Significance of Serum and Ascitic Fluid Bacterial DNA in Culture Negative Non-Neutrocytic Ascites

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Abstract: Spontaneous bacterial peritonitis (SBP) is a severe complication of liver disease. A significant proportion of patients have culture-negative ascites, despite having similar signs, symptoms and mortality to those with SBP. A high index of suspicion should exist for SBP in patients with cirrhosis and ascites. Bacterial translocation (BT) from intestinal wall to blood and other extra-intestinal sites is considered the key step in the pathogenesis of SBP in liver cirrhosis. This study aimed to identify bacterial DNA and its significance as a marker of bacterial translocation in patients with advanced cirrhosis and culture-negative non-neutrocytic ascites (CNNA). Sixty three patients with cirrhosis and CNNA were included in the study. The corresponding bacterial DNA (BactDNA) was identified in blood and ascitic fluid (AF) samples using Polymerase chain reaction (PCR) technique. The BactDNA was detected in AF and/or blood samples in 39.6% of patients (25/63). It was found in blood sample of 48% of patients (12/25), AF sample in 6/25 patients (24%), and in simultaneously both blood and AF samples in 7/25 patients (28%). *Escherichia coli* is the main bacterial species detected in 72% of samples, *Klebsiella pneumoniae* was detected in 16% , *Staphylococcus* aureus in 8% and diphteroid in 4% of BactDNA positive cases. No, significant differences were detected between both BactDNA positive and negative groups as regard serum or AF biochemical parameters, except for creatinine. Also, the clinical data were not statistically different between groups, except for previous episodes of gastrointestinal (GI) bleeding, hepatic encephalopathy and band ligation. After 12 months of follow up, spontaneous bacterial peritonitis (SBP), hepatorenal syndrome (HRS), gastrointestinal bleeding and death were more frequent in BactDNA positive group. In conclusion: the detected BactDNA in our patients with CNNA might be an alternative diagnostic tool for early diagnosis and prompt treatment of AF infection to ameliorate the serious complications and poor prognosis of such patients.

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1. Introduction

Bacterial infections are frequent in patients with liver cirrhosis and have major impact on their short and long term morbidity and mortality The ascitic infections is identified in the absence of secondary causes such as bowel perforation or focal inflammatory processes **(Rimola *et al.*, 2000)**. Once bacteria reach a critical concentration in the gut lumen, they "spill over", and escape the gut, "translocating" to mesenteric lymph nodes. Then they can enter lymph, blood, and eventually ascitic fluid. If the ability of the ascitic fluid to motivate macrophages and neutrophils in killing the errant bacteria is deficient, uncontrolled growth occurs causing spontaneous bacterial peritonitis (SBP). Thus SBP is the result of failure of the gut to contain bacteria and failure of the immune system to kill the virulent bacteria once they have escaped the gut (**Runyon *et al.*, 1994)**. SBP is diagnosed on basis of polymorphnuclear (PMN) cell counts greater than 250 cells/ mm³ in ascitic fluid, regardless the result of the ascites culture**.** Conversely, patients of culture negative and PMN cell count less than 250 cells/mm^3 was identified as culture negative non- neutrocytic ascites (CNNA) **(Rimola *et al.*, 2000).**

SBP develops in 20 - 60% of hospitalized cirrhotic patients with ascites. The mortality rate is 20 to 40% despite clearance of bacteria from the AF in 70-90% of cases. Without prophylactic antibiotics, the recurrence rate at 1 year is about 70%. **(Gines *et al.*, 1990).** The infecting organisms are usually enteric gram-negatives which have translocated from the bowel. Completely asymptomatic cases have been reported in as many as 30% of patients **(Rogers *et al.*, 2010).** Therefore, the detection of bacterial DNA (bactDNA) may be considered a marker of bacterial translocation, particularly in the cases of cirrhosis and culture negative non-neutrocytic ascites (CNNA); with PMN <250 mm³ and a negative ascitic fluid culture **(Such *et al.*, 2002).** The diagnosis of bacterascites is based on a positive ascitic fluid culture, an ascitic PMN count <250 cells/mm³ and lack of symptoms and signs suggestive for SBP, for

example fever, abdominal pain, icterus or hepatic encephalopathy **(Sheer & Runyon, 2005).**

The low rates of positivity of bacterial culture in ascites samples is possibly caused by the relatively small concentration of bacteria in ascitic fluid, such as occurs in cerebrospinal fluid **(Rimola *et al.*, 2000). Runyon & Canawati (2008)** have demonstrated that inoculation of ascites directly into blood culture bottles at the bedside increases the sensitivity of bacterial culture to 90%. However, in general practice, ascites culture is negative in approximately 40% of patients with SBP **(Rimola *et al.*, 1995; Such *et al.*, 1998; Such *et al.*, 1999).**

Early diagnosis of SBP along with prompt initiation of appropriate antibiotic therapy would allow targeted intervention to prevent the development of SBP, or where peritonitis is already present, treatment with the most appropriate therapy. Further access to such data would allow the effective monitoring of treatment efficacy **(Rogers *et al.*, 2010)** and could be helpful in overall patient's survival **(Barclay, 2003; Kamani *et al.*, 2008).** Mortality rates in SBP have declined dramatically, largely due to earlier detection and improved therapy **(Zapater *et al.*, 2008).**

Polymerase chain reaction (PCR) is a molecular diagnostic technique based on the enzymatic amplification of a specific sequence of DNA with production of millions of copies **(Jordan *et al.*, 2000).** Thus, this technique has the potential increase for sensitivity **(Qian *et al.*,2001;Rantakokko-Javala *et al.*,2001).** The use of universal PCR primers targeting conserved DNA regions in bacteria, such as 16S ribosomal RNA (rRNA) sequences, is a promising means for identifying bacteria in clinical samples **(Chesky *et al.*,2000; Nikkari *et al.*, 2002).**

Aim of the study:

This study aimed at detection of bacterial DNA (bactDNA) in the serum and ascitic fluid by polymerase chain reaction (PCR) and its significance in patients having liver cirrhosis and culture negative non- neutocytic ascites (CNNA).

2. Patients and Methods:

Sixty three patients, 43 males and 20 females with age range from 52-65 years having liver cirrhosis with ascites, and who were admitted to the Hepatology Department - National Liver Institute (NLI) –Menoufiya University, were included in the study. The study was done in the period from September 2009 to July 2010.All patients were subjected to proper history taking and clinical examination, laboratory investigations including urine analysis and urine culture, chest x ray, abdominal ultrasound and upper endoscopy.Patients were culture negative and PMN cell count was less than 250 cells/mm^3 .Culture negative non-neutrocytic ascites (CNNA) was identified in the presence of a negative culture and PMN cell count no greater than 250 cells/mm^3 **(Rimola *et al.*, 2000).** The study was approved by the local ethical committee at the NLI, Menoufiya University and a written informed consent was obtained from all patients included in the study.

Exclusion criteria were:- the presence of a culture-positive blood or AF, patients showing two or more of criteria of systemic inflammatory response syndrome according to previously published criteria (temperature >38°C or <36°C, heart rate >90 beats/minute, respiratory rate >20 breaths/minute, white blood cells <4000 or >12,000/ mm^3, ascitic fluid neutrophilic count >250/ mm^3) (**Rangel-Frausto *et al.*, 1995)**, upper gastrointestinal bleeding (UGIB), or intake of antibiotics in the preceding 2 weeks before study, hepatocellular carcinoma, or portal vein thrombosis and transjugular intrahepatic portosystemic shunt (TIPS).

Follow-up of patients:

Clinical and analytical data of all patients were recorded on inclusion in the study together with previous clinical history. Applied therapy and diagnosis were recorded on discharge from the hospital during follow-up.

Sampling and laboratory tests:

Ten ml of blood was obtained for haematologic (complete blood count), coagulation (prothrombin time), blood chemistry (serum albumin, urea, creatinine, total bilirubin, direct bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, ascitic fluid total protein, albumin) and hepatitis markers (HCV, HBs Ag and total HBc Ab, and autoantibodies for autoimmune disease).

Paracentesis was performed under aseptic conditions. Ascitic fluid samples were examined for total leucocytic count (TLC) using Sysmex-K 21 counter (Japan) and polymorphnuclear leucocytes (PMN) by manual count and by using a bedside test for detection of leukocytes leucoesterase enzyme (Multistix SG10 Bayer Diagnostics Bridgend, UK). Ascitic fluid total protein, albumin, and LDH were Analyzed by COBAS Intrgra-400 (Roche -Germany). Urine culture was also done to rule out UTI and it was also proved useful since asymptomatic bacteruria has been suggested to predispose to the development of spontaneous bacterial peritonitis.

Both ascitic fluid and blood samples (7-10 ml of each) were innoculated at bedside in aerobic and anaerobic blood culture bottles (Oxoid blood culture

system), then incubated at 37 C for at least 7 days or until the positive signal was detected (according to manufacturer's instructions). Subcultures, both aerobically and anaerobically were done on blood agar and MacConkey's agar plates **(Runyon *et al.*, 1988).** Serum and AF samples were also inoculated in pyrogen free cork-screw capped 2 ml vials stored at -70°C until DNA extraction was done for molecular detection of bacterial DNA.

DNA Isolation:

A total of 200 µL of serum or AF was incubated in a lysozyme/proteinase K buffer for 2 hours and applied into QIAamp Spin Columns (QIAampDNA Mini Kit; QIAGEN, Hilden, Germany). Samples were centrifuged at full speed. DNA was finally eluted with 50 uL of 70°C preheated water **(Llovet *et al.*, 1994).** Positive and negative controls were included in each PCR run. Positive control DNA was obtained by extraction of bacterial DNA from overnight broth culture of Staphylococcus aureus (ATCC 25213) and Escherichia coli (ATCC 35320) according to **Fang and Hedin (2003),** whereas sterile distilled water was used as a negative control.

DNA amplification:

A PCR reaction for the universal amplification of a region of the 16S ribosomal RNA gene was used as described by **Such *et al.*(2002).** The primers located at positions 7-27 and 531-514 of *Escherichia coli* numbering are universal eubacterial primers that amplify any known bacterial 16S ribosomal RNA gene.

Universal eubacterial primers located at positions 7–27 and 531–514 (E. coli numbering) were used to amplify any known bacterial 16S rRNA gene (forward primer 5'-GAGTTTGATCATGGCTCAG-3' and reverse primer 5'-ACCGCGACTGCTGCTGGCAC-3' **(Llovet *et al.*,1993).** 10 µl of template was added into a reaction mix containing 10 mM Tris buffer (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 800 µM of deoxynucleoside triphosphate mixture (dNTP), 50 pmol of each primer (Operon, USA), and 1.25 units of Taq polymerase (Promega Corporation, Madison, WI, USA) to complete a final volume of 100 µL. PCR was performed using Roche Thermal Cycler. The amplification protocol was 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 60 seconds for 35 cycles followed by a final extension at 72ºC for 10 min.

Ten microliters of amplified products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized using UV transilluminator. The sizes of PCR products were estimated according to the migration pattern of a 50-bp DNA ladder (Fermentas, Germany). A band of about 540 base pairs was obtained from different bacterial cultures corresponding to positive controls and positive samples corresponding to the specific amplification of the prokaryotic 16S ribosomal RNA gene. Figure (1) represents a sample of agarose gel electrophoresis of PCR products.

Statistical analysis:

Data are expressed as mean ± SD. The SPSS computer program version 11.0 was used for statistical analysis. Student's t-test for parametric data and the Mann Whitney test for nonparametric data were used. Chi square (X^2) was used to compare qualitative variables. All tests were two-tailed and p-values <0.05 were considered significant.

3. Results:

Sixty three patients were included in this study, 43 males and 20 females with age range 52-65 years. The patients characteristics and demographic data are shown in table (1). No statistically significant difference in baseline clinical data and basic hemodynamics were found between the two groups except for previous episodes of upper GI bleeding, hepatic encephalopathy and band ligation. where p value was <0.05.The upper endoscopy was done for all patients of both groups at the time of index admission did not reveal recent upper GI bleeding and there were no differences between both groups with regard to severity of portal hypertension-related endoscopic findings. Encephalopathy also showed significant difference, more in group 1 (BactDNA positive). Despite Child Pugh score was more in BactDNA positive group, it did not reach statistical significance (Table 1).

No significant difference was found between both groups as regards liver functions, urea, electrolytes and CBC parameters. Only serum creatinine showed significant difference, p value was <0.05. (Table 2). In table (3) ,no significant differences found between studied groups with regard to the results of ascitic fluid examination namely, total protein, albumin, LDH,TLC and PMN.

Among 25 bact DNA positive patients, bacterial species isolated were as following: *Escherichia coli (E.coli)* was found in 18/25 (72%) patients, of them 9 (36%) in serum, 3 (12%) in AF and 6 (24%) in both. *Klebsiella pneumoniae* was found in 4/25 (16%) patients, one case (4%) in serum, 2 (8%) in AF and one (4%) in both, *Staphylococcus aureus* was found in 2/25 (8%) patients,1 (4%) in serum and 1 (4%) in AF and diphteroid was isolated in serum of one (4%) patient only out of 25 (Table 4).

After 12 months of follow up, development of SBP, HRS, upper GI bleeding and death were

occurred in 44%, 36%, 12%, and 32%, respectively in BactDNA positive compared to 13.2%, 7.9%, 5.3% and 10.5% in BactDNA Negative cases which is significantly higher in the former group (Table 5).

Table (1): Clinical data of patient groups

Variables	*BactDNA positive group (N=25)*	**BactDNA negative group (N=38)**	**P value**
Age (years) M±SD	62.7 ± 8.9	58.5 ± 10.2	>0.05
GenderM/F& (Male %)	17/8 (68%)	26/12 (68.4%)	>0.05
Temperature (°C) M±SD	37.8 ± 0.4	36.9 ±0.4	>0.05
Respiratory rate(breaths/min) M±SD	17±3	15±2	>0.05
Mean arterial BP (M±SD)	88± 9	84±5	>0.05
Heart rate (beats/min) M±SD	82±7	78 ±6	>0.05
Abdominal pain (No %)	7 (28%)	14 (36.8%)	>0.05
Jaundice (No %)	9 (36%)	16 (42%)	>0.05
Previous episodes of UGI bleeding (No %)	15 (60%)	10 (26.3%)	<0.05*
Previous banding ligation (No %)	11 (44%)	8 (21.1%)	<0.05*
Previous episodes of encephalopathy (No %)	13 (52%)	11 (28.9%)	<0.05*
Previous therapeutic Paracentesis (No %)	10 (40%)	13 (34.2%)	>0.05
Child Pugh score: Class B (No %) Class C (No %)	 8 (32%) 17 (68%)	 15 (39.5%) 23 (60.5%)	>0.05

Table (2): Biochemical data of blood examination of the studied groups.

Parameters	*BactDNA positive group (N=25)* M ± SD	**BactDNA negative group (N=38)** M ± SD	P value
ALT (U/L)	66.5 ± 10.5	68.4 ± 13.5	>0.05
AST (U/L)	72.7 ± 11.2	80.5 ± 12.3	>0.05
S. bilirubin (mg/dl)	3.7 ± 1.5	3.2 ± 0.8	>0.05
S. albumin (gm/dl)	2.4 ± 0.6	2.8 ± 1.01	>0.05
Creatinine (mg/dl)	2.7 ± 0.8	1.9 ±0.7	<0.05*
Urea (mg/dl)	63.7 ± 7.6	75.1 ± 10.4	>0.05
S. sodium (mEq/L)	132.5 ± 4.2	129.1 ± 2.5	>0.05
S. potassium (mEq/L)	5.5 ± 0.4	5.3 ± 0.2	>0.05
TLC ($x10^3$/ mm^3)	8.6 ± 2.6	9.6 ± 3.3	>0.05
HB (gm/dl)	9.8 ± 0.16	10.3 ± 0.35	>0.05
Platelets ($x10^3$/ mm^3)	126 ± 28	132 ± 43	>0.05

Table (3): Data of ascitic fluid examination of the studied groups.

Parameters	*BactDNA positive group (N=25)* M ± SD	**BactDNA negative group (N=38)** M ± SD	P value
AF total protein (gm/dl)	1.7 ± 0.61	1.5 ± 0.42	>0.05
AF albumin (gm/dl)	0.65 ± 0.41	0.73 ± 0.38	>0.05
AF LDH (U/L)	154 ± 38	166 ± 44	>0.05
AF TLC / mm^3	211 ± 56	178 ± 32	>0.05
AF PMN (cells / mm^3)	103 ± 44	85 ± 30	>0.05

Table (4): Data of serum and AF BactDNA in DNA positive cases (N=25)

Organism isolated	*Escherichia coli*	*Klebsiella. pneumoniae*	*Staphylococcus aureus*	*Diphteroid*
Serum BactDNA	9/25 (36%)	1/25 (4%)	1/25 (4%)	1/25 (4%)
AF BactDNA	3/25 (12%)	2/25 (8%)	1/25 (4%)	0
Both serum & AF BactDNA	6/25 (24%)	1/25 (4%)	0	0
Total number	18/25 (72%)	4/25 (16%)	2/25 (8%)	1/25 (4%)

Table (5): Prognosis of overall patients within 12 months of follow up

Variables	*BactDNA positive group (N=25)*	**BactDNA negative group (N=38)**	**P value**
Development of SBP (No %)	11 (44%)	5 (13.2%)	<0.01**
Development of HRS (No %)	9 (36%)	3 (7.9%)	<0.001**
Upper GI Bleeding (No%)	3 (12%)	2 (5.3%)	<0.01**
Death rate (No %)	8 (32%)	4 (10.5%)	<0.010**

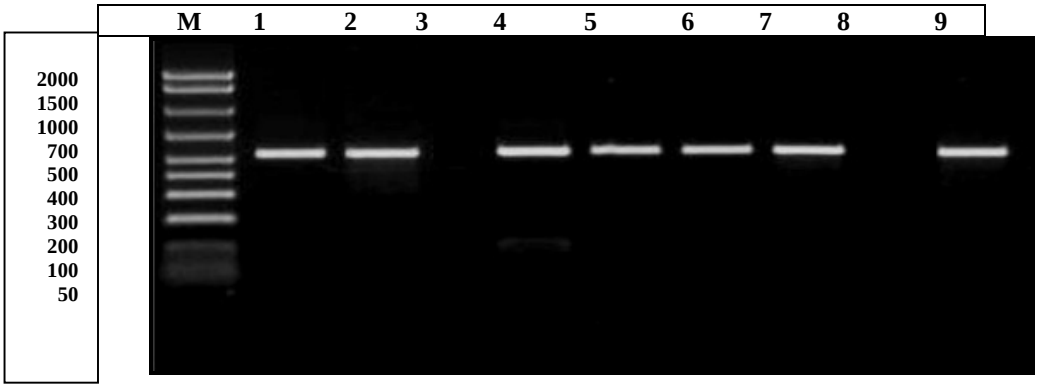

Figure (1) Amplification of PCR products on agarose gel (M band: molecular weight ladder (50-2000 bp), band 1, 2, 4, 5, 6, 7: positive samples (540 bp), band 8: negative control, band 9: positive control

4. Discussion:

Spontaneous bacterial peritonitis (SBP) is defined by the presence of >250 PMN/mm^3 in ascites in the absence of an intra-abdominal source of infection or malignancy (**Rimola *et al.*,2000)**. Asymptomatic presence of BactDNA in both the blood and AF in patients with advanced and decompensated cirrhosis has been found in the study done by **Such *et al.*** (**2002)**.

In our study which was done on 63 patients presented with symptoms such as low grade fever and some of them had abdominal pain. They were admitted to the hospital suspected of having SBP and diagnostic paracentesis was done for all patients. PMN was found less than 250 cells/mm^3 in all patients and serum and AF cultures were found negative (CNNA). No significant difference was found between both groups as regards liver functions, urea, electrolytes, CBC and AF parameters, except serum creatinine was significantly increased in BactDNA positive cases. This finding was in agreement with **Kamani *et al.* (2008),** who reported increased serum creatinine in patients with AF infection regardless positive or negative culture.

Furthermore, our results revealed that BactDNA was detected in AF and/or blood samples in 25/63 (39.7%) and was negative in 38/63 (60.3%).Among the 25 patients having BactDNA positive,12(48%) of them was detected in serum,6 (24%)of them in AF and 7(28%) in simultaneously both serum and AF.

Our results are in accordance with the conclusion of **Zapater *et al.* (2008)** and **Bruns *et al.* (2009)** where both studies agree that identification of ascitic BactDNA is an appropriate alternative to bacterial ascites culture for pathogen identification in patients at risk for SBP. The investigations published by **Francés and colleagues (2004)** have shown that some patients with cirrhosis have bacterial DNA in their serum and ascitic fluid, and that the DNA is always present simultaneously in both body fluids.

Beate *et al.* (2010) study results showed that 18 of 151 patients (12%) had SBP according to the classic definition. Bacterial DNA was detected in five of these 18 patients (3%), whereas in 13 patients (9%), bacterial DNA was detected without standard SBP. Seven patients (5%) had culture-positive SBP, only in two of them bacterial DNA was detected. In the DNA-positive ascites group, none of the assessed parameters was significantly associated with the bacterial DNA positivity. The authors of this study found no correlation between detection of bacterial DNA in ascites and SBP (PMN count>250/mm^3).

Presence of bacterial DNA in patients with decompensated cirrhosis is associated with marked activation of peritoneal macrophages, as evidenced by nitric oxide synthesizing ability, together with enhanced cytokine production (**France´s *et al.*, 2004). Runyon (2003)** reported that ascitic fluid state should no longer be considered as either sterile or infected, but instead to be part of a spectrum that includes both polymicrobial colonisation and clinically evident infection.

In our current study urine analysis was done for all patients and did not show signs of infection and urine culture found negative. So, UTI was ruled out as a cause of infection. Urine culture may also prove useful, since asymptomatic bacteruria has been suggested to predispose to the development of spontaneous bacterial peritonitis.

There was also no clinical signs suggestive of respiratory tract infection and chest-X ray done for all patients did not reveal any infiltrate to rule out respiratory tract infection.

In the study done by **Such *et al.* (2002),**they detected bactDNA in serum and AF in 32% of all patients studied, and this likely represents single clone episodes of translocation and systemic seeding.

E. coli is the most frequently identified bacteria. This goes hand in hand with our results which revealed that E.coli is the most frequent bacterial species detected among bactDNA positive, 18/25(72%). and this might reflect on choosing the appropriate antibiotic where offending agent guides antibiotic therapy.

We started our patients on Cefotaxime IV 2 gm every 8 hours for 5 days, after paracentesis was done and serum and AF culture samples were taken, as an empirical treatment because they already had clinical manifestations suggestive of SBP such as fever, abdominal pain, poor response of ascites to diuretic therapy, signs of unexplained or worsening encephalopathy and new onset or worsening renal impairment. Our patients actually had clinical improvement 2-3 days after starting the antibiotic.

Empiric therapy of suspected SBP must be initiated as soon as possible to maximize the patient's chance of survival. The recommendations mentioned below are consistent with a 2009 guideline issued by the American Association for the Study of Liver Diseases(AASLD). The main indication for empiric therapy is the otherwise unexplained presence of one or more of the following findings that are characteristically seen in SBP : temperature greater than 37.8ºC, abdominal pain and/or tenderness, a change in mental status, ascitic fluid PMN count ≥250 cells/mm3.

Runyon (2003) showed that despite improvements in the analysis of ascitic fluid a significant proportion of patients who have clinical manifestations suggestive of SBP, including elevated levels of ascites PMN, have culture-negative ascites. This results in empirical antibiotic treatment for SBP often being initiated without the identification of infective agents.

We know that culture negativity is present in significant proportion of patients may be 40-65% of SBP. So, BactDNA may be an alternative diagnostic tool for early diagnosis and prompt treatment of any suspicion of ascitic fluid bacterial infection where we should start giving antibiotic before pathogen identification.

In our study some of patients had symptoms like abdominal pain and low grade fever. Despite these symptoms suspecting SBP, ascitic fluid PMN was found not in favour of diagnosis of SBP and all patients were culture negative in AF. The question now; is that detection of bacterial DNA in ascites of patients with liver cirrhosis is of clinical or diagnostic relevance? We gave our patients 3rd generation cephalosporin (cefotaxime) being suspected of having SBP and after PMN came less than250 cells/mm^3 (classic definition of SBP) is it wise to continue on the antibiotic and if so to whom should we continue to bactDNA positive only or both positive and negative patients? we continued on the antibiotic for 5 days based on AASLD guidelines mentioned previously.

After 12 months of follow up to our patients, development of SBP, HRS, upper GI bleeding and death were occurred in 44%, 36%, 12%, and 32% respectively in BactDNA positive compared to 13.2%, 7.9%, 5.3% and 10.5% in BactDNA Negative cases which is significantly higher in the former group.

Kamani *et al.* (2008) showed that patients with SBP have a higher mortality than CNNA. Independent predictors of mortality in SBP are raised serum creatinine and a positive blood culture. Even though bacterial cultures of ascitic fluid are negative in up to 65% of cases; BactDNA has been frequently detected in episodes of SBP as well as in CNNA. This finding was in agreement with our study where serum creatinine was significantly higher in bact DNA positive than bactDNA negative cases, whereas both groups were culture negative. This could be one cause of high rate of mortality in the former group.

Rogers *et al.* (2010) concluded that cirrhotic patients with CNNA and bacterial DNA have a significantly higher level of serum and AF TNF-alpha and higher risk of HRS, SBP and mortality compared to those without bacterial DNA, suggesting that bacterial DNA and TNF-alpha are implicated in these complications of liver cirrhosis.

Francés and colleagues (2004) also showed that patients who subsequently develop SBP have a higher baseline ascitic fluid TNF level than patients who do not develop SBP which occurs when the organism is more virulent than the bacteria that were killed by host immune defences at the stage of colonisation, when immune defences weaken, or a combination of these events. It is the peritoneal macrophage that is the first line of defence against bacterial colonisation of ascitic fluid. SBP occurs when macrophages fail to kill the bacteria and the second line of defence is called in the neutrophils.

Conclusion: Identification of bacterial DNA in serum and AF has its significant clinical and diagnostic relevance in suspicion of SBP and its prompt therapy specially in CNNA. It might be an alternaive diagnostic tool to AF bacterial culture in early diagnosis and prompt treatment with subsequent improvement of survival which needs further investigations.

Our patients who found positive for bacterial DNA had risks of SBP, hepatorenal syndrome, and death higher than those of DNA negative patients.We reccommend if excessive morbidity and/or mortality are documented in bactDNA positive patients, the next step would be to conduct a

randomised controlled trial of selective intestinal decontamination versus placebo in the DNA positive group and determine if hepatorenal syndrome and death can be prevented.

SBP develops in a significant proportion of individuals shown to have bacteria in their ascites suggesting the presence of bacteria prior to the development of an elevated PMN count or other evidence of local or systemic infection, which may represent an intermediate stage on the way to infection. However, it is recommended to conduct a study looking for the development of a rapid, sensitive and accurate method to detect and characterise bacteria in ascites would provide an opportunity to make an appropriate intervention before an infection has been established.

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