Journal of Molecular Biomarkers & Diagnosis

Abed et al., J Mol Biomark Diagn 2019, 10:1
DOI: 10.4172/2155-9929.1000411

Research Article Open Access

The Role of 16s Ribosomal RNA in Diagnosing Spontaneous Bacterial Peritonitis

Sally Abed[1], **Mohamed Egezy**[1], **Tarek Sheta**[2] **and Maysaa El-Sayed Zaki**[3*]

[1]*Tropical Medicine Department, Mansoura University of Medicine, Mansoura, Egypt*

[2]*Gastroenterology Medicine Department, Mansoura University of Medicine, Mansoura, Egypt*

[3]*Faculty of Medicine, Department of Clinical Pathology, Mansoura University of Medicine, Mansoura, Egypt*

[*]**Corresponding author:** Maysaa El-Sayed Zaki, Faculty of Medicine, Department of Clinical Pathology, Mansoura University, Egypt, Tel: +0020502258877; E-mail: may_s65@hotmail.com

Rec Date: December 28, 2018, **Acc Date:** February 07, 2019, **Pub Date:** February 11, 2019

Abstract

Background: Cancer, the accurate clinical diagnosis of spontaneous bacterial peritonitis (SBP) is challenging due to frequent absence of the symptoms and signs which are non-specific. The laboratory diagnosis of SBP depends mainly on the ascetic fluid neutrophil count. Therefore, it is recommended to inoculate the ascitic fluid into blood culture but cultures are time consuming and have a limited value to urgently direct the initiation of specific effective antibiotic treatment.

Aim of study: To evaluate the role of 16s ribosomal RNA in early diagnosis of SBP.

Patients and methods: The present study was cross-sectional study that was carried out in Mansoura University hospital from January 2016 till March 2017. The study included 120 patients complaining of chronic hepatitis C. Each patient was subjected to full clinical history including symptoms of spontaneous bacterial peritonitis such as fever, constipation and abdominal pain. The severity of liver affection was classified according to Child score. Complete liver function tests were performed. The peritoneal fluid was divided to three samples, one sample for total leucocytic count by hemocytometer, the other sample was centrifuged and the sediment was cultured on blood agar, MacConkey agar and Sabouraud's dextrose agar at 37C for 24-48 hours DNA Extraction the sediment pellet of the peritoneal fluid was subjected to DNA extraction by QIAampDNAM; ini kit (QIAGEN, Germany) according to the manufacturer's instruction.

Results: The culture positive cases were 21 patients 20 of them were positive for 16sRNA and only 1 patient was negative for 16sRNA. While 16s RNA was positive in 26 patients in which 6 were negative culture. The WBCS count was >250/mm^3 in 31 patients of which 26 patients were positive for 16s RNA and 21 were culture positive. The 16sRNA had higher sensitivity (81.25%), while the culture the sensitivity was (67.7%).

Conclusion: In the current study we assessed the 16s ribosomal RNA detection by PCR and it was more rapid and sensitive than bacterial cultures to confirm bacterial infection of the ascetic fluid even after cultures with bedside inoculation of the ascetic fluid samples.

Keywords: Spontaneous bacterial peritonitis; PCR; 16s rRNA

Introduction

Spontaneous bacterial peritonitis (SBP) is a serious acute and life threatening ascetic fluid infection occurring in about 10-30% of ascetic cirrhotics. It occurs in absence of a defined surgical source of infection [1].

Spontaneous bacterial peritonitis occurs as a result of impaired host immune response in cirrhoticsand bacterial translocation throughout the gut wall. The enteric organisms represent about 90% of the isolated organisms in SBP. An alternative proposed mechanism for SBP is hematogenous transmission of infection to the ascetic fluid [2-4].

At least 92% of SBP cases are monomicrobial [4]. The most frequent pathogens responsible for SBP are the Gram negative bacteria specially the *E coli* [1,5] but recent studies have shown an increased incidence in SBP cases due Gram positive cocci infection which is suggest to be associated with the frequent use of prophylactic antibiotics, the frequent long term hospitalization with exposure to invasive maneuvers and nosocomial infections [5-7].

The accurate clinical diagnosis of SBP is challenging due to frequent absence of the symptoms and signs which are non-specific [8]. The laboratory diagnosis of SBP depends mainly on the ascetic fluid neutrophil count. Spontaneous bacterial peritonitis is defined as a count >250/mm in absence of secondary bacterial peritonitis and the detection of the causative agent for SBP depends routinely on the ascetic fluid cultures [1,8].

The ascetic fluid neutrophil count may not rise to this threshold in SBP cases due to many factors in cirrhotics including the immune deficiency, hypersplenism and SBP caused by Gram positive cocci [9].

Conventional bacterial culture methods effectively detect bacteria in less than 50% of ascites samples with an elevated PMN count (>250/mm^3) [3,10]. Therefore, it is recommended to inoculate the ascitic fluid into blood culture bottles at the patient's bedside in order to increase the sensitivity of the bacterial culture [1,8,10,11].

Unfortunately, cultures are time consuming and have a limited value to urgently direct the initiation of specific effective antibiotic treatment [2,9]. In addition, cirrhotic patients with clinical features of ascetic fluid infection and neutrophil count >250 may have negative cultures a variant of SBP named culture-negative neutrocytic ascites. Bacterascites is a different variant with a positive culture and a neutrophil count <250. In both culture-negative neutrocytic ascites and Bacterascites the initiation of antibiotic treatment is recommended [8,12].

Many studies have been done to investigate the value of different nucleic acid amplification tests for detection of the bacterial DNA in the acetic fluid and its role in the early diagnosis of SBP [13-18]. Diagnostic tests that target the bacterial 16S rRNA gene can offer many advantages; the highly conserved sequences of the gene allow broad-range detection of almost any eubacterial species, while the hypervariable sequences can be exploited for species-level identification [12-18]. In culture-negative, non-neutroceytic ascites the detection of the 16S rRNA gene in both blood and ascetic fluid simultaneously is a marker of bacterial translocation [17] and can be a predictor of one year mortality [18].

Patients and Methods

The present study was cross-sectional study that was carried out in Mansoura University hospital from January 2016 till March 2017. The study included 120 patients complaining of chronic hepatitis C with ascites. The study was approved by Faculty of Medicine Mansoura ethical committee. Each participated patient signed an informed written consent.

Each patient was subjected to full clinical history including symptoms of spontaneous bacterial peritonitis such as fever, constipation and abdominal pain. The degree of hepatic affection was determined by clinical examination and pathological classifications. The severity of liver affection was classified according to child score. Complete liver function tests were performed for each patient. Paracentesis was performed under complete antiseptic techniques and 30 mL volume of the ascetic fluid was obtained for laboratory investigations.

Immediately, ten milliliters of the ascitic fluid was inoculated in Bactec blood culture bottle. The peritoneal fluid was subjected for total leucocytic count by hemocytometer and the remaining sample was centrifuged and DNA was extracted from the deposit and was kept frozen at -20°C for molecular analysis of 16S rRNA.

The positive blood culture bottles were subjected to subculture on blood agar, at 37°C for 24 hours and colonies were identified by gram stain, biochemical reaction for identification of bacterial species and antibiotics susceptibility by disc diffusion method.

DNA extraction

The sediment pellet of the peritoneal fluid was subjected to DNA extraction by QIAampDNAMini kit (QIAGEN, Germany) according to the manufacturer's instruction.

Amplification for 16S rRNA

Amplification of the extracted DNA was performed by the use of Qiagen amplification kit according to the protocol reported previously. The primers used and the amplification procedures and the fragment bp size are summarized in Table 1.

Gene	Primer sequences	Amplification	bp
U1	5/-CCAGCAGCCGCGGTAATACG-/3	Cycling of 1 min 94°C, 1 min 55°C, 2 min 72°C	960 bp
U2	5/-ATCGG(C/T)TACCTTGTTACGACTTC-/3		
ITS1	5/-TCCGTAGGTGAACCTGCGG-/3	Cycling of 1 min 94°C, 1 min 55°C, 2 min 72°C	600 bp
ITS4	5/-TCCTCCGCTTATTGATATGC-/3		

Table 1: The primers used, the amplification procedures and the fragment bp size.

Results

The number of patients included in this study was 120 (93 males and 27 females) with age 46.6 ± 16.2 (mean ± SD)108 of patients were positive for HCV-antibidies, five patients were positive for HBs-Ag and seven patients were tested negative for both Ascites was graded into mild in 42 patients (35%), moderate in 24 patients (20%) and severe in 54 patients (45%). Child score was calculated 18 patients were Child A, 36 patients were child B and 66 were Child C (Table 2).

Culture was positive in 21 patients, 16s rRNA was positive in 26 patients and WBCS was >250/mm^3 in peritoneal fluid in 31 patients. In the 21 patients with positive culture 14 patients had *Escherichia coli*, 5 patients had *Klebsiella spp.* while only 2 had *Staphylococcus aureus* (Tables 3 and 4).

Fever was found in 22 (88%) of patients, abdominal pain in 16 patients (64%) and constipation in 15 patients (60%). The degree of ascites was mild in 11 patients (44%), moderate in 3 patients (12%) and severe in 11 patients (44%). The child score was C in 12 patients (48%), B in 7 patients (22%) and A in 6 patients (24%) (Table 5).

The culture positive cases were 21 patients 20 of them were positive for 16sRNA and only 1 patient was negative for 16s RNA. While 16sRNA was positive in 26 patients in which 6 were negative culture (Table 6).

The WBCS count was >250/mm^3 in 31 patients of which 26 patients were positive for 16s RNA and 21 were culture positive (Table 7).

The 16s RNA had higher sensitivity (81.25%), while the culture the sensitivity was (67.7%) (Table 8).

Data	Values
Age	46.616.2 (mean ± SD)
Sex	
Male	93 (77.5%)
Female	27 (32.5%)
Ascites	
Mild	42 (35%)
Moderate	24 (20%)
Severe	54 (45%)
HBsAg	5 (4.2%)
HCV-IgG	108 (90%)
Fever	41 (34.2%)
Abdominal pain	48 (40%)
Tenderness	29 (24.2%)
Constipation	37 (30.8%)
Child classification	
A	18 (15%)
B	36 (30%)
C	66 (55%)
Albumin (gm/dl) Mean ± SD	3.03 ± 0.5
Total bilirubin (mg/l) Mean ± SD	3.4 ± 1.5
Direct bilirubin (mg/l) Mean ± SD	2.1 ± 1.5
ALT (IU/l) Mean ± SD	55.1 ± 20.1
ALT (IU/l) Mean ± SD	40.01 ± 14.5
INR Mean ± SD	1.4 ± 0.6

Table 2: Clinical and laboratory findings of the studied patients.

Presence of bacterial pathogens	No. (%)
Culture	21 (17.5%)
16s rRNA	26 (21.7%)
WBCs >250/mm^3 in peritoneal fluid	31 (25.8%)

Table 3: Detection of presence of bacterial pathogens in peritoneal fluid by culture, 16s rRNA, and WBCs (n=120).

Discussion

Spontaneous bacterial peritonitis is a serious complication in patients with cirrhotic ascites with a high recurrence rate and a poor prognosis [8,19]. The rapid and accurate laboratory diagnosis of SBP is challenging with no single, rapid, sensitive, specific and simple test to confirm the diagnosis and detect the causative agent [20-22].

Species	No. (%)
Escherichia coli	14 (66.7%)
Klebsiella spp.	5 (23.8%)
Staphylococcus aureus	2 (9.5%)

Table 4: Bacterial species Isolated by culture (n=21).

Clinical presentation	No. (%)
Degree of ascites	
Mild	11 (44%)
Moderate	3 (12%)
Severe	11 (44%)
fever	22 (88%)
Abdominal pain	16 (64%)
Tenderness	9 (36%)
Constipation	15 (60%)
Child classification	
A	6 (24%)
B	7 (22%)
C	12 (48%)

Table 5: Clinical presentation in spontaneous bacterial peritonitis (n=25).

Comparison		Culture Positive	Culture Negative	Total
		No. (%)	No. (%)	No. (%)
16s rRNA	Positive	20 (76.9%)	6 (23.1%)	26 (100%)
	Negative	1 (1.1%)	93 (98.9%)	94 (100%)

Table 6: Comparison between culture and 16s rRNA.

Although identifying the pathogen (s) plays a major role in the management of infectious diseases, it takes several days to identify the casual bacteria of SBP by the bacterial cultures. In addition, ascitic fluid cultures are negative in approximately 10–60% of patients with clinical manifestations of SBP and PMNS above 250/mm [2,3,23].

Routine bacterial cultures of the ascetic fluid have about 40% sensitivity in SBP diagnosis and bedside inoculation of the ascetic fluid samples in blood culture bottles markedly increases the sensitivity of the cultures [2,3,10,11,24], but unfortunately even with bedside inoculation in patients with clinical manifestations of SBP and PMNS above 250/mm different studies has shown highly variable sensitivities ranging from 40 to 70% [1,8,21,25].

We aimed to study the 16s ribosomal RNA PCR testing for more accurate, rapid and simple confirmation of the bacterial infection in SBP.

Comparison	WBCs Positive >250/mm^3	WBCs Negative<250/mm^3	Total
	No. (%)	No. (%)	No. (%)
16s rRNA			
Positive	26 (100%)	0 (0%)	26 (100%)
Negative	5 (5.3%)	89 (94.7%)	94 (100%)
Culture			
Positive	21 (100%)	0 (0%)	21 (100%)
Negative	10 (10.1%)	89 (89.9%)	99 (100%)

Table 7: Comparison by 16srRNA and WBCs counts.

Comparison	Sensitivity	Specificity	Positive predicative value	Negative predictive value	Accuracy
16s RNA					
%	81.25%	100.00%	100.00%	93.68%	95.04%
CI-95%	63.56% -92.79%	95.94% - 100.00%	--	87.82% - 96.83%	89.52% - 98.16%
Culture					
%	67.70%	100%	100%	89%	91.70%
CI-95%	48.63% to 83.32%	95.94% to 100.00%	--	84.24% to 93.68%	85.21% to 95.93%

Table 8: Comparison of 16srRNA detection by PCR and WBCs ≥ 250/mm^3.

In our study 31 patients (25.8% of the study population) were diagnosed as SBP based on the clinical manifestations and a PMNs above 250/mm in the ascetic fluid samples. The 16s ribosomal RNA PCR was positive in 26 patients (21.6% of the study population) and the bacterial culture was positive in 21 patients (17.5% of the study population) and from these data the rates of neutrocytosis, positive cultures and the rates of bacterial DNA detection in the ascetic fluid in our study were comparable with the rates in other published studies [11-17,26-28].

We compared the sensitivity of the 16s ribosomal RNA PCR and the bacterial cultures to prove bacterial infection in neutrocytic ascites. The 16s ribosomal RNA PCR can detect bacterial infection in clinically evident SBP patients having a PMNs above 250/mm with a sensitivity of 81.25%, while bacterial cultures are less sensitive than the 16s ribosomal RNA PCR with a sensitivity of 67.7%. These results agree with previous studies showing higher sensitivity for bacterial DNA detection than the cultures to prove ascetic fluid bacterial infection [11-17].

The 16s ribosomal RNA PCR was negative in 1/21 (4.7%) of the culture positive neutrocytic cases. while the 16s ribosomal RNA PCR was positive in 6/10 (60%) of the culture negative neutrocytic cases These results are comparable to those of many previous studies depending on the bacterial DNA detection in the ascetic fluid of cirrhotics [11-13,16,17]. It is suggested that the culture negative neutrocytic ascites is associated with bacterial infection resistant to ordinary cultures under standard conditions or present at low concentrations [12].

All the culture positive cases in our study were monomicrobial, the *Escherichia coli* was positive in 14/21 (66.7%) of the positive cultures, *Klebsiella spp*. was positive in 5/21 (23.8%) of positive cultures, while *Staphylococcus aureus* was positive in 2/21 (9.5%) of the positive cultures, so our data agree with other previous studies proving that the *E coli* and other coliforms such *Klebsiella spp* are the most frequent pathogens detected in SBP with and increasing incidence of SBP infections by the Gram positive cocci [1,5-7,29].

Conclusion

In conclusion, Spontaneous bacterial peritonoitis remains one of the most serious complications of cirrhotic ascites. The bacteriological diagnosis of SBP is challenging and bacterial cultures are time consuming and have variable results. Many authors studied the bacterial DNA detection as an alternative useful tool to diagnose SBP. In the current study we assessed the 16s ribosomal RNA detection by PCR and it was more rapid and sensitive than bacterial cultures to confirm bacterial infection of the ascetic fluid even after cultures with bedside inoculation of the ascetic fluid samples.

References

1. Wiest R, Krag A, GerbesA (2012) Spontaneous bacterial peritonitis: Recent guidelines and beyond. Gut 61: 297-310.
2. Runyon BA (2009) Management of adult patients with ascites due to cirrhosis: An update. Hepatology 49: 2087–2107.
3. Runyon BA, Canawati HN, Akriviadis E (1988) A optimization of ascitic fluid culture technique. Gastroenterology 95: 1351–1355.
4. Parsi MA, Atreja A, Zein NN (2004) Spontaneous bacterial peritonitis: Recent data on incidence and treatment. Cleve Clin J Med 71: 569-576.
5. Cholongitas E, Papatheodoridis GV, Lahanas A, Xanthaki A, Kastellanou CK, et al. (2004) Increasing frequency of Gram-positive bacteria in spontaneous bacterial peritonitis. Liver Int 25: 57-61.
6. Campillo B, Richardet JP, Kheo T, Dupeyron C (2002) Nosocomial spontaneous bacterial peritonitis and bacteremia in cirrhotic patients: Impact of isolate type on prognosis and characteristics of infection. Clin Infect Dis 35: 1-10.
7. Alexopoulou A, Papadopoulos N, Eliopoulos DG, Alexaki A, Tsiriga A, et al. (2013) Increasing frequency of gram-positive cocci and gram-negative multidrug-resistant bacteria in spontaneous bacterial peritonitis. Liver Int 33: 975-981.
8. European Association for the Study of the Liver (2010) EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. J Hepatol 53: 397–417.
9. Wei FF, Zhang X, Xu Z, Zhang S (2014) New markers to explore spontaneous bacterial peritonitis. J Mol Biomark Diagn 5: 200.
10. Siersema PD, Marie S, Zeijl JH, Bac DJ, Wilson JHP (1992) Blood culture bottles are superior to lysis-centrifugation tubes for bacteriological diagnosis of spontaneous bacterial peritonitis. J Clin Microbiol 30: 667–669.
11. Mostafa MS, El-Seidi EA, Kassem AM, Shemis MA, Saber M, et al. (2011) Detection of ascitic fluid infections in patients with liver cirrhosis and ascites. Arab J Gastroenterol 12: 20–24.
12. Hardick J, Won H, Jeng K, Hsieh YH, Gaydos CA, et al. (2012) Identification of bacterial pathogens in ascitic fluids from patients with

suspected spontaneous bacterial peritonitis by use of broad-range PCR (16S PCR) coupled with high-resolution melt analysis. J Clin Microbiol 50: 2428–2432.

13. Bruns T, Sachse S, Straube E, Assefa S, Herrmann A, et al. (2009) Identification of bacterial DNA in neutrocyticandnon-neutrocytic cirrhotic ascites by means of a multiplex polymerase chain reaction. Liver Int 8: 1206 –1214.
14. El-NaggarMM, Khalil el-SA, El-Daker MA, Salama MF (2008) Bacterial DNA and its consequences in patients with cirrhosis and culture negative, non-neutrocytic ascites. J Med Microbiol 57: 1533–1538.
15. Frances R, Zapater P, González-Navajas JM, Muñoz C, Caño R, et al. (2008) Bacterial DNA in patients with cirrhosis and noninfected ascites mimics the soluble immune response established in patients with spontaneous bacterial peritonitis. Hepatology 47: 978–985.
16. Soriano G, Esparcia O, Montemayor M, Guarner-Argente C, Pericas R, et al. (2011) Bacterial DNA in the diagnosis of spontaneous bacterial peritonitis. Aliment Pharmacol Ther 33: 275–284.
17. Such J, Francés R, Muñoz C, Zapater P, Casellas JA, et al. (2002) Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, non-neutrocytic ascites. Hepatology 36: 135–141.
18. Zapater P, Francés R, Navajas JMG, Hoz MA, Moreu R, et al. (2008) Serum and ascitic fluid bacterial DNA: A new independent prognostic factor in non-infected patient and cirrhosis. Hepatol 48: 1924 –1931.
19. Rimola A, Tsao GGG, Navasa M, Piddock LJV, Planas R, et al. (2000) Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: A consensus document. J Hepato 32: 142–153.
20. Moore KP, Aithal GP (2006) Guidelines on the management of ascites in cirrhosis. Gut 55: vi1–vi12.
21. Tsao GG, Lim JK (2009) Management and treatment of patients with cirrhosis and portal hypertension: Recommendations from the department of veterans affairs hepatitis C resource center program and the national hepatitis C program. Am J Gastroenterol 104: 1802–1829.
22. Barreales M, Fernandez I (2011) Spontaneous bacterial peritonitis. Rev Esp Enferm Dig 103: 255–263.
23. Fernández J (2002) Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. Hepatology 35: 140–148.
24. Wu SS, Lin OS, Chen YY, Hwang KL, Soon MS, et al. (2001) Ascitic fluid carcinoembryonic antigen and alkaline phosphatase levels for the differentiation of primary from secondary bacterial peritonitis with intestinal perforation. J Hepat 34: 215–221.
25. Moore KP, Aitha GP (2006) Guidelines on the management of ascites in cirrhosis. Gut 55: vi1–vi12.
26. Hörner R, Salla A, Oliveira LOD, Forno NLFD, Righi RA, et al. (2010) Spontaneous bacterial peritonitis caused by *Streptococcus bovis*: Case report and review of the literature. Braz J Infect Dis 14: 294 –296.
27. Serste T, Bert F, Guibout VL, Chauvet C, Marcon E, et al. (2011) Detection of bacterial DNA in serum and ascitic fluid of asymptomatic outpatients with cirrhosis and non-neutrocyticascites. Liver Int 4: 494–498.
28. Frances R, Benlloch S, Zapater P, González JM, Lozano B, et al. (2004) A sequential study of serum bacterial DNA in patients with advanced cirrhosis and ascites. Hepatology 39: 484–491.
29. Cheong HS, Kang CI, Lee JA, Moon SY, Joung MK, et al. (2009) Clinical significance and outcome of nosocomial acquisition of spontaneous bacterial peritonitis in patients with liver cirrhosis. Clin Infect Dis 48: 1230–1236.