

## Research Article



## Ascitic Fluid Calprotectin - Novel Marker for Detection of Spontaneous Bacterial Peritonitis in Cirrhotic Patients

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### ABSTRACT

Spontaneous bacterial peritonitis (SBP) is characterized by infection of ascitic fluid (AF) in cirrhotic patients, in absence of inflammatory lesions and surgically treatable conditions like acute appendicitis, kidney abscess etc. The said condition is very fatal, as high mortality rate is associated with it. Early clinical detection, confirmative laboratory diagnosis and timely management are very much needed, to save life of SBP patients. In this study, evaluation of ascitic fluid calprotectin for diagnosis of SBP in cirrhotic patients was studied. Total 50 cirrhotic ascitic fluid cases were included in the study. All the cases were divided into two groups: SBP and non-SBP. The 28 cases were included in SBP group, as the SBP was confirmed by increased polymorphonuclear leukocytes (PMNLs) count in ascitic fluid ( $\geq 250$  cells/mm<sup>3</sup>) along with microbiologically positive ascitic fluid bacterial culture. In the remaining 22 non-SBP cases, PMNLs count in ascitic fluid was less than 250 cells/mm<sup>3</sup> and ascitic fluid bacterial culture was negative. For all the cases, calprotectin levels ascitic fluid were measured using enzyme-linked immunosorbent assay (ELISA) method. Ascitic fluid calprotectin cut off of 515 ng/ml level showed a sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of 96.4 %, 90.9 %, 94.0 %, 93.1 % and 95.2 % respectively in diagnosis of SBP. The present study concluded that ascitic fluid calprotectin is valuable marker, for rapid diagnosis of SBP, in cirrhotic patient ascitic fluid.

**Keywords:** Ascitic fluid calprotectin, SBP, PMNLs.

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There are two gold standard laboratory tests to confirm SBP – ascitic fluid PMNLs  $>250/\text{mm}^3$  and microbiological culture positivity for ascitic fluid bacterial cultures. However, culture positivity is not seen in all SBP cases.<sup>6</sup> The ascitic fluid manual microscopic PMNLs counting method is simple, accurate method, but it is time consuming. Also, there is diagnostic accuracy limitation, for ascitic fluid automated cell counters PMNLs counting method.<sup>7</sup>

For early detection of SBP, several studies were conducted for ascitic fluid level of, various markers such as- C-reactive protein (CRP),<sup>8</sup> lactoferrin,<sup>9</sup> homocysteine<sup>10</sup> and calprotectin<sup>11</sup> etc. The present study was aimed to assess the accuracy of ascitic fluid calprotectin as a diagnostic marker of SBP.

### MATERIALS AND METHODS

The study was carried out in tertiary care hospital pathology laboratory for 7 months period from January 2022 to July 2022. The ascitic fluid samples with patient having clinical features of liver cirrhosis with ascites were included in this study. All the patients were divided into two groups- SBP group and Non-SBP group. The SBP group included patients with cirrhotic ascites and SBP diagnosis confirmed by Ascitic fluid PMNL count  $> 250$  cells/mm<sup>3</sup> along with positive or negative Ascitic fluid bacterial culture. Non-SBP group labeled patients were suffering with cirrhotic ascites and ascitic fluid PMNL count were  $< 250$  cells/mm<sup>3</sup> and ascitic fluid bacterial cultures were negative. The following patients were excluded from the study- cirrhotic patients on antibiotics since one week,

### INTRODUCTION

Ascitic fluid formation is one of the common complications in liver cirrhosis patients. The liver cirrhotic patients have generally impaired immune system and are hence prone for infection. SBP is ascitic fluid infection, in previously sterile ascitic fluid in cirrhotic patients. In 30 % cases, the infection is spread from gut bacteria.<sup>1</sup> The inflammatory lesion and abscess in the abdominal cavity are the exclusion criteria, to label the case as SBP case.<sup>2,3</sup>

The patients of SBP presents with clinical features of abdominal pain and fever, hepatic encephalopathy, liver or kidney function impairment, gastrointestinal tract bleed etc. Around 10% cases are asymptomatic in SBP. Hence, diagnostic paracentesis become mandatory for all ascitic fluid cases.<sup>1</sup> The incidence of hospital admission due to SBP ranges from 10 % to 30 %. The mortality rate for SBP ranges from 40 to 70% and it may decrease with rapid timely management of SBP.<sup>4, 5</sup> Hence, simple, rapid, accurate laboratory method to detect SBP is very much needed.



history of abdominal surgery in last three months, history of inflammatory bowel disease, history of hematological and autoimmune disorders and presence of intra-abdominal infected lesions.

Ascitic fluid calprotectin was measured by ELISA method. Data were statistically analyzed using SPSS software. The receiver operating characteristic (ROC) curve analysis was done and used to find out, the cut off value of the calprotectin, for detection of SBP. Ascitic fluid calprotectin level accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was studied for diagnosis of SBP.

**Table 2:** Validity of ascitic fluid calprotectin in detection of SBP

Variables	Cut off point	AUC	Sensitivity	Specificity	Accuracy	PPV	NPV
Ascitic fluid calprotectin (ng/ml)	515	0.967	96.4 %	90.9 %	94.0 %	93.1 %	95.2 %

AUC - area under curve; NPV - negative predictive value; PPV- positive predictive value

The optimum ascitic fluid calprotectin level cut off point, for the diagnosis of SBP, from the ROC curve analysis, was 515 ng/ml with a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of 96.4 %, 90.9 %, 93.1 %, 95.2 % and 94.0 % respectively, with an AUC of 1.000 (P<0.001). (Table 2)

Calprotectin is acute-phase inflammatory protein. It is a calcium and zinc-binding protein from S100 protein family. It is detected almost exclusively in neutrophils. As a reaction to inflammatory conditions, it is released in circulation and its concentration in the body fluids (serum, urine, saliva, feces, ascitic fluid etc.) is directly proportional to the influx of neutrophils.<sup>7,12,13</sup> Calprotectin also has immunomodulatory and antimicrobial effects.<sup>14</sup> Calprotectin is mainly expressed by neutrophils and macrophages, and rarely by lymphocytes.<sup>15</sup> Jansen PL et al observed that diuretic therapy may increase the ascitic TLC count, but does not cause changes in the PMNLs count.<sup>16</sup> Hence, ascitic fluid calprotectin reliable marker to predict SBP in the high PMNLs count cases and thus acts as a useful marker for diagnosis of SBP.

The high levels of calprotectin in ascitic fluids of cirrhotic patients give high possibility of mortality in such cases.<sup>14</sup> In liver cirrhosis patients, the higher fecal calprotectin levels were observed; hence, the SBP diagnosis was done with fecal calprotectin in the past.<sup>17</sup>

The ELISA and point-of-care (POC) lateral flow assay can be utilized for measurement of ascitic calprotectin. In view of accuracy, ELISA method is generally preferred over other methods for ascitic calprotectin measurement and hence, ELISA method was followed in this study.<sup>7</sup>

In the many studies in the past, the optimum cut off for ascitic fluid calprotectin, were statistically obtained, to obtain best accuracy for detection of ascitic fluid SBP. In the present study, with ROC analysis; the ascitic fluid calprotectin cut off of 515 ng/ml, gave accuracy of 94 %.

## RESULTS AND DISCUSSION

In the 50 ascitic fluid samples cases, 36 were male and 14 were female patients ranging in age from 22 to 57 years. As per etiology, 28 were SBP cases and remaining 22 were Non- SBP cases. (Table 1)

**Table 1:** Etiological distribution of ascitic fluids

Ascitic Fluid	Number of cases (n)
SBP Cases	28
Non- SBP cases	22
Total	50

Abdel-Razik A. et al found that with a cutoff 445 ng/ml, ascitic fluid calprotectin had 95.4 % sensitivity and 85.2 % specificity for detecting SBP.<sup>18</sup> Fernandes SR et al showed that, with ascitic fluid calprotectin cutoff of 157 ng /ml, there was 87.8% sensitivity and 97.9% specificity for detecting SBP.<sup>11</sup> Burri E et al reported that ascitic calprotectin (cut off value - 630 ng /ml) had 94.8% sensitivity, 89.2% specificity for diagnosing SBP.<sup>7</sup> Selim FO et al. showed that the 90.91% sensitivity, 95.45% specificity of ascitic fluid calprotectin was obtained with cut-off value of 620 ng /ml for diagnosing SBP.<sup>19</sup>

The present study had limitation of small sample size. More studies with larger sample size are needed, for establishing optimum cut- off of ascitic fluid calprotectin, for diagnosing SBP.

## CONCLUSION

The ascitic fluid calprotectin (cut off 515 ng /ml) is a reliable marker with excellent statistical accuracy, for conformation of SBP diagnosis in cirrhotic ascites patients. With the results obtained in the present study, it is recommended that in addition to ascitic fluid PMNLs count, ascitic fluid calprotectin can be used as a marker in the diagnosis of SBP. More studies worldwide are needed for standardization and agreement on cut-off value ascitic fluid calprotectin for detection of SBP.

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