Calprotectin Measurement in Ascitic Fluid: A New Test for the Rapid Diagnosis of Spontaneous Bacterial Peritonitis

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Abstract

Introduction: Spontaneous bacterial peritonitis (SBP) is diagnosed by the presence of ≥250 polymorph nuclear cells (PMN) per milliliter (ml) in ascitic fluid. However, the procedure is time-consuming, operator-dependent, and not always readily available. Calprotectin is a cytosolic protein in neutrophile leukocytes that is released in response to inflammatory processes. In the GI tract calprotectin in feces is considered a valid marker of intestinal inflammation.

Aims and Methods: The study aimed to assess the role of calprotectin concentrations in ascites for the diagnosis of SBP. Forty patients [mean age 50±2 years, 10 (25%) females] with HCV liver cirrhosis. Who were referred for paracentesis were included in this study; the following investigations were performed: Serum-ascites albumin gradient, ascitic PMN cell count (microscopic hand-count), bacterial cultures and cytological analysis. In addition, ascitic calprotectin was measured using an enzyme-linked immunosorbent assay (ELISA).

Results: Ascitic calprotectin values measured by ELISA correlated well with PMN cell count (p-value=0.001, rho—F0.729). Calprotectin levels of patients with PMN ≥250/m1 (N=8, 20%) were higher than in patients with PMN <250/ml (N=32, 80%). Ascitic calprotectin values correlated well with albumin level (p-value=0.001, rho=−0.716).

Conclusion: Measurement of calprotectin in ascites might be a valuable surrogate marker for PMN cell count. It might be valuable for the rapid diagnosis of SBP.

Key Words: Calprotectin — Ascites — Spontaneous bacterial peritonitis.

Introduction

SBP is the infection of the ascitic fluid that occurs in the absence of a visceral perforation and in the absence of an intra-abdominal inflammatory focus such as abscess, acute pancreatitis or cholecystitis. For SBP diagnosis, the number of polymophonuclear leukocytes (PMN) from the ascitic fluid obtained by paracentesis must exceed 250 cells/mm³ and from bacteriological cultures only one germ must be isolated (5-7). Because SBP is in most cases a monomicrobial infection, the presence of more microorganisms in the culture (>1), must raise the suspicion of secondary peritonitis [1].

Calprotectin is a 36-kDa calcium and zinc binding protein and constitutes approximately 60% of soluble cytosol proteins in human neutrophil granulocytes [2]. It comprises two heavy chains of 14 kDa and one light chain of 8 kDa. This protein has also been called MRP8, MRP14,2 cystic fibrosis-associated antigen [3] calgranulin [4] and S100 [5]. Finally, its name was changed to reflect its calcium binding and protective properties [6].

The name calprotectin has not met with uniform agreement as it actually refers to a heteromeric complex of two subunits, S100A8 and S100A9. It has been suggested that these may be differentially regulated in disease states [7] but free subunits are not found in biological samples unless the protein is heated in the presence of detergents to dissociate the non-covalent bonds between them. Calprotectin is a multipotent biologically active molecule [8]. It has been suggested that calprotectin plays an important role in myeloid cell metabolism [9]. When externalized to cells, calprotectin has immunomodulatory [10] and antimicrobial effects [11].

Ascitic fluid calprotectin levels are significantly elevated in malignant ascites and spontaneous bacterial peritonitis, compared with ascites secondary to alcoholic liver disease and viral hepatitis. High ascitic calprotectin levels in cirrhotic patients appear to be predictive of high mortality [12].

Aim of the study: To evaluate the possible role of calprotectin concentrations in ascitic fluid for the diagnosis of SBP.
Material and Methods

This study was conducted at the Tropical Medicine and Gastroenterology Department, Qena Faculty of Medicine from February 2012 — May 2012. Forty patients with hepatitis C related cirrhosis with ascites admitted to the department were recruited. Exclusion criteria were all causes of ascites other than cirrhotic ascites.

*All patients were subjected to:*  
- Thorough history taking.  
- Complete physical examination.  
- Laboratory investigations including: (AST, ALT, bilirubin, serum albumin, prothrombin time and concentration and complete blood count).  
- Ascitic fluid study was done for diagnosis of SBP and determination of ascitic albumin level.  
- Ascitic calprotectin were detected by simple ELISA technique using polyclonal antibodies for capture and affinity purified. The calprotectin test was supplied by BioActiva Diagnostica GmbH, Germany.  
- Abdominal ultrasonography: For assessment of liver and spleen size, portal and splenic vein diameter and the degree of ascites.

Results

This study included 40 patients with liver cirrhosis and ascites with their mean age 50±2 years. Eight (20%) of them showed SBP and 32 (80%) patients without SBP. Demographic and laboratory data for patients are illustrated in Table (1).

![Fig. (1): Ascitic calprotectin and PMN cell count in patients with and without SBP.](image)

![Fig. (2): Correlation between ascitic calprotectin and PMN cell count (p-value=0.001, rho=W.729).](image)

**Table (1): Demographic and laboratory data for patients.**

<table>
<thead>
<tr>
<th>Patients (n=40)</th>
<th>Value</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>50±2</td>
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**Gender:**  
- Male, n (%) 30 (75%)  
- Female, n (%) 10 (25%)

**SBP:**  
- Yes, n (%) 8 (20%)  
- Bilirubin (mg/dl) 2.87±1.102  
- Albumin (g/dl) 2.29±0.55  
- ALT (U/L) 47.12±30.50  
- AST (U/L) 57.80±50.88  
- Prothrombin time (sec) 17.108±3.45  
- Prothrombin conc. (%) 56.120±11.7

Data expressed as mean±SD
Calprotectin is a surrogate marker of neutrophil turnover and is elevated in a number of inflammatory conditions [13]. It is detectable in plasma, urine, feces, cerebrospinal fluid, saliva, synovial fluid, empyema supernatant, [13] and colonic biopsies [14]. Calprotectin has been studied in a variety of infective and inflammatory conditions, but more recently it has received increasing attention in gastroenterology. Its functional aspects have been reviewed recently by Johne et al. [13].

The emerging role for measurement of this protein in the field of gastroenterology is because of the fact that, although non-specific, it is elevated in a number of organic gastroenterological conditions. Measurement in different samples provides diagnostic and prognostic information for a number of commonly encountered organic diseases.

In the current study calprotectin was significantly higher in patients with SBP than those without SBP and this agreed with Elbanna study [15]. This increase of calprotectin can be explained by the release of calprotectin from activated neutrophil, so calprotectin had antimicrobial [10] and immunmodulatory actions [11]. Moreover, the elevated calprotectin correlated to severity of liver cirrhosis as there was significant positive correlation with serum bilirubin and an inverse correlation with serum albumin and prothrombin concentration. These correlations with severity of liver cirrhosis can be explained by impaired reticuloendothelial function. SBP is thought to result from a combination of factors inherent in cirrhosis and ascites, such as prolonged bacteremia secondary to compromised host defenses, intrahepatic shunting of colonized blood, and defective bactericidal activity within the ascitic fluid [16]. Contrary to earlier theories, transmucosal migration of bacteria from the gut to the ascitic fluid is no longer considered to play a major role in the etiology of SBP [17].

With respect to compromised host defenses, patients with severe acute or chronic liver disease are often deficient in complement and may also have malfunctioning of the neutrophilic and reticuloendothelial systems [18].

As for the significance of ascitic fluid proteins, it was demonstrated that cirrhotic patients with ascitic protein concentrations below 1g/dL were 10 times more likely to develop SBP than individuals with higher concentration [19]. It is thought that the antibacterial, or opsonic, activity of ascitic fluid is closely correlated with the protein concentration [20].

**Discussion**

Calprotectin is found in cells, tissues and fluids throughout the body [13]. However, it is mainly a myelomonocytic protein constituting approximately 5% of total proteins in neutrophils [2].
Conclusion: Calprotectin might be valuable for rapid diagnosis of SBP and also for detection of severity of liver cirrhosis.

References