Role of Serial Ascitic Fluid Analysis and Other Prognostic Factors in Spontaneous Bacterial Peritonitis

Avanish Kumar Bhardwaj¹, Dwijen Das², Prithwiraj Bhattacharjee³, Giridhari Kar⁴, Bhaskar Kanti Nath⁵

¹Registrar, Department of Medicine, Silchar Medical College & Hospital, Silchar, Assam, India, ²Associate Professor, Department of Medicine, Silchar Medical College & Hospital, Silchar, Assam, India, ³Professor, Department of Medicine, Silchar Medical College & Hospital, Silchar, Assam, India, ⁴Professor & Head, Department of Medicine, Silchar Medical College & Hospital, Silchar, Assam, India, ⁵Assistant Professor, Department of Medicine, Silchar Medical College & Hospital, Silchar, Assam, India

Abstract

Introduction: Cirrhosis of the liver is one of the commonly encountered liver disorders in clinical practice caused by a wide variety of etiologies. It is defined anatomically as a diffuse process of nodule formation and fibrosis in the liver. Spontaneous bacterial peritonitis (SBP) is defined by infection of the previously sterile ascitic fluid (AF), without any apparent intra-abdominal source of infection. SBP is one of leading cause of death in decompensated liver disease which need prompt diagnosis and treatment. There is increasing toll of alcoholic liver disease, and its complications in Assam in recent times. However, information regarding the clinical profile and bacteriological profile of SBP and its impact on outcome of cirrhotic patients is still lacking in many aspects in this part of the country.

Aims and Objectives: This study was undertaken with an idea to evaluate the significance of serial AF analysis and other biochemical markers to determine prognosis in cirrhotic patients in this region.

Materials and Methods: Patients admitted with hepatic cirrhosis with ascites were studied during the period from July 2013 to July 2014. All patients included in the study were confirmed hepatic cirrhosis either by liver biopsy or ultrasound with ascites presented with features of the SBP.

Results: Total 50 patients of age group >12 years, were included and studied thoroughly with regards to both history and clinical examination. Higher total leukocyte count (>13,700/mm³), serum creatinine (>2 mg/dl) and low serum albumin (<2.2 g/dl), and AF protein (<1.086 g/dl) were significantly associated with increased mortality. Reduction in polymorphonuclear leukocyte count in serial AF analysis showed reduction in mortality.

Conclusion: Increased total leukocyte count, low serum albumin, increased serum creatinine levels, and low AF protein are associated with poor prognosis. Serial AF cell count is helpful in predicting prognosis and can be used to monitor treatment.

Key words: Ascites, Ascitic fluid, Biopsy, Liver cirrhosis, Peritonitis, Prognosis

INTRODUCTION

Cirrhosis of the liver is one of the commonly encountered liver disorders in a clinical practice caused by a wide variety of etiologies. It is defined anatomically as a diffuse process of

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nodule formation and fibrosis in the liver. The pathological hallmark of cirrhosis is the development of scar tissue that replaces the normal parenchyma, blocking the portal flow of blood through the organ and disturbing the normal functions of the liver. Patients with cirrhosis may have compensated liver disease and may manifest as anorexia, weight loss, weakness, fatigue, and even osteoporosis as a result of vitamin D malabsorption and subsequent calcium deficiency. Decompensation of liver function may result in clinical symptoms such as jaundice, pruritus, gastrointestinal bleed, ascites, coagulopathy, and mental status changes and complications such as SBP, hepatic encephalopathy, and variceal bleeding from portal hypertension.

Corresponding Author: Dr. Dwijen Das, Department of Medicine, Silchar Medical College & Hospital, Ghungoor, Silchar, Cachar - 788 014, Assam, India. Phone: +91-9435181953. E-mail: drdwijendas@yahoo.co.in

In most of the patients of cirrhosis terminal events are spontaneous bacterial peritonitis (SBP), hepatic encephalopathy, gastro intestinal bleeding, hepatorenal syndrome, hepatocellular carcinoma, and other infection due to decrease immunity.³ Established cirrhosis has 10 years mortality rate between 34% and 66%.⁴ The treatment is mainly directed against the prevention and management of the complications. Therefore, prompt and early diagnosis of these complications is the mainstay for prolonging life in cirrhotic patients. The SBP is a severe complication appearing in 8-22% of hepatic cirrhosis with ascites.⁵

SBP

The SBP is by definition an infection of the previously sterile ascitic fluid (AF), without any apparent intraabdominal source of infection. Polymorphonuclear (PMN) cell count of more than 250/mm³ in AF is currently considered diagnostic of SBP and warrant the prompt start of antibiotic treatment.²

AF Defense Mechanisms

The arrival of bacteria to the AF does not guarantee that infection will develop. In fact, cirrhotic AF is capable of humoral self-defense, mainly on the basis of the effectiveness of the complement system. Patients with adequate activity of this vital bactericidal system usually do not develop AF bacterial infections. However, it has been demonstrated that patients with low C3 component of complement in AF (<13 mg/dl) and/or a protein level of <1 g/dl, are predisposed to this infection. The complement levels may be deficient because of increased consumption of these components or because of impaired synthesis. Most of the bacteria that colonize AF are intestinal Gram-negative bacteria. The presence of lipopolysaccharides in their cell wall activates the alternative pathway of complement. If the complement levels are inadequate to effectively kill the bacteria, the infection will not develop.

Predisposing factors for SBP^{6,7}

- 1. Child-Pugh class C cirrhosis
- 2. AF protein level <1 g/dl or complement C3 level <13 mg/dl
- 3. Gastrointestinal bleeding
- 4. Urinary tract infection
- 5. Intestinal bacterial overgrowth
- 6. Iatrogenic causes- urinary bladder, intravascular catheters
- 7. Previous SBP.

The severity of the liver disease is probably the most important factor. Almost 70% of patients who develop SBP are Child-Pugh class C, with the remainder being

Class B.⁶ A serum total bilirubin level of >2.5 mg/dl is an independent predictive factor of SBP. A direct correlation between total protein level, complement components, and opsonic activity explains why an AF total protein level of <1 g/dl is a risk factor for the development of infection.⁷

Alcohol consumption is rising all over the country, especially in the Northeastern part of India. Global information system on alcohol and health 2011 report shows high prevalence of alcohol consumption in this part of the country.

In Assam, 58% of the youth used at least one substance and 27.4% were concurrent users of both alcohol and tobacco.⁸

The survey, conducted by the Registrar General of India across 284 districts found that men from Chhattisgarh love their drink, recording the highest percentage prevalence at 31.6%, followed by Jharkhand (24.6%) and Assam (23.8%). When it comes to women, Assam ranked first. Around one in 10 adult women in Assam reported drinking alcohol followed by Jharkhand (8.2%), Chhattisgarh (7.4%) and Odisha (4.5%).9

The SBP is one of leading cause of death in decompensated liver disease which need prompt diagnosis and treatment. Studies have shown that causative bacteriological agent and empirical therapy guidelines are different in different part of world and even different in same region on different time periods. 10,11

As this part of country has the increasing toll of alcoholic liver disease, the number of cirrhosis patients attending the Silchar Medical College and Hospital (SMCH) also has been increasing every year. SMCH is the only tertiary care center of Southern Assam having catchment area of whole Barak Valley, Manipur, Tripura, Mizoram, India. However, information regarding the clinical profile and bacteriological profile of SBP and its impact in cirrhotic patients is still lacking in many aspects in this part of country.

This study was undertaken with an idea to evaluate the significance of serial AF analysis and other biochemical markers to determine the prognosis in cirrhotic patients in this region.

Study Design

Hospital-based, single centered, and observational study.

MATERIALS AND METHODS

The present study was carried out on patients admitted to medicine ward, SMCH, Silchar, Assam. Patients admitted for chronic liver disorder, and its complications were studied during the period from July 2013 to July 2014. Ultrasonography machine was used to diagnose cirrhosis of liver and ascites giving special reference to caudate lobe, portal vein, and spleen. All patients with hepatic cirrhosis, confirmed by ultrasound or liver biopsy, with ascites were included in the study.

SBP was diagnosed based on following criteria.

AF PMN leukocytes count >250 cells/mm³.

OR

AF total cells count >500 cells/mm³ with >50% neutrophils.

AND

Absence of a primary source of infection.

About 50 patients of SBP were studied, diagnosed by above described guidelines, result of the present study are compared with other studies as follows.

Investigations such as complete blood count, serum protein and fraction, serum bilirubin, alanine transaminase, aspartate transaminase, fasting blood glucose, lactate dehydrogenase, AF analysis including culture sensitivity and acid-fast bacilli staining, hepatitis B surface antigen (HbsAg), anti-hepatitis C virus, prothrombin time/international normalized ratio were done.

Serial AF Cell Count

In patients suspected of SBP, ascitic tap was done before the therapy (day 0), 48 h and then every 5th day after starting antibiotic treatment until the final outcome of the disease. This was referred to as serial AF cell count. The fluid was analyzed for total protein, sugar, and the total and differential leukocyte count.

Intravenous cefotaxime 2 g every 8 h were used as treatment of SBP.

RESULTS

Total 50 patients of age group >12 years, diagnosed as SBP were studied thoroughly with regards to both history and clinical examination. The majority of patients (72%) were alcoholics while 12% were HBsAg positive. In 16% of patient's etiology could not be determined and may be cryptogenic or nutritional (Tables 1 and 2).

Outcome was grave with 32% of mortality. Most of patients died due to SBP and hepatic-encephalopathy while some of the patients died due to hematemesis, hepatorenal syndrome and other complications of cirrhosis (Figure 1).

Mean total leukocyte count was above 13,700/mm³ in 32% of cases. Higher total leukocyte count was significantly associated with increased mortality.

Mean serum creatinine levels were 1.05 in survived group while it was 2.0 in patients who expired. Elevation of creatinine >2 was associated with increased mortality and was a poor prognostic marker.

Mean AF protein level was 1.29 g/dl with the majority of patients survived. AF protein levels <1.086 g/dl is poor prognostic sign. Mean serum albumin is 2.212 g/dl in

Table 1: Symptom distribution in SBP patients

Symptoms	N (%)
Abdominal distension	50 (100)
Abdominal pain	29 (58)
Fever	31 (62)
Jaundice	35 (70)
Altered sensorium	20 (40)
Hemetemesis/melena	17 (34)
Oliguria	20 (40)

SBP: Spontaneous bacterial peritonitis

Table 2: Signs distribution of patients

Sign	N (%)
Fever	31 (62)
Icterus	35 (70)
Asterixis	26 (52)
Hypotension	14 (28)
Abdominal tenderness	36 (72)
Ascites	50 (100)
Hepatomegaly	5 (10)
Splenomegaly	15 (30)

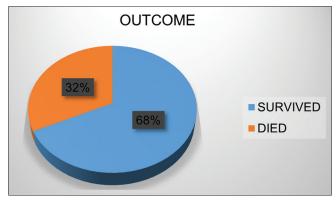


Figure 1: Outcome in the present study

survived while serum albumin <1.9 g/dl is poor prognostic sign (Table 3).

Table 3: Comparison of investigations between the patients of SBP and those who died of it

Investigations		P value			
	Surv	ived	Died		
	Mean	SD	Mean	SD	
Total count	10168.32	5751.82	13765	5588.75	0.044*
Total bilirubin	4.75	3.61	6.32	4.29	0.2151
SGOT	145.61	112.69	202.75	195.64	0.145
SGPT	182.44	211.3	205.75	197.39	0.706
Serum albumin	2.212	0.43	1.9	0.37	0.0134*
Serum creatinine	1.05	0.28	2	1.5	0.0231*
AF protein	1.29	0.33	1.086	0.3	0.04337*

^{*:} Significant p value is < 0.05, SD: Standard deviation, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, AF: Ascitic fluid, SBP: Spontaneous bacterial peritonitis

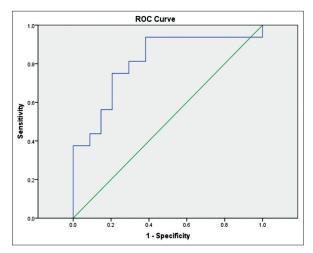


Figure 2: The receiver operating characteristics (ROC) for ascitic fluid cells at 0 h to predict the death, area under ROC curve: 0.809

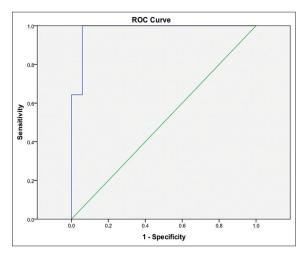


Figure 3: The receiver operating characteristics (ROC) for ascitic fluid cells at 48 h to predict the death, area under ROC curve: 0.979

AF Cell Count at the Time of Diagnosis and Serial AF Cell Count (Tables 4-9 and Figures 2-4)

Mean AF PMN cell count at the time of diagnosis was 492/mm³ in patients of SBP who survived while it was 1721/mm³ in patients who died. Thus, a high AF PMN count at the time of diagnosis was associated with poor prognosis.

AF PMN count of >600/mm³ at time of diagnosis indicated poor prognosis with sensitivity of 87.5% and specificity of 78%.

Table 4: Comparison of AF (PMN) cells count between the patients who died and survive

Investigations			Outcom	е	
	Survived Died			P value	
	Mean	SD	Mean	SD	
AF cells (PMNs) 0 h	492.76	215.96	1721.68	1740.25	0.013*
AF cells (PMNs) 48 h	311	76.42	1235.75	1336.72	0.014*
AF cells (PMNs) 5 days	80.82	77.91	447.88	585.85	0.025*

PMN: Polymorphonuclear, AF: Ascitic fluid, SD: Standard deviation, *=Significant p value is < 0.05

Table 5: % change in AF cell count in 48 h of therapy between the patients who died and survived

Investigations	Outcome (% change)		
	Survived	Died	
AF cells (PMNs) 48 h	36.78	28.22	
AF cells (PMNs) 5 days	83.60	65.97	

PMN: Polymorphonuclear, AF: Ascitic fluid

Table 6: Prediction of death based on AF cells (PMN) at 0 h

AF cells at 0 h	Sensitivity (%)	Specificity	Accuracy	LR+	LR-
>450	100	61.76	74	2.54	0
>500	87.5	61.76	70.00	2.22	0.2
>550	87.5	67.64	74	2.62	0.18
>600	87.50	75.52	78.00	3.30	0.17
>650	81.20	79.41	80.00	3.94	0.23
>750	68.75	85.28	80	4.67	0.36
>800	65.25	88.23	78.00	4.80	0.49

PMN: Polymorphonuclear, AF: Ascitic fluid

Table 7: Prediction of death based on AF cells (PMN) at 48 h

AF cells at	Sensitivity	Specificity	Accuracy
48 h cut-off	(%)	(%)	(%)
>200	87.50	3.00	30
>250	87.50	17.65	40
>300	87.50	52.94	44
>350	87.50	82.35	84
>450	87.50	94.15	88
>600	80.36	97.05	88

PMN: Polymorphonuclear, AF: Ascitic fluid

AF PMN count of >450/mm³ at 48 h predicted poor prognosis with sensitivity of 87.5% and 94.15%.

A fall in AF cell count from the time of diagnosis to, at 48 h was associated with good prognosis. Achievement of AF PMN cell count of <450/mm³ or >28% reduction at 48 h of treatment was associated with good outcome.

In present study, the 5th day data shows that ascitic PMN cell count <200 associated with good outcome.

AF Culture and Sensitivity (Figure 5)

AF culture did not show any growth in 56% of cases while 38% showed *Escherichia coli*, 8% showed *Klebsiella*, and 2% showed of *Peptostreptococcus*, and 4% showed *Staphylococcus aureus*. **8% patients had mixed flora in culture. Out of 22 cases which showed positive culture result, in 17 (77.27%) cases isolated organism showed sensitivity to cefotaxime (Figure 6).

Only 8% of patients had shown blood culture positive with 2% showing *Klebsiella*, 4% patients showing growth of *E. coli* and 2% *S. aureus*.

DISCUSSION

About 50 patients of SBP was studied, diagnosed by above described guidelines, result of present study are compared with other studies as follows:

Factors Predicting Mortality in SBP

Increased total leukocyte count, creatinine >2 mg/dl and low serum albumin <2.2 g/dl and low AF protein <1.086 g/dl was significantly associated with increased mortality in the present study. Increased serum bilirubin

Table 8: Prediction of death based on AF cells at 5th day

AF cells at 5 th day cut off value	Sensitivity (%)	Specificity (%)	Accuracy (%)
>50	100	0	37.5
>100	100	20	50
>150	100	65	78.13
>200	100	85	90.63
>250	75	100	90.63

AF: Ascitic fluid

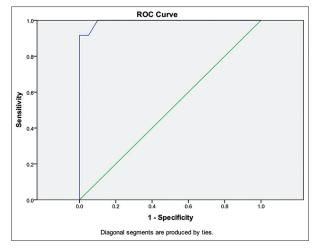


Figure 4: The receiver operating characteristics (ROC) for ascitic fluid cells at 5 days to predict the death, area under ROC curve: 0.994

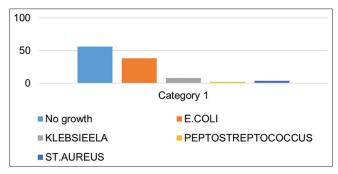


Figure 5: Ascitic fluid culture in present study

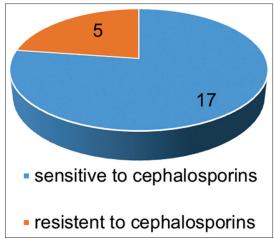


Figure 6: Sensitivity to cefotaxime to isolated organism

Table 9: Prediction	of death based on	AF cells (PMN)
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AF cells	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	Area under ROC
AF cells (PMNs) 0 h	>600	87.5	73.52	59.09	89.28	78	0.809
AF cells (PMNs) 48 h	>450	87.5	94.15	87	94.11	88	0.979
AF cells (PMNs) 5 days	>200	100	85	80	100	93.63	0.994

PMN: Polymorphonuclear, AF: Ascitic fluid, NPV: Negative predicative value, PPV: Positive predicative value, ROC: Receiver operating characteristics

above 6.32 mg/dl was also associated with increased mortality, but it was not statistically significant.

Even studies by Filik *et al.*¹² and Rawat *et al.* showed association of increased total count, increased bilirubin levels and high creatinine levels with mortality.

According to study by Filik *et al.* decreased serum albumin and AF protein was related to mortality similar to the present study but in study by Rawat *et al.* fails to find any such correlation.

Sort et al.¹³ 1999, Kamani et al.¹⁴ 2008 also concluded that increased creatinine and low serum albumin is poor prognostic factor.

AF protein plays an important role in developing SBP in these patients. Patients with AF protein <1 g/dl are frequently predisposed to SBP. In Runyon *et al.*¹⁵ series the patients with AF protein <1 g/dl were more predisposed to the development of SBP. In the series of Amarapurkar¹⁶ the mean AF protein was 0.78 ± 0.24 g/dl in patients of SBP.

In the present series the mean AF protein was 1.086 ± 0.3 g/dl indicating the role of low AF in developing SBP.

Low AF protein was significantly related to mortality (Table 10).

AF Biochemical Analysis (Table 11)

AF cell count

In the present series, the AF cell count at the time of diagnosis, as well as that done at 48 hours was significantly related to outcome. A very high cell count at the time of diagnosis was associated with increased mortality. The AF cell count done at 48 h was also related significantly to outcome. The % fall in AF cell count from 0 h to 48 h was also related significantly to mortality (28% in the present study which is comparable to previous studies).

According to study by Rawat *et al.* the AF cell count at the time of diagnosis was not significantly related to mortality. This may have been due to less number of patients studied. While the AF count done at 48 h was significantly related to outcome. The attainment of AF cell count of < 250/mm³ during the course of treatment was also associated with better outcome, which was consistent with the results of present study (Rawat, Bhatnagar¹7, Lady Harding College, New Delhi, Diamond APICON 2005). AF PMN count of >700/mm³ at time of diagnosis and > 450/mm³ at 48 h was associated with poor prognosis. A progressive fall in AF cell PMN count at 24, 48 h, and 5 days as compared to AF cell count at the time of diagnosis was associated with good prognosis. Achievement of AF PMN cells count

of <200/mm³ at 5 days of treatment was associated with good outcome (Krishnamurthy and Patil 2008)¹⁸. These all results are comparable to the present study.

A fall of AF cell count <250/mm³ can be used as a guide for duration of antibiotic therapy in treatment of SBP (Rawat *et al.*, Bhatnagar). In present study also patient who attained AF PMN cell count <200/mm³ had good outcome. Hence, AF PMN<200/mm³ can be used to guide the duration of therapy in SBP (Table 12).

Summary

- 1. Total leukocyte count >13,700/mm³, serum albumin <2.2 g/dl were associated with increased mortality and served as poor prognostic marker in SBP.
- An AF PMN count >600/mm³ at diagnosis, >450/mm³ at 48 h of treatment and >250/mm³ at the 5th day of treatment was associated with poor prognosis.
- 3. >28% reduction in AF cell PMN count after 48 h of treatment denoted successful antibiotic therapy and good prognosis.

Table 10: Comparison in prognostic factor in SBP in different studies

Study	Poor prognostic factors
Filik et al., Rawat et al.	1. High bilirubin
	2. High TLC
	High creatinine
Sort et al., Kamani et al.	1. High creatinine
	Low serum albumin
Present study	1. High TLC
·	2. High bilirubin
	3. High creatinine
	4. Low serum albumin
	Low AF protein
Agrawal et al.	1. Low AF protein

AF: Ascitic fluid, TLC: Thin-layer chromatography, SBP: Spontaneous bacterial peritonitis

Table 11: Comparison of value of AF protein predisposing SBP

Study	Runyon et al.	Amarapurkar et al.	Present study
Value of AF protein predisposing SBP (g/dl)	<1	<1	<1.08

SBP: Spontaneous bacterial peritonitis, AF: Ascitic fluid

Table 12: Comparison of serial AF count (PMN) for poor prognosis in SBP studies

Study PMN count	Rawat et al.	Krishnamurthy et al.	Present study
0 h	-	>700	>600
48 h	>250	>450	>450
Further analysis	>250	>200	>200

SBP: Spontaneous bacterial peritonitis, PMN: Polymorphonuclear, AF: Ascitic fluid

4. In the present series, the mean AF protein was 1.086 g/dl indicating low AF protein being the risk factor in developing SBP.

Drawback of the present study is small sample size. Further study, with big sample size will definitely give better guidance in these aspects.

CONCLUSION

All patients of cirrhosis of the liver with ascites should be screened for SBP as no specific symptoms can define the SBP. As many patients may be asymptomatic. SBP carries a very high mortality and should be treated aggressively. Increased total leukocyte count, low serum albumin, increased creatinine levels and low AF protein is associated with poor prognosis. Once SBP is diagnosed, serial AF cell count is helpful in predicting prognosis and can be used to monitor treatment.

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