Introduction

Ascites is the most common complication of liver cirrhosis and it develops as a consequence of portal hypertension and splanchnic vasodilatation [1].

Spontaneous bacterial peritonitis (SBP) is one of the main infectious complications of cirrhosis and occurs in 8–30% of hospitalized patients with ascites [2]. The 1-year probability of development of the first episode of SBP in end-stage liver disease patients with ascites is ∼10% [3].

It is defined as an infection of the previously sterile ascitic fluid in the absence of a visceral perforation and in the absence of an intra-abdominal inflammatory focus such as abscess, acute pancreatitis, or cholecystitis [4]. Also, the number of polymorph nuclear leukocytes (PMN) from the ascetic fluid obtained by paracentesis must exceed 250 cells/mm³ and only one germ must be isolated in the bacteriological cultures [5].

In the setting of cirrhosis, several abnormalities have been described in the humoral and cellular bactericidal systems including decreased serum levels of complement factors, impaired chemotaxis, poor function of phagocytes activity of neutrophils, and decreased function of Fc-g-receptors in macrophages [6].

One of the earliest signs of infection is the acute-phase response. Acute-phase response may include the changes in the concentration of many plasma proteins, known as the acute-phase proteins that are synthesized almost exclusively in the liver; most are glycosylated [7].

Inflamatory markers, such as C-reactive protein (CRP) and procalcitonin, and white blood cells (WBCs) could be used easily for the diagnosis and follow-up of several morbidities [8].

A 116-amino acid prohormone of calcitonin, PCT, is normally synthesized in the C cells of the thyroid gland. Other sources of PCT include liver and inflammatory
The aim of this study was to measure and compare the levels of PCT, high-sensitive CRP (hsCRP), C3, and complement 4 (C4) in patients with SBP and patients without SBP and to assess their role in detecting patients at high risk of developing SBP.

Patients and methods
This case–control study was carried out on 30 patients; 10 of these patients had cirrhotic ascites and had been admitted with SBP (cases) and 20 patients had cirrhotic ascites with no existing evidence of SBP (controls). Cases were recruited from among the attendants of the Tropical and Internal Medicine Departments of Al Zahra University Hospital, Cairo, Egypt, from January 2013 to August 2013.

Inclusion criteria for cases
Adult patients who presented with cirrhotic ascites and were admitted with SBP diagnosed on the basis of clinical (fever, abdominal tenderness, and prehepatic coma manifestations) and laboratory diagnostic criteria for SBP: ascetic fluid PMNs greater than 250/mm³ and culture positive (culture-positive SBP), ascetic fluid PMNs greater than 250/mm³ and culture negative (culture-negative SBP), and ascitic fluid PMNs less than 250/mm³ and culture positive [12].

Inclusion criteria for controls
Adult patients who presented with cirrhotic ascites and were admitted with no existing evidence of SBP assessed by clinical and laboratory evaluation.

Exclusion criteria for cases and controls
Patients with secondary causes of intra-abdominal sepsis (ascitic fluid protein >2.5 g/dl), patients with tuberculous peritonitis, patients with right-sided heart failure and diabetes mellitus, patients with renal impairment, rheumatoid arthritis, systemic lupus erythematosus, hepatocellular carcinoma, and grade IV encephalopathy were excluded.

After explaining the purpose of the study and obtaining the consents of both cases and controls, data were collected through a personal interview.

The studied cases were subjected to the following:

Clinical evaluation
Full assessment of history was performed, with a special focus on recent onset of fever, abdominal pain, nausea, vomiting, and diarrhea, symptoms suggestive of hepatic encephalopathy, symptoms suggestive of associated infection anywhere in the body, especially urinary tract infection, previous episodes of ascetic fluid infection and history of prophylaxis, history of iatrogenic procedures (intravenous catheters — urinary catheters), lack of response to diuretics, history of diagnostic or therapeutic paracentesis, and history of gastrointestinal bleeding.

Imaging studies
Pelvic-abdominal ultrasonography examination was performed, with a special focus on signs of hepatic decompensation in the form of signs of encephalopathy, signs of hypoalbuminemia (white nails, muscle wasting, bilateral lower limb edema, and ascites), and abdominal tenderness.

Laboratory investigations
Routine laboratory investigations
Blood sample collection: after overnight fasting, 6 ml of venous blood samples were collected by venipuncture under aseptic conditions from each patient. They were divided as follows:

(1) A volume of 2 ml was placed in a vacutainer tube containing EDTA for complete blood picture (CBC) using an automated cell counter model SysmexKx N 21, (Sysmex Corporation, Kobe, Hyogo, Japan) differential leukocyte count, and erythrocyte sedimentation rate measurement.

(2) A volume of 2.7 ml was placed in a vacutainer tube containing 0.3 ml of 3.8% sodium citrate for the measurement of prothrombin time using Sysmex A500 (Sysmex Corporation, Kobe, Hyogo, Japan).

(3) The remaining was placed in a tube with no anticoagulant and centrifuged within 30 min of collection at 4000 rpm for 10 min and the serum from all blood samples was separated and used for measurement of complement 3 (C3) and complement 4 (C4) concentrations using ELISA method.
for fasting blood sugar, liver, and kidney function tests using a Cobas C-311 auto analyzer (Roche Diagnostics, Indianapolis, Indiana, USA).

The remaining serum was aliquoted and stored at -20°C for subsequent detection of serum PCT, hs-CRP, C3, and C4.

Ascitic fluid collection: ascitic fluid samples were collected by diagnostic abdominal paracentesis from the patients under complete aseptic conditions. The abdominal wall in the right lower quadrant two fingers breadths cephalic and two fingers breadths medial to the anterior superior iliac spine was the preferred site in most cases [13]. Each sample was subjected to the following:

1. Direct inoculation of ~10 ml into anaerobic and aerobic BactAlert blood culture bottles. These bottles were then placed in an automated BacT/Alert 3D culture system. Bottle incubation and subsequent testing were carried out according to the manufacturer's protocol (bioMerieux, Durham, North Carolina, USA).

2. Ten to 20 ml was placed in a sterile container for direct microscopic examination, gram-stained film, and culture on routine laboratory media including blood, MacConkey, Mannitol salt agar plates, and thioglycollate broth. Culture-positive samples were identified using Gram stain, biochemical reactions, and an automated micro-dilution method using the Micro Scan Walk-Away system (Siemens Healthcare Diagnostics Inc., West Sacramento, California, USA). All culture and identification media were supplied by Oxoid (Basingstoke UK).

3. Approximately 3 ml was placed in an EDTA tube for estimation of the total cell count and PMN count using an automated cell counter model SysmexKx N 21.

4. Approximately 10 ml was placed in a clean centrifuge tube and centrifuged at 4000 rpm for 10 min. Ascitic fluid supernatants were used for assay of total proteins, glucose, lactate dehydrogenate levels, and ascitic fluid albumin using a Cobas C-311 auto analyzer (Roche Diagnostics) and the serum–ascites albumin gradient was calculated. The remaining supernatants were aliquoted and stored at -20°C for the subsequent detection of serum PCT, hs-CRP, C3, and C4.

Specific investigations

Serum and ascitic fluid levels of PCT, hs-CRP, C3, and C4 were measured by an enzyme-linked immunosorbent assay technique using an SLT Spectra ELISA reader (SLT Lab Instruments, Salzburg, Austria).

Statistical methods

Statistical Package for the Social Sciences (SPSS) TM, Version 17, produced by IBM SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, United States.

1. Descriptive statistics: mean and SD were calculated to measure the central tendency and dispersion of quantitative data.

2. Analytic statistics: comparison of groups was carried out using the Kruskal–Wallis test for comparison of nonparametric data between more than two groups. The correlation coefficient was calculated to determine the association between two variables. The level of significance was considered at a $P$ value of less than 0.05 and insignificance at a $P$ value greater than 0.05.

Results

The present study was carried out on 30 selected patients who presented with cirrhotic ascites; they were classified into two groups. Group I included 10 patients with cirrhotic ascites with SBP (cases). According to the Child–Pugh scoring system for cirrhosis [18], six patients were Child C (60%) and four patients were Child B (40%). Group II included 20 patients with cirrhotic ascites without SBP (controls). Thirteen patients were Child B (65%) and seven patients were Child C (35%). All patients had posthepatitis C cirrhosis.

Table 1 shows the demographic data of the groups studied. Group I included five women (50%) and five men (50%); their ages ranged from 45 to 77 years, with mean ± SD 56.7 ± 9.42. Group II included eight women (40%) and 12 men (60%); their ages ranged from 50 to 69 years, with mean ± SD 54.5 ± 4.95. There was no statistically significant difference in age and sex between the groups studied.

Table 2 shows the comparison between the two groups in the CBC and liver function tests. For the CBC,
the mean ± SD of WBCs count was 8.79 ± 5.536 and 5.85 ± 4.069 in group I and group II, respectively. There was an increase in the total leukocyte count in group I in comparison with group II, with no statistically significant difference.

For the RBCs count, the mean ± SD was 3.42 ± 0.5308 and 3.56 ± 0.7223 in group I and group II, respectively. There was no statistically significant difference between the groups studied.

For the hemoglobin level, the mean ± SD was 10.73 ± 1.3622 and 10.74 ± 1.6423 in group I and group II, respectively. There was no statistically significant difference between the groups studied.

For the platelet count, the mean ± SD was 88.5 ± 73.188 and 110.7 ± 62.483 in group I and group II, respectively. There was a decrease in the platelet count in group I in comparison with group II, with no statistically significant difference.

For the liver function test, the mean ± SD of the total bilirubin level was 3.56 ± 2.278 and 2.12 ± 1.1242 in group I and group II, respectively, with an increase in the serum level of total bilirubin in group I in comparison with group II, with a statistically significant difference between the groups studied \((P < 0.05)\).

For the serum albumin level, the mean ± SD was 2.49 ± 0.4254 and 2.44 ± 0.5113 in group I and group II, respectively. There was no statistically significant difference between the groups studied.

For prothrombin time, the mean ± SD was 22.33 ± 8.234 and 18.03 ± 3.548 in group I and group II, respectively. There was a statistically significant difference between the groups studied \((P < 0.05)\).

Table 3 shows the serum levels of C3, C4, hsCRP, and PCT. For the serum C3 level, the mean ± SD was 3.38 ± 2.12 and 2.04 ± 1.98 in group I and group II, respectively. There was no statistically significant difference between the groups studied. For the serum C4 level, the mean ± SD was 0.36 ± 0.25 and 0.36 ± 0.29 in group I and group II, respectively. There was no statistically significant difference between the groups studied. For serum hs-CRP, the mean ± SD was 18.76 ± 6.37 and 16.80 ± 5.97 in group I and group II, respectively. There was no statistically significant difference between the groups studied Figure 1.

For the serum PCT level, the mean ± SD was 136.79 ± 58.14 and 147.78 ± 58.65 in group I and group II, respectively. There was no statistically significant difference between the groups studied Fig. 2.

Table 4 shows a comparison of the studied groups in ascitic fluid levels of C3, C4, hs-CRP, and PCT. For the ascitic fluid C3 level, the mean ± SD of C3 was 0.21 ± 0.14 and 0.46 ± 1.01 in group I and group II, respectively. There was no statistically significant difference between the groups studied. For the ascitic fluid C4 level, the mean ± SD was 1.84 ± 1.69 and 2.07 ± 1.93 in group I and group II, respectively. There was no statistically

<table>
<thead>
<tr>
<th>Variables</th>
<th>n (%)</th>
<th>χ²-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>5 (50.00)</td>
<td>8 (40.00)</td>
</tr>
<tr>
<td>Males</td>
<td>5 (50.00)</td>
<td>12 (60.00)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>56.7 ± 9.42</td>
<td>54.5 ± 4.95</td>
</tr>
<tr>
<td>Range</td>
<td>45–77</td>
<td>50–69</td>
</tr>
</tbody>
</table>

SBP, spontaneous bacterial peritonitis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SBP</th>
<th>Non-SBP</th>
<th>Independent t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>8.79 ± 5.536</td>
<td>5.85 ± 4.069</td>
<td>1.653 0.109</td>
</tr>
<tr>
<td>RBC</td>
<td>3.42 ± 0.5308</td>
<td>3.56 ± 0.7223</td>
<td>-0.527 0.603</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>10.73 ± 1.3622</td>
<td>10.74 ± 1.6423</td>
<td>-0.017 0.987</td>
</tr>
<tr>
<td>Platelets</td>
<td>88.5 ± 73.188</td>
<td>110.7 ± 62.483</td>
<td>-0.867 0.393</td>
</tr>
<tr>
<td>Serum bilirubin</td>
<td>3.56 ± 2.278</td>
<td>2.12 ± 1.1242</td>
<td>2.34 0.027*</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>2.49 ± 0.4254</td>
<td>2.44 ± 0.5113</td>
<td>0.266 0.792</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>22.33 ± 8.234</td>
<td>18.03 ± 3.548</td>
<td>2.016 0.054*</td>
</tr>
</tbody>
</table>

RBC, red blood cell; SBP, spontaneous bacterial peritonitis; WBC, white blood cell; *Significance difference \((P < 0.05)\).

<table>
<thead>
<tr>
<th>Serum</th>
<th>SBP</th>
<th>Non-SBP</th>
<th>Independent t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>3.38 ± 2.12</td>
<td>2.04 ± 1.98</td>
<td>1.713 0.098</td>
</tr>
<tr>
<td>C4</td>
<td>0.36 ± 0.25</td>
<td>0.36 ± 0.29</td>
<td>-0.011 0.991</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>18.76 ± 6.37</td>
<td>16.80 ± 5.97</td>
<td>0.830 0.414</td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>136.79 ± 58.14</td>
<td>147.78 ± 58.65</td>
<td>-0.485 0.631</td>
</tr>
</tbody>
</table>

C3, complement 3; C4, complement 4; hs-CRP, high-sensitive C-reactive protein; SBP, spontaneous bacterial peritonitis.

<table>
<thead>
<tr>
<th>Ascitic</th>
<th>SBP</th>
<th>Non-SBP</th>
<th>Independent t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>0.21 ± 0.14</td>
<td>0.46 ± 1.01</td>
<td>-0.764 0.452</td>
</tr>
<tr>
<td>C4</td>
<td>1.84 ± 1.69</td>
<td>2.07 ± 1.93</td>
<td>-0.319 0.752</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>1.96 ± 1.15</td>
<td>2.98 ± 5.90</td>
<td>-0.541 0.593</td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>162.43 ± 82.51</td>
<td>180.51 ± 93.70</td>
<td>-0.517 0.609</td>
</tr>
</tbody>
</table>

C3, complement 3; C4, complement 4; hs-CRP, high-sensitive C-reactive protein; SBP, spontaneous bacterial peritonitis.
significant difference between the groups studied. For the ascitic fluid hsCRP level, the mean ± SD was 1.96 ± 1.15 and 2.98 ± 5.90 in group I and group II, respectively. There was no statistically significant difference between the groups studied. For the ascitic fluid PCT level, the mean ± SD was 162.43 ± 82.51 and 180.51 ± 93.70 in group I and group II, respectively. There was no statistically significant difference between the groups studied Figure 4.

Table 5 shows a comparison of the groups studied in neutrophil counts in the ascitic fluid. The median number was 700/mm³, with a range of 400–1800, and 100/mm³, with a range of 50–150, in group I and group II, respectively. There was a highly statistically significant increase in group I in comparison with group II (P=0.00) Figure 5.

Table 6 shows the cut-off value, sensitivity, specificity, positive predictive value, and negative predictive value for ascitic fluid PMN count using the receiver operating characteristic curves.

Discussion

Our study was carried out on 30 patients who presented with cirrhotic ascites; 10 of these patients had been diagnosed with SBP by ascitic fluid PMNs greater than 250/mm³ and culture negative (culture-negative SBP) and 20 patients did not have SBP. All of them

Table 5 Comparison between the two studied groups in neutrophil counts in the ascitic fluid

<table>
<thead>
<tr>
<th></th>
<th>Median (IQR)</th>
<th>Mann-Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>SBP: 700 (400–1800)</td>
<td>Non-SBP: 100 (50–150)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; SBP, spontaneous bacterial peritonitis.
had posthepatitis C cirrhosis. Early detection of SBP is very valuable for patients as the mortality rate among untreated patients is around 50% [19]. The diagnosis of SBP was established by ascitic fluid analysis. The most common marker of infection is an ascitic fluid PMN cell count of 250/mm³ or higher [20] and in a meta-analysis, the negative likelihood ratio for SBP if the PMN cell count was greater than 250/mm³ was 0.2 [21]. These data were in agreement with our results as we found that an ascitic fluid PMN cell count higher than 200/mm³ had a sensitivity and specificity of 100% in the diagnosis of SBP patients.

Although ascitic fluid culture is an important diagnostic test, the use of culture results for the diagnosis of SBP would delay therapy, and ascitic fluid culture is negative in as many as 60% of patients [20]. These findings were in agreement with our results as we found that an ascitic fluid PMN cell count higher than 200/mm³ had a sensitivity and specificity of 100% in the diagnosis of SBP patients.

Our results of C3 in the serum of cirrhotic patients of both groups, those with SBP and those without SBP, were high, with no significant difference between two groups; also, C3 in ascitic fluid of patients showed a low normal level in both groups, with a greater decrease in the serum of patients without SBP, whereas C4 in the serum was within the normal level in both groups and higher in ascitic fluid in patients without SBP than in those with SBP, but with no significant statistical difference.

These results were in agreement with those of Rabinovitz et al. [23], who carried out their study on 14 patients with end-stage liver disease and ascites, and the diagnosis of SBP was made by a positive ascitic fluid culture (three patients) and/or an ascitic fluid neutrophil count greater than 250 cells/mm³ (all patients); they concluded that serum immunoglobulin and complement levels and the cell-mediated immune system activity were similar in patients with advanced liver disease who develop and do not develop SBP. Thus, these parameters cannot be used as predictors for the development of SBP.

However, these results are not in agreement with the findings of Kamal et al. [24], who carried out their study on 45 cirrhotic patients; 25 of these patients had SBP. They found a low level of ascitic fluid C3 in cirrhotic patients with SBP compared with patients without SBP, probably because C3 plays an important role in the local defense of ascitic fluid.

The explanation for the low level of C3 and C4 in cirrhotic patients may be complement consumption or reduced production because of a decrease in the number of functioning hepatocytes. As the liver is the major site of synthesis of most of the complement components, the low serum complement level has been proposed to be induced by the defective synthesis of the components [25].

Also, in the present study, we found an increase in the level of hsCRP in the serum of both groups, with no significant difference between them, but a normal level of hs-CRP in the ascitic fluid in both groups. Our study found an increase in the PCT level in patients without SBP compared with those with SBP, with no significant difference, whereas the PCT level in ascitic fluid was higher in the SBP group compared with the group without SBP, but with no significant difference.

These results were not in agreement with those obtained by Yuan et al. [26], who carried out their study on 42 patients with chronic hepatitis B liver disease with SBP and another 42 patients without SBP. The concentrations of PCT and hs-CRP and the WBC count in the serum were significantly higher in the chronic severe hepatitis B patients with SBP than in those without SBP. Furthermore, there were significant correlations between PCT and other inflammatory markers such as the WBC count and hs-CRP. However, PCT and hs-CRP concentrations were found to be better than the WBC count for prediction of chronic severe hepatitis B with SBP, and the accuracy of the PCT concentration was not significantly different from that for the hs-CRP concentration.
Spahr et al. [27] carried out a study on 20 patients with cirrhosis; 10 of these patients had SBP and another 10 patients did not have SBP. They measured the plasma and ascitic fluid levels of PCT, hsCRP, and interleukin-6. PCT level in plasma, but not in ascitic fluid, was significantly higher in patients with SBP compared with the controls. Interleukin-6 levels in ascitic fluid were similar between groups. hsCRP concentrations were higher both in plasma and in ascitic fluid of patients with SBP compared with the controls. Thus, in SBP, the measurement of PCT is not an accurate diagnostic test, possibly because of the absence of systemic inflammatory response syndrome in this condition. In addition, the diagnostic value of hsCRP is limited by the wide overlap between values; these results are in agreement with our result.

**Conclusion**

C3 and C4, hsCRP, and PCT serum and ascitic fluid levels are not accurate markers for the diagnosis of SBP, whereas an ascitic fluid polymorph leukocyte count higher than 200/mm³ is a rapid, sensitive, and specific test for the diagnosis of SBP.

**Acknowledgements**

**Conflicts of interest**

There are no conflicts of interest.

**References**