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Full Length Research Paper

Changes in the profiles of bacteria causing spontaneous bacterial peritonitis: A recent twelve-year study

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During the last 20 years, there has been the recognition of prompt diagnosis and appropriate initiation treatment of Spontaneous Bacterial Peritonitis (SBP). It has been suggested that the profile of the infections has changed, and severe infections caused by resistant bacteria species have started to emerge. In this study, 166 cirrhotic patients, with SBP, were retrospectively evaluated through positive ascitic fluid and blood culture from the period of January 1996 to January 2008. The study period was dichotomized into two 6-year periods; periods A (01/1996 to 01/2002) and B (01/2002 to 01/2008). 166 (23%) of 721 patients with positive ascitic fluid and blood culture were diagnosed during 1996-2008. Gram-positive bacteria were found to be the cause of SBP in patients of period B than A (37/102 or 36.3% vs 12/64 or 18.8%, p = 0.016). Fungi were the cause of SBP in 3 (4.7%) of the 64 patients during period A and in 6 (5.9%) of the 102 patients during period B. Although it seemed that the numbers and species increased, statistical data indicated that there was no significant difference (p = 1.000). Our retrospective review suggested that bacterial isolates from SBP in cirrhotic patients with ascites had shown an increased incidence of SBP caused by Gram-positive bacteria over the last twelve years.

Key words: Spontaneous bacterial peritonitis, gram-positive bacteria and infection.

INTRODUCTION

Spontaneous Bacterial Peritonitis (SBP) is defined as the infection of a previously sterile ascitic fluid, without any apparent intra-abdominal source of infection. These were documented in 10 - 30% of the patients with cirrhotic ascites and they were the most significant complications in patients with end-stage liver disease (Rimola et al., 2000). Patients with cirrhosis and ascites showed a higher susceptibility to bacterial infections because of their inadequate defense mechanisms. It is generally considered that the main pathogenic mechanism, by which SBP develops, is bacterial translocation. Over 70% of the SBP episodes were produced by Gram-negative enteric bacilli-*Escherichia coli* and *Klebsiella pneumonia*, which were the frequently isolated micro-organisms (Garcia-Tsao et al., 2001; 2004).

Unfortunately, there have been suggestions that the profile of the infections has changed, and severe infections caused by resistant bacteria species have started to emerge (Fernández et al., 2002; Singh et al., 2003). Moreover, an increasing incidence of SBP caused by Gram-positive bacteria in cirrhotic patients with ascites has been observed by different authors (Cholongitas et al., 2005; Campillo et al., 1998). This recent changes in its microbial etiology may have several important implications for the management and treatment of SBP and suggestions have been made for verifying the efficacy of current guidelines. The aim of our study is to evaluate the possible changes of isolated bacteria in our cirrhotic patients with SBP in two hospitals over the last twelve years.

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MATERIALS AND METHODS

Patients

The medical records of 721 SBP-diagnosed patients with decom pensated cirrhosis and ascites admitted to Tangdu Hospital and

Xijing Hospital was retrospectively reviewed between January 1, 1996 and January 1, 2008. The diagnosis of cirrhosis was established on the basis of clinical examination, biochemical test and instrumental examination and/or liver biopsy. The severity of the liver disease in each patient was classified at entry according to the Child-Pugh scores (Pugh et al., 1973). The study period was dichotomized into two 6-year periods: periods A (01/1996 to 01/2002), and B (01/2002 to 01/2008). The ages of the patients were between 18 to 75 years old, patients were excluded if tested positive to Human Immunodeficiency Virus (HIV) infection, heart failure or hepatocellular carcinoma.

Methods

Laboratory tests including complete blood count, prothrombin activity (PTA) and routine liver and kidney biochemistry tests were performed in all cirrhotic patients on the day of admission and whenever they developed relative symptoms or signs for SBP during hospitalization. Abdominal paracentesis was performed in the first 24 h after the admission of patients, using aseptic technique. The samples of ascitic fluid were immediately taken to biochemical, cytological and microbiological laboratory to be analyzed.

Blood samples were taken for simultaneous determinations of some biochemical parameters. Ascitic fluids were collected for the determination of glucose, protein, albumin, white blood cell and total cell count, with standard biochemical methods. Results were expressed as mean ± standard deviation. Aerobic and anaerobic cultures were performed, using a sterile technique during the whole period of the study. Samples of 10 ml of ascitic fluid and blood were inoculated into aerobic and anaerobic blood culture bottles (Hemoline performance biphasique, Bio Merieux, France and Oxoid SIGNAL blood culture system, Oxoid, Hampshire, UK) for bacteriological examination at the patient's bedside and before the administration of antibiotic (Runyon et al. 1988). Antibiotic susceptibility was assessed using the disc diffusion method, with tablets of Neosensitabs (Rosco Diagnostica, Taastrup, Denmark), based on the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 1990). All organisms isolated in positive cultures were tested for antimicrobial susceptibility.

A bacterium was considered resistant when the minimal inhibitory concentration of antibiotic was higher than 2, 8 and 2/38 mg/ml, respectively (National Committee for Clinical Laboratory Standards, 1990).

Diagnostic criteria

The diagnosis criteria for SBP involved a combination of positive ascitic fluid culture and an ascitic polymorphonuclear (PMN) leukocyte count of >250 cells/mm^3 with no evidence of intra-abdominal source of infection. The diagnosis of culture-negative neutrocytic ascites (CNNA) was based on a negative ascitic bacterial culture, PMN count >250 cells/mm^3, there was no antibiotics given within 7 days and no evident intra-abdominal source of infection or an alternative explanation for the elevated PMN count. The diagnosis of bacterascites was based on a positive ascitic culture, an ascitic PMN count <250 cells/mm^3, and a lack of symptoms or signs of SBP (Sheer and Runyon, 2005; Guarner and Soriano, 1997).

Statistical analysis

Statistical analysis was conducted using SPSS ver. 16.0 for Windows (SPSS, Chicago, IL). Categorical variables were compared using the chi-square or Fisher's exact test where appropriate. Continuous data were compared using the t-test or the Mann-Whitney test, the Kruskal-Wallis test was used for multiple comparisons, when appropriate. Quantitative variables with a normal distribution were expressed as mean values ± standard deviation and those with a non-normal distribution as median values (range). Significance level was two-sided and set to less than 0.05.

RESULTS

During the entire study period, SBP was diagnosed in 721 patients. Both blood culture and ascitic fluid culture positive patients were found (23%) of them. The mean age of the 166 culture-positive SBP patients was 50 ± 10 years, whereas 119 (71.7%) cases were males. The cause of cirrhosis was hepatitis B virus in 120 (72.3%), including one case with HBV/HEV co-infection, hepatitis C virus in 23 (13.9%), alcoholic abuse in 12 (7.2%), primary biliary cirrhosis in 5 (3.0%) and cryptogenic cirrhosis in 6 (3.6%). 18 patients (10.8%) had Child-Pugh class A, 51 patients (30.7%) had Child-Pugh class B, while 97 patients (58.4%) had Child-Pugh class C.

Sixty four patients (38.6%) were admitted during 1996 - 2002 (period A) and the remaining 102 (61.4%) patients were admitted during 2002 - 2008 (period B). Patients admitted during period A and period B did not significantly differ in their epidemiological and clinical characteristics (Table 1).

One hundred and six patients (38 during period A and 68 during period B) were on antibiotics when SBP was diagnosed, but no patient with culture-positive SBP was receiving prophylactic antibiotic therapy when admitted.

Gram-positive bacteria were found to be the cause of SBP and were significantly present in patients of period B than A (37/102 or 36.3% vs 12/64 or 18.8%, p = 0.016). Organisms isolated from the ascitic fluid of the patients during the whole period of the study (1996 - 2008), as shown in Table 2.

E. coli (gram -ve), were the most frequently grown bacterium from the ascetic fluid (n = 66). They account for 39.8% of all isolated agents, followed by *Klebsiella ozytoca* (gram -ve, n=18); accounting for 10.8%; *S. aureus* (gram +ve, n=12) 7.2%; *S. epidermidis* (gram +ve, n = 9) 5.4 and 4.2% *Group D streptococci* (gram +ve, n=7) and *Enterobacter cloacae* (gram -ve, n = 7) respectively (Table 2).

In period A, *E. coli* was the most frequently grown bacterium from the ascitic fluid (n = 34, 53.1%), followed by *K. ozytoca* (gram -ve, n=7, 10.9%); *Flavobacterium breve* (gram -ve, n = 3, 4.7%) and *S. aureus* (gram +ve, n = 3, 4.7%).

Although the percentages changed, the order continuously remained the same in period B. Isolated organisms from the whole study period in culture-positive spontaneous bacterial peritonitis lists were ranked as *E. coli* (gram -ve) in 66 cases by 39.8% in the first place, followed by *K. ozytoca* (gram -ve) in 18 cases by 10.8%, *S. aureus* (gram +ve) in 12 cases by 7.2%, and *S. epidermidis* (gram +ve) in 9 cases by 5.4%.

Table 1. Baseline characteristics of patients with culture-positive spontaneous bacterial peritonitis during 1996 - 2002 and 2002 - 2008.

Patient characteristics	Period 1996 – 2002 (N = 64)	Period 2002 – 2008 (N = 102)	P- value
Age (years)	50±11	49±10	0.52
Gender, male/female	45/19	74/28	0.76
Cause of cirrhosis, n (%)			0.94
HBV-related cirrhosis	48(75%)	72(70.6%)	
HCV-related cirrhosis	8(12.5%)	15(14.7%)	
Alcoholic cirrhosis	4(6.3%)	8(7.8%)	
Other causes	4(6.3%)	7(6.9%)	
Child-Pugh class, n (%)			0.32
A	6(9.4%)	12(11.8%)	
B	24(37.5%)	27 (26.5%)	
C	34(53.1%)	63(61.8%)	
Past use of antibiotics, n (%)	38(59.4%)	68(66.7%)	0.34
Immunosuppression, n (%)	18(28.1%)	26(25.5%)	0.71
White blood count ($\times10^9$/L)	4.68±1.96	4.97±1.74	0.33
Platelet count ($\times10^9$/L)	55.05±33.26	54.63±27.27	0.93
Prothrombin activity, (%)	36.79±16.30	36.82±14.67	0.99
Serum total protein, g/L	61.25±7.83	62.04±7.22	0.51
Serum albumin, g/L	29.90±5.14	29.12±5.46	0.36
Serum bilirubin, umol/L	121.29±106.09	116.46±90.65	0.76
ALT (U/L)	54.66±30.40	63.82±47.92	0.13
AST (U/L)	102.66±56.39	92.61±47.32	0.24
Serum creatinine, umol/L	83.47±50.30	97.84±53.18	0.10
Urea nitrogen, mmol/L	6.26±4.47	7.12±4.12	0.21
Uric acid, umol/L	170.88±77.38	155.96±74.18	0.22
Ascitic fluid			
Total cell count ($\times10^6$/L)	565(31-14880)	690.5(45-9002)	0.48
White blood count ($\times10^6$/L)	73.5(12-4640)	82.5(14-5402)	0.50
PMN cell count ($\times10^6$/L)	209.58(5.16-8060.58)	387.32(12.15-7111.58)	0.99
Protein, g/L	21.75(1.9-13280.1)	19.75(2.2-19870.4)	0.56
Chlorinum, mmol/L	103.88±9.07	101.44±9.53	0.11
Glucose, mmol/L	7.78±2.78	7.45±2.97	0.47
Isolated bacteria from ascitic fluid, n (%)			0.016
Gram-positive bacteria	12(18.8%)	37(36.3%)	
Gram-negative bacteria	49(7.6.6%)	59(57.8%)	
Fungi-positive bacteria	3(4.7%)	6(5.9%)	1.000

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; Immunosuppression: diabetes mellitus, corticosteroid therapy. Quantitative variables are expressed as mean values ± standard deviation or as median values (range).

Fungi were the cause of SBP in 3 (4.7%) of the 64 patients during period A and in 6 (5.9%) of the 102 patients during period B, although it seemed that the numbers and species increased, statistical data indicated that there was no significant difference (p = 1.000).

Antibiotic susceptibility of multi drugs was observed in major pathogenic bacteria (*E. coli, K. ozytoca, S. aureus, S. epidermidis*) isolated from culture-positive SBP patients (Table 3).

DISCUSSION

Patients with cirrhosis were predisposed to infection due

Table 2. Isolated bacteria from ascitic fluid of cirrhotic patients during 1996 - 2002 and 2002 - 2008.

Species	Period 1996-2002 (N=64)		Period 2002-2008 (N=102)		Total (N=166)	
	n	%	n	%	n	%
Gram-negative bacteria						
Escherichia coli	34	53.1	32	31.4	66	39.8
Klebsiella ozytoca	7	10.9	11	10.8	18	10.8
Enterobacter cloacae	2	3.1	5	4.9	7	4.2
Corynebacterium	2	3.1	4	3.9	6	3.6
Flavobacterium breve	3	4.7	2	2.0	5	3.0
Xanthomonas maltophilia	1	1.6	2	2.0	3	1.8
Pseudomonad	0	0	3	2.9	3	1.8
Gram-positive bacteria						
Staphylococcus aureus	3	4.7	9	8.8	12	7.2
Staphylococcus epidermidis	2	3.1	7	6.9	9	5.4
Group D streptococci	2	3.1	5	4.9	7	4.2
Streptococcus mitis	2	3.1	3	2.9	5	3.0
Streptococcus pneumoniae	2	3.1	2	2.0	4	2.4
Staphylococcus cohnii	1	1.6	2	2.0	3	1.8
Micrococcus luteus	0	0	2	2.0	2	1.2
Methicillin resistant staphylococcus aureus (MRSA)	0	0	2	2.0	2	1.2
Staphylococcus heamolyticus	0	0	2	2.0	2	1.2
Staphylococcus warneri	0	0	2	2.0	2	1.2
Bacillus cereus	0	0	1	1.0	1	0.6
Fungi						
Cryptococcus neoformans	1	1.6	4	3.9	5	3.0
Mould	1	1.6	1	1.0	2	1.2
Torulopsis glabrata	1	1.6	1	1.0	2	1.2

Table 3. Antibiotic susceptibility of major pathogenic bacteria during 1996 - 2002 and 2002 - 2008 (%).

Antibiotics	*Escherichia coli*		*Klebsiella ozytoca*		*Staphylococcus aureus*		*Staphylococcus epidermidis*	
	Period 1996-2002	Period 2002-2008	Period 1996-2002	Period 2002-2008	Period 1996-2002	Period 2002-2008	Period 1996-2002	Period 2002-2008
Ciprofloxacin	50.2	42.9*	27.3	21.4	69.2	65.3	68.6	65.0
Amikacin	90.1	89.3	33.2	31.5	85.6	84.3	84.8	50.3*
Penbritin	45.4	20.1*	16.3	17.5	71.6	21.4*	72.3	18.1*
Cefazolin	47.5	33.3*	69.0	25.0*	69.6	68.5	70.2	65.0
Cefoxitin	95.5	94.3	86.1	78.2	98.6	97.5	97.7	97.2
Cefotaxime	66.4	37.5*	50.2	46.3	85.0	82.5	85.4	85.4
Ceftazidime	84.3	79.6	85.2	61.7*	86.2	81.4	86.5	82.3
Cefepime	86.6	51.2*	85.1	64.1*	86.4	83.7	87.4	86.3
Tienam	97.0	95.6	98.3	97.6	93.2	89.2	94.1	92.3

*$p < 0.05$.

to an impaired immune function together with an increased passage of bacteria from the gut (bacterial translocation). This was facilitated by altered intestinal immunity and bacterial overgrowth. The organisms that cause SBP were predominantly enteric. In most cases (approximately 70 - 80%), SBP are caused by Gram-negative bacteria, with *E. coli* accounting for nearly 50% of these, followed by *K. pneumoniae*, *S. pneumoniae*, and other streptococcal species including *enterococci*. However, during the last decade, practice in hepatology

has considerably changed and this may have influenced the epidemiology of bacterial infections in liver diseases (Dupeyron et al., 1998).

The management of cirrhotic patients with spontaneous bacterial peritonitis has improved greatly, an early diagnosis of SBP can be made through routine examination on admission to hospital and whenever patients develop signs of infection or impairment in their clinical condition. In addition, new invasive procedures have been developed and are extensively used for specific complications of cirrhosis, which may also be associated with the infections. Moreover, long-term administration of antibiotic prophylaxis in cirrhotic patients may promote the carriage of bacterial infections because of multi-resistant gram-positive bacteria (Fernández et al., 2002; Campillo et al., 2001). All these features indicated that the microbial etiology and spectrum of SBP in cirrhosis may have changed under the circumstances.

The periods documented in our study correspond to the introduction of SPB prophylaxis with norfloxacin in clinical practice. Since the year 2002, a large proportion of cirrhotic patients admitted into our department are already receiving norfloxacin preventive treatment on arrival, which considerably reduces the frequency of Gram negative spontaneous bacterial peritonitis. Since that time we have also observed fewer Gram negative infections coupled with severe infections caused by resistant Gram positive species.

Our findings further supported these suggestions since the proportion of isolated Gram-positive bacteria have increased significantly. Gram-positive bacteria were responsible for the majority of our culture-positive SBP cases in recent years. The incidence of culture-positive SBP in this study was 23% (166/721), which was lower than the results from other studies in hospitalized cirrhotic patients with ascites (Cholongitas et al., 2005; Park et al., 2007). This difference may be due to the differences in cirrhotic etiology and also, the fact that the positive rate of clinical bacterium culture remained lower in the Chinese main land notwithstanding diagnostic method enhancement and the renewal of medical instruments.

Most of the present cases (72.3%, n = 120) were caused by viral hepatitis infections, especially hepatitis B virus, followed by hepatitis C infection (13.9%, n = 23), while alcohol abuse caused cirrhosis, which accounted for only 7.2% (n = 12). This was also lower than the reports from other studies (Singh et al. 2003; Angeloni et al., 2008) and also demonstrated that the cirrhotic etiology in China is somewhat different. HBV caused liver cirrhosis which still prevails as the predominant agent of the hepatopathy.

Gram-negative bacteria were the predominant pathogens associated with SBP in our study (65.1%). Although *E. coli* and *K. ozytoca* were the most common Gram-negative pathogens, *S. aureus* accounted for 7.2% (12/166) of the Gram-positive bacteria, which increased from 4.7% to 8.8% in period B. Emergence of Gram-positive bacteria including *S. aureus* as significant pathogens in SBP in recent years has been noted in other studies as well (Dupeyron et al., 2001; Campillo et al., 2002). An overall rising incidence of *S. aureus* as a nosocomial pathogen and the employment of quinolones as prophylaxis for SBP are largely proposed to account for these changes (Campillo et al. 2002).

As the profile of the infections changes, severe infections caused by resistant Gram-positive species have started to emerge. Generally prophylaxis antibiotic use and abuse in infection led to the emergence of multi-drug resistant bacteria. Fatal cases of spontaneous bacterial peritonitis caused by drug-resistant species (two *methicillin resistant S. aureus*, one *vancomycin-resistant enterococci*) have been recently observed.

For a long time, quilonones have been used for the prevention of bacterial infections in cirrhotics and nosocomial prophylaxis at our institution, as it determines a marked reduction in the incidence of nosocomial bacterial infections without the development of opportunistic infections or significant side effects. Firstly, although, norfloxacin, ciprofloxacin and ofloxacin are effective drugs for the prevention of bacterial infections in cirrhotic patients, they show a broader antimicrobial spectrum and higher systemic absorption characteristics that may be prone to the development of infections caused by gram-positive cocci or drug resistant gram-negative bacilli in long-term treatments. Moreover, the infections that cirrhotic patients develop while on prophylaxis with quilonones are usually caused by gram-positive cocci, and there also had been reported a high incidence of infections caused by gram-negative bacilli resistant to norfloxacin (mainly *E. coli*) and *enterococci* in long-term treated patients (Novella et al. 1997; Ortiz et al. 1999). Our study further proved that, in the later period, increased resistance of *E. coli* to antibiotics appeared not only in ciprofloxacin, but also in beta-lactam penbritin, through the first generation cephalosporins cefazolin, third generation cephalosporins cefotaxime and fourth generation cephalosporins cefepime. In addition, some of the recently cultured gram-positive staphylococcus had shown resistance to broad-spectrum penbritiin or aminoglycosides amikacin. Bacteria resistance was in accordance with the frequency of drug administration, especially ampicillin, some cephalosporins and fluoroquinolones. Fluoroquinolones in levofoloxacin resistance rate lower than ciprofloxacin, and may be related to the former in the low frequency of usage. It may also consider a similar rotation of the use of drugs to reduce bacterial resistance rates.

Table 3 shows the status quo of antibiotic susceptibility to major pathogenic bacteria in patients with cirrhosis. *E. coli* is sensitive to ceftazidime, cefepime, cephalosporin and tienam but not to other commonly used antibiotics. The other three bacteria, although of much resistance to the commonly used antibiotics, still remain highly sensitive to ceftazidime, cefepime, cephalosporin and tienam. Therefore, ceftazidime, cefepime, cephalosporin

and tienam can still be classified as highly efficient broad-spectrum antibiotics for serious infections, which can play a great therapeutic effect. In the third generation cephalosporins cefotaxime resistance is generally higher than ceftazidime, partly because of the regional drug habit preferred by the third generation cephalosporins cefotaxime. This results to bacterial resistance, which is one of the mechanisms for ESBLs-producing bacteria. *E. coli*, *K. pneumoniae* and *Enterobacter cloacae* are the most commonly gram-negative bacilli producing extended-spectrum β-lactamases (ESBLs), whose resistance rates have also increased year by year. Moreover, the study also found two strains of *E. coli* sensitive to ceftazidime cephalosporin but resistant to cefepime. Such strains may be ESBLs-producing strains, and may have other resistance mechanisms. In addition, different regions experience different medication which must have led to differences in drug resistance.

Development of fungal infections is extremely uncommon during antibiotic prophylaxis in patients with cirrhosis. The presence of isolated fungi raises the suspected presence of secondary bacterial peritonitis when more than one organism is isolated from ascites. Our study confirmed the culture results by excluding this possibility, while, the difference was not statistically significant ($p = 1.000$). Exact explanation on any new fungi isolated from ascites was not given (Novella et al., 1997; Ginés et al., 1990; Kerr et al., 1963). In recent years, internal fungal infection is increasingly becoming a major hospital pathogen; one of the reasons may be the development of interventional therapy, immunosuppressive agents and the use of broad-spectrum antibiotic. Clinical studies have also suggested that the finding of fungi in intra-abdominal specimens are likely to be associated with the development of fungal infection, especially in the context of surgery for acute pancreatitis and in patients with recurrent perforation or leakage at anastomotic sites (Calandra et al., 1989; Christou et al., 2007).

Conclusion

Our retrospective review of patients with SBP suggested that bacterial isolates from spontaneous bacterial peritonitis in cirrhotic patients with ascites had shown an increasing incidence of SBP caused by Gram-positive bacteria over the last twelve years. Also, our observations confirmed that bacterial isolates from spontaneous bacterial peritonitis have shown an increasing level of resistance to standard antibacterial agents. This may have some implications in their management, and should be taken into account in empirical antibiotic treatment, especially for patients with a history of infections.

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