total (n=156)	CNNA (n=69.2%)	SBP (n=30.8%)	*P* value
Age (years)	49.01±14.514	47.82±14.17	51.69±15.069	0.125
Male	119	85 (n=78.7%)	34 (n=70.8%)	0.286
Admission days	21.29±20.689	20.2±18.007	23.75±25.779	0.325
Previous antibiotic treatment	101	68 (n=62.96%)	33 (n=68.75%)	0.485
Complicated hepatorenal syndrome	31	21 (n=19.44%)	10 (n=20.8%)	0.841
Complicated hepatic encephalopathy	19	11 (n=10.2%)	8 (n=16.67%)	0.253
Infection days in hospital	35	20 (n=18.5%)	15 (n=31.25%)	0.079
Diagnosis to AFI (months)	23.47±34.834	25.16±37.683	19.69±27.341	0.367
In-hospital mortality	19	8 (n=7.4%)	11 (n=22.9%)	0.006
Subjective symptoms at admission				
Abdominal pain	62	47 (n=43.5%)	15 (n=31.25%)	0.148
Drowsy mental status	33	21 (n=19.44%)	12 (n=25%)	0.433
Dyspepsia	108	75 (n=69.44%)	33 (n=68.75%)	0.931
Vital signs				
Systolic blood pressure (mmHg)	123.17±81.171	116.44±14.501	138.31±144.61	0.121
Diastolic blood pressure (mmHg)	69.59±10.797	69.52±10..009	69.75±12.337	0.902
Temperature (°C)	36.93±0.680	36.93±0.71	36.95±0.611	0.851
Heart rate (b.p.m.)	83.32±12.383	84.59±13.657	80.46±8.305	0.054
Serum analysis				
WBC count (/mm^3)	8.50±6.562	9.16±7.272	6.30±4.121	0.062
Neutrophils count (/mm^3)	69.45±17.072	70.30±18.099	67.54±14.493	0.353
Platelet (10^9/L)	95.03±80.838	100.50±87.671	82.71±61.876	0.206
ALT (IU/L)	122.43±215.449	135.15±244.16	93.83±127.099	0.270
AST (IU/L)	141.27±203.413	148.1±222.047	125.88±154.463	0.530
Albumin (g/dL)	33.55±42.667	36.3±51.009	27.36±4.874	0.228
The rate of Alb and globulin (A/G)	0.99±0.455	1.03±0.515	0.91±0.267	0.157
Total bilirubin	203.47±610.56	167.71±178.51	283.93±1070.361	0.274
Prothrombin time (INR)	22.14±10.451	22.52±11.651	21.27±6.985	0.495
BUN	9.30±11.189	8.71±6.954	10.63±17.326	0.326
Cr	86.83±64.876	88.69±70.149	82.65±51.492	0.593
Sodium (mmol/L)	148.90±138.888	144.77±118.845	158.20±177.011	0.579
Potassium (mmol/L)	7.24±38.587	4.23±0.815	4.00±0.692	0.093
Ascitic fluid analysis				
WBC count (/mm^3)	4363.87±11573.756	2314.98±3966.537	5274.±13579.811	0.141
PMN count (/mm^3)	2917.2±7680.892	3567.51±9021.775	1454.0±2470.968	0.113
Protein (g/dL)	15.78±9.343	16.61±10.156	13.93±6.927	0.099
Child-Pugh score	10.99±1.24	10.9±1.16	11.2±1.39	0.115

Note: Values in parentheses are percentages.

Results

Patient and disease characteristics

Baseline characteristics of the 156 patients with cirrhosis, who experienced the first episode of SAI, are presented in **Tables 2** and **3**. The mean age of all patients at the time of admission was 49.01 years. The youngest patient was 19 years old, and the oldest patient was 81 years old. Among these patients, 85 (78.7%) patients were male, 108 (69.2%) patients had CNNA, and 48 patients (30.8%) had SBP. Patients with CNNA and SBP did not differ in terms of age, gender, admission days, complication of hepatorenal syndrome (HEP),

Table 3. Correlation factors of in-hospital morality during the first episode of SAI

	Wals	*P* value	OR value	95% CL
Serum analysis				
WBC count (/mm^3)	3.06	0.08	1.319	0.967-1.798
Neutrophils count (/mm^3)	0.012	0.914	1.003	0.949-1.060
Neutrophils (10^9/L)	1.777	0.182	1.013	0.994-1.031
ALT	2.547	0.110	1.022	0.995-1.049
AST	3.409	0.065	0.992	0.983-1.001
Albumin (g/dL)	0.285	0.594	1.052	0.872-1.270
A/G	0.999	0.318	0.311	0.031-3.073
Prothrombin time (INR)	2.173	0.140	0.940	0.866-1.021
Total bilirubin	2.165	0.141	0.998	0.996-1.001
BUN	3.679	0.55	0.952	0.905-1.001
Cr	0.064	0.800	0.998	0.980-1.016
Sodium (mmol/L)	0.016	0.900	1.002	0.973-1.031
potassium(mmol/L)	0.007	0.933	1.018	0.673-1.540
Ascitic fluid analysis				
WBC count (/mm^3)	0.998	0.318	1.000	1.000-1.000
PMN count (/mm^3)	0.275	0.6	1.000	1.000-1.000
Protein (g/dL)	0.536	0.464	0.957	0.851-1.077
Child-Pugh score	6.168	0.013	0.328	0.136-0.791

Note: Values in parentheses are percentages.

Table 4. Causes of cirrhosis and in-hospital mortality in all kinds of cirrhotic patients

Causes of Cirrhosis	Patients	In-hospital mortality	Value
Hepatitis virus liver cirrhosis	117	16	*P*=0.763
Alcoholic liver cirrhosis	18	1	
Hepatitis virus and alcoholic liver cirrhosis	9	1	
Cirrhosis with other reasons	12	1	

previous antibiotic treatment, vital signs and subjective symptoms at the time of admission, serum and ascitic fluid analysis, and Child-Pugh score. However, in-hospital mortality due to the first episode of SAI was significantly higher among patients with SBP (11 patients, 22.9%) compared to patients with CNNA (eight patients, 7.4%; *P*=0.006). Among the 156 patients, 117 patients were caused by hepatitis virus, 18 patients were caused by excessive alcohol, nine patients were caused by hepatitis virus and excessive alcohol, and 12 patients were related with other reasons. Baseline characteristics of the patients were compared to determine the difference between SBP and CNNA. Age, male, admission days, previous antibiotic treatment, complications, infection days in the hospital, the days of diagnosis AFI, in-hospital mortality, subjective symptoms at admission, vital signs, serum analysis, ascitic fluid analysis and Child-Pugh score were chosen as baseline characteristics in patients. In the present study, patients with CNNA had lower in-hospital mortality than patients with SBP (7.4 *vs.* 22.9%), and the difference was statistically significant (*P*=0.006). Other baseline characteristics were similar between SBP and CNNA.

Prognostic factors

Laboratory results (including the results of serum and ascitic fluid, Child-Pugh score) were analyzed to discover the correlation factors of in-hospital morality during the first episode of SAI in **Table 3**. Serum analysis included white blood cell (WBC) count, neutrophil and platelet count, the values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, albumin/globulin (A/G), blood urea nitrogen (BUN), creatinine (Cr), sodium, and potassium, and prothrombin time. Ascitic fluid analysis included WBC count, PMN count and the levels of protein. From the analysis using conditional logistic regression, only the Child-Pugh score remained as a significant predictor of survival (*P*=0.013; OR: 0.328; 95% CI: 0.136-0.791); while no association was observed with CNNA and SBP during the first episode of SAI (*P*=0.115). Furthermore, the difference of in-hospital mortality was not statistically significant between patients related with hepatitis virus, alcoholic or other reasons in **Table 4** (*P*=0.763).

Microbiological results of SBP patients

Table 5 shows the spectrum of microorganisms isolated from ascitic fluid in patients, in which

Table 5. Isolated bacteria in ascitic fluids of patients with spontaneous ascites infection

Isolated bacteria	Positive culture (n=48)	In-hospital mortality	Value
Gram-negative bacterium	32 (66.67%)	8 (25%)a	*P*=0.994
Gram-positive bacterium	15 (31.25%)	3 (20%)b	
Escherichia coli	15 (31.25%)	7 (46.67%)c	*P*=0.193
Staphylococcus aureus	7 (14.58%)	1 (14.29%)d	

66.67% (32 patients) of the isolated pathogen was Gram-negative bacterium (GNB), and 31.25% (15 patients) was gram-positive bacterium (GPB). *Escherichia coli* was the most common pathogen (15 patients, 62.1%), followed by *Staphylococcus aureus* (seven patients, 14.58%). GNB (72.73%) had a highest in-hospital mortality compared to GPB (27.27%), and *Escherichia coli* had a higher in-hospital mortality (seven patients, 46.67%), when compared with *Staphylococcus aureus* (one patient, 14.29%). However, all these differences were not significant (*P*=0.994, *P*=0.193).

Discussion

Since the phrase 'culture-negative neutrocytic ascites' was first proposed as a variant of SBP in 1984 [7], some groups have investigated the clinical features and prognosis of CNNA, and compared this to SBP, in patients with hepatitis virus-related liver cirrhosis were similar [7-11]. It was reported that when the patients with CNNA were left untreated, after some time, positive bacteriological evidence can be found in one-third of these cases [9]. Although several previous studies have compared the clinical characteristics and mortality between SBP and CNNA, results have varied to some extent. Runyon and Hoefs [7] found no significant discriminating variables, including mortality, between these two groups; and concluded that CNNA may be the same infectious disease as SBP. Pelletier *et al*. [8] revealed that serum creatinine, blood culture positivity, and mortality were significantly higher in patients with SBP; while ascitic fluid white blood cell (WBC) and PMN counts were significantly lower in those with CNNA. Terg *et al.* [9] concluded that CNNA and SBP were the same disease, because laboratory and clinical variables did not differ between these. However, al Amri *et al*. [10] revealed that Child-Pugh scores, serum total bilirubin and mortality were significantly higher in patients with SBP than in patients with CNNA; and concluded that SBP carried a higher mortality than CNNA. In our study, in-hospital mortality was significantly lower in patients with CNNA than in patients with SBP (7.4 *vs.* 22.9%, *P*=0.006). However, the difference in serum creatinine, serum total bilirubin, WBC count and PMN count were not statistically significant between patients with CNNA and SBP. We did not include blood culture in our study, because approximately half of the patients did not take the examination for blood culture. Considering so many similarities in baseline characteristics, it appears that CNNA is the same type of infectious disease as SBP. At present, it has been generally accepted that patients with neutrocytic ascites should receive prompt empirical antibiotic treatment, without waiting for the results of the ascitic fluid culture; and that a delay in antibiotic treatment could result in death due to overwhelming infection. The overall in-hospital mortality (12.2%) was slightly higher in contrast to a previous study (7.7%) in Korea [15]. According to the investigation of correlation factors for in-hospital morality during the first episode of SAI, only the Child-Pugh score remained as a significant predictor of survival (*P*=0.013). The number of patients with liver cirrhosis caused by hepatitis was higher than that in patients caused by other reasons. However, in-hospital mortality was not significantly different between the causes of cirrhosis. From this investigation, we may deduce that no matter what kind of cirrhosis, in-hospital mortality was only related to the Child-Pugh score. Diagnostic paracentesis with ascitic fluid culture was very common in patients with CNNA or SBP. In previous studies, approximately 80% of the isolated organisms were gram-negative bacterium; and gram-positive bacterium infection was uncommon [16, 17]. In our study, 66.67% (32 patients) were infected by GNB and 31.25% (15 patients) were infected by GPB. The two most common isolated bacteria were *Escherichia coli* and *Staphylococcus aureus*. This was similar to Tolga Yakar's study [18]. Although *Escherichia coli* infection lead to a higher level of in-hospital mortality than *Staphylococcus aureus*, it did not show a statistically significant difference between these two bacteria (*P*>0.05). In recent decades, norfloxacin, which is a long-term

selective intestinal decontamination used in patients with cirrhotitis and SAI, was introduced in 1987 by Rimola A [19]. At present, it is widely used for primary and secondary prophylaxis in SBP and spontaneous bacteremia in cirrhosis. Norfloxacin prevents infections through GNB, but it has no effect on GPB. Therefore, GPB infections have an upward tendency in current studies. However, GNB (*Escherichia coli*) infection was more dangerous, because most GPBs have had drug resistance.

Conclusion

Patients with CNNA revealed lower in-hospital mortality than those with SBP, but the CNNA can turn out to be SBP. In-hospital mortality was only related to the patient's liver function, and not the causes of cirrhosis or other reasons. The therapy for spontaneous ascite infections need to consider Gram-positive bacteria infection.

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Disclosure of conflict of interest

None.

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