



# Biomarkers for Spontaneous bacterial peritonitis

**Amr Ahmed Hamed, Khairy Hammam Morsy, Asmaa Naser Mohammad , Ali Abdel aziz<sup>1</sup> , Reem Abd El Hamed**

\* Tropical Medicine and Gastroenterology Department, Faculty of Medicine, Sohag University

## Abstract:

For cirrhotic ascitic individuals, spontaneous bacterial peritonitis is a potentially fatal outcome. Other tests for early spontaneous bacterial peritonitis diagnosis were looked upon, despite the fact that an ascitic polymorphonuclear leukocytes count of 250 cells/mm<sup>3</sup> remains the gold standard for the diagnosis of spontaneous bacterial peritonitis. This article compiles and evaluates recent studies on spontaneous bacterial peritonitis diagnostic indicators such as procalcitonin, calprotectin, and homocysteine. Many ascitic cytokines and chemokines, such as tumor necrosis factor-alpha, macrophage inhibitory protein-1 beta, interleukin-1 beta, interleukin-8, interleukin-10, and the soluble receptors of tumor necrosis factor-alpha, are more prevalent in patients with spontaneous bacterial peritonitis than in those without spontaneous bacterial peritonitis. Patients with spontaneous bacterial peritonitis had significantly higher serum high sensitivity C-reactive protein levels than those with sterile ascites. Ascites lactoferrin is a biomarker that can be used to diagnose and predict spontaneous bacterial peritonitis, and it was found to be lower in all spontaneous bacterial peritonitis patients who responded well to antibiotic therapy. Ascitic Calprotectin can distinguish between spontaneous bacterial peritonitis and non-spontaneous bacterial peritonitis patients, according to numerous research. Patients with spontaneous bacterial peritonitis had significantly higher ascitic and serum homocysteine levels than patients without spontaneous bacterial peritonitis. Instead of the second paracentesis, it might be used to assess the infection's eradication.

**Keywords:** Spontaneous bacterial peritonitis; Procalcitonin; Calprotectin; Homocysteine

## Abbreviations:

CRP: C-reactive protein, ELISA: enzyme-linked immunosorbent assay, Hcy: Homocysteine, hs-CRP: high-sensitivity C-reactive protein, PCT: procalcitonin, PMNs: Polymorphonuclear leukocytes, SBP: spontaneous bacterial peritonitis, TNF-alpha: tumor necrosis factor-alpha

## Introduction:

In people with cirrhosis, bacterial infections are frequent and can increase

the risk of complications and mortality. The most frequent type, accounting

for around 25% of cases, is spontaneous bacterial peritonitis (SBP) <sup>(1)</sup>.

SBP is bacterial ascites infection without an intraabdominal source of infection (e.g., viscus perforation, abscess), intraabdominal inflammatory focus (as acute pancreatitis), or cancer. When ascitic fluid neutrophils exceed 250/mm<sup>3</sup>, SBP is diagnosed. It has been proven that late diagnosis of SBP is linked with a higher death rate. As a result, a precise marker for early SBP diagnosis would be extremely beneficial <sup>(2)</sup>.

Due to the lysis of the Polymorphonuclear leukocytes (PMN) during transfer to the laboratory, false-negative results can occur. The operator's skill in manually counting ascitic fluid PMNs can influence the diagnosis. As a result, discovering new biomarkers may aid in the diagnosis and treatment of SBP <sup>(3)</sup>.

The current study examines and summarizes past investigations of serum and ascitic fluid laboratory indicators for SBP diagnosis.

### **Traditional Biomarkers in Ascites:**

#### **1-PMN Count:**

The PMN count is the gold standard clinical measure. If the PMN count is greater than 250 cells/mm<sup>3</sup>, SBP is diagnosed <sup>(4)</sup>. SBP diagnosis is difficult to achieve clinically because, unlike other kinds of peritonitis, SBP can be asymptomatic. Even though the 250 PMN/mm<sup>3</sup> cut-off is widely utilized, it is known that false-positive or false-negative results are possible <sup>(5)</sup>.

A twenty-five percent reduction in PMN count after two days of therapy indicates a satisfactory response <sup>(4,5)</sup>. Some investigations revealed no difference between manual and automated PMN counting <sup>(6,7)</sup>.

### **2- Total Protein Content**

Total ascitic protein ought to be assessed since the low level of total protein in ascites may be a risk factor for SBP. Patients should take primary antimicrobial prophylaxis if their total ascitic protein level is less than 1.5 g/L in order to prevent SBP <sup>(4)</sup>.

Recent research, however, has found no link between low ascitic protein and SBP <sup>(8,9)</sup>. These data cast doubt on the use of ascitic protein content as a reliable indicator of SBP.

### **Ascitic biomarkers which have not yet been used clinically:**

#### **1-Bacterial DNA:**

Blood-culture bottles are the most effective way of identifying the causal organism in SBP, however, they are only positive in roughly half of the cases. Just 40% of bacterial ascites and low ascitic PMN levels (bacterascites) require antibiotic treatment, as the majority of these individuals recover spontaneously <sup>(4)</sup>.

Due to the low accuracy of ascites cultures in SBP diagnosis, molecular amplification of bacterial DNA has been widely explored as another method of identifying the organism causing SBP. *Such and colleagues* suggested this approach for the first time when they discovered similar bacterial DNA, predominantly *Escherichia coli*, in serum and ascites in nine of twenty-eight cirrhotic ascitic patients. However, bacterial DNA was found in roughly 30% of individuals without SBP in the prior investigation, indicating inadequate specificity <sup>(10)</sup>. The low specificity of bacterial DNA was discovered in subsequent research <sup>(11-13)</sup>. Bacterial DNA is not employed as an SBP marker because of its limitations.

### **2-Procalcitonin (PCT):**

Procalcitonin, a prohormone of calcitonin is produced by the thyroid gland C cells. The enzyme-linked immunosorbent assay (ELISA) (14) or immunofluorescence (15) can be used to quantify procalcitonin. In healthy adults, the reference value is around 0.5 ng/mL. Procalcitonin is a C-reactive protein (CRP) in the acute phase. Microbial toxins can directly release procalcitonin during infection, or it can be released indirectly via humoral factors or cell-mediated response. As a result, it can detect systemic bacterial diseases (16).

#### **Procalcitonin in SBP patients**

It is controversial if PCT is accurate in the early diagnosis of bacterial disease in cirrhotic patients, particularly SBP. (17). SBP patients had higher serum procalcitonin than those with non-infected ascites, according to two investigations (14,18). There were no significant differences in only the study of *Lesińska et al* (15).

### **3-Leukocyte Esterase Reagent Strips:**

A quick and affordable method to identify SBP is with leukocyte esterase reagent strips, which track the activity of leukocyte esterase in body fluids. They were originally used for urine analysis, but they are currently utilized to treat the majority of bodily fluid infections (19).

The sensitivity of the strips for the diagnosis of SBP ranged from 45-100%, with 90-100% specificity, 42-100% positive predictive value, and 93-100% negative predictive value, according to a review of 23 studies by *Oey et al.* from 2002-2015. While the strips exhibited varying sensitivity,

they had a high negative predictive value, according to the studies (20).

Two more subsequent studies found that the strips were effective in excluding SBP (19,21).

### **4-Lactoferrin:**

A protein called lactoferrin binds to iron in PMNs before releasing it during degranulation. Because lactoferrin is stable and does not degrade if kept at room temperature for an extended period, it is perfect for use in medicine (22).

According to *Parsi et al.*, ascites lactoferrin could be a diagnostic and predictive test for SBP. All SBP patients who responded to antibiotic therapy had lower lactoferrin levels. (3).

Ascitic lactoferrin was also recommended as a marker for SBP, according to another 2 studies (23,24).

### **5-Inflammatory Cytokines:**

Many ascitic cytokines and chemokines, such as interleukin-1 beta (25), interleukin-8 (26), interleukin-10 (27), macrophage inhibitory protein-1 beta (15), tumor necrosis factor-alpha (TNF-alpha) (28), and the soluble receptors of TNF-alpha are more prevalent in patients with SBP than in those without SBP (29).

One study on 20 patients found no difference in interleukin-6 levels between those with SBP and those without (30), in contrast to four investigations involving 63 patients (31), 47 patients (25), and 425 patients (28), respectively, which showed a significant increase in interleukin-6 in SBP patients.

An Egyptian study conducted on 425 cirrhotic patients, 61 of whom had SBP and found that ascitic interferon gamma-induced protein 10 kDa, inter-

leukin-6, and TNF-alpha can be good diagnostic SBP markers(28).

### **6-High-Sensitivity C-Reactive Protein:**

The high-sensitivity C-reactive protein (hs-CRP) can identify significantly lower serum CRP values in SBP patients than in prior techniques<sup>(32)</sup>. Patients with SBP had significantly higher serum hs-CRP levels than those with sterile ascites. Also, these levels reduced after 48 hours of antibiotic treatment, suggesting that serum hs-CRP could be a useful measure for predicting treatment response.

### **Novel markers:**

#### **1-Calprotectin :**

Calprotectin was discovered in the cytoplasm of neutrophil granulocytes as an antibacterial protein<sup>(33)</sup>. Then it was discovered to be a promising inflammatory marker. Furthermore, interactions with endothelial cells have a role in inflammatory cells migration, and its capacity to cause zinc arrest can disrupt physiological balance<sup>(34)</sup>.

Numerous investigations have demonstrated that ascitic Calprotectin levels are significantly higher in SBP patients than in individuals without the illness<sup>(35-40)</sup>.

Ascitic Calprotectin/albumin ratio can be employed in SBP diagnosis and prognosis, according to *Makhlouf et al.*<sup>(24)</sup>. According to *Lutz et al.*, ascitic Calprotectin/protein ratio is stronger than Calprotectin for SBP diagnosis<sup>(37)</sup>.

#### **2-Homocysteine (Hcy):**

In the metabolism of Hcy, the liver is crucial. A multi-step process produces Hcy from methionine. To make S-adenosyl methionine, methionine first acquires an adenosine group from

ATP, which is mediated by S-adenosyl methionine synthetase. The methyl group is then transferred to an acceptor molecule by S-adenosyl methionine (e.g. DNA methyltransferase, nor-epinephrine). The adenosine is degraded to produce L-Hcy, which is then changed by tetrahydrofolate into L-methionine or L-cysteine<sup>(41)</sup>.

Even when there are no evident clinical signs of infection, ascitic patients may nevertheless have high levels of circulating endotoxin. These endotoxins raise NO levels, which deactivates methionine synthase and causes Hcy to build up in cells and extracellular space. Hcy buildup in cells and the extracellular environment increases the vasoactive endothelium-relaxing factor, which causes circulatory dysfunction in these people<sup>(42)</sup>

Hcy was recommended by *Abdel-Razik et al.* for SBP diagnosis since ascitic and serum Hcy were considerably greater in SBP than in non-SBP individuals. The researchers discovered that ascitic Hcy is linked to the hepatic condition, ascitic PMN, ascitic protein, and inflammatory markers like CRP. Instead of the second paracentesis, it might be used to assess the infection's eradication<sup>(43)</sup>.

Ascitic Hcy as a novel marker for SBP diagnosis was also advocated by *Samir et al.*<sup>(45)</sup>, *Abd Ellatif et al.*<sup>(44)</sup>, and *Ahmed et al.*<sup>(40)</sup>.

### **Conclusion:**

SBP can cause life-threatening consequences for cirrhotic patients with ascites. While an ascitic PMN count of 250 cells/mm<sup>3</sup> is the standard method for detecting SBP, other laboratory tests are crucial for making an early diagnosis and gauging how well

the first course of treatment will work. This article gathers and assesses current research on SBP markers. Larger studies with more participants will be needed to evaluate the effectiveness of markers for the rapid recognition of SBP.

## REFERENCES

- 1-Piano S, Singh V, Caraceni P, Maiwall R, Alessandria C, Fernandez J et al. Epidemiology and effects of bacterial infections in patients with cirrhosis worldwide. *Gastroenterology* 2019;156:1368–1380.
- 2- Lee JM, Han KH, Ahn SH, et al. Ascites and spontaneous bacterial peritonitis. *J Gastroenterology Hepatology* 2009;24(9):1494-503.
- 3-Parsi MA, Saadeh SN, Zein NN, Davis GL, Lopez R, Boone J, et al. Ascitic fluid lactoferrin for diagnosis of spontaneous bacterial peritonitis. *Gastroenterology*. 2008;135:803–7
- 4-Runyon BA. Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis 2012. *Hepatology* Baltim MD. 2013;57:1651–3.
- 5-Wiest R, Krag A, Gerbes A. Spontaneous bacterial peritonitis: recent guidelines and beyond. *Gut*. 2012;61:297–310.
- 6-Angeloni S, Nicolini G, Merli M, Nicolao F, Pinto G, Aronne T, Attili AF, Riggio O. Validation of automated blood cell counter for the determination of polymorphonuclear cell count in the ascitic fluid of cirrhotic patients with or without spontaneous bacterial peritonitis. *Am J Gastroenterol* 2003; 98: 1844-1848
- 7-Cereto F, Genescà J, Segura R. Validation of automated blood cell counters for the diagnosis of spontaneous bacterial peritonitis. *Am J Gastroenterol* 2004; 99: 1400
- 8-Schwabl P, Bucsics T, Soucek K, Mandorfer M, Bota S, Blacky A, et al. Risk factors for the development of spontaneous bacterial peritonitis and subsequent mortality in cirrhotic patients with ascites. *Liver Int Off J Int Assoc Study Liver*. 2015;35:2121–8
- 9-Terg R, Casciato P, Garbe C, Cartier M, Stieben T, Mendizabal M, et al. Proton pump inhibitor therapy does not increase the incidence of spontaneous bacterial peritonitis in cirrhosis: a multicenter prospective study. *J Hepatol*. 2015;62:1056–60.
- 10-Such J, Francés R, Muñoz C, Zapater P, Casellas JA, Cifuentes A, et al. Detection and identification of bacterial DNA in patients with cirrhosis and culture-negative, non neutrocytic ascites. *Hepatology* Baltim MD. 2002;36:135–41.
- 11-Sugihara T, Koda M, Maeda Y, Matono T, Nagahara T, Mandai M, et al. Rapid identification of bacterial species with bacterial DNA microarray in cirrhotic patients with spontaneous bacterial peritonitis. *Intern Med Tokyo Jpn*. 2009;48:3–10.
- 12-Soriano G, Esparcia O, Montemayor M, Guarner-Argente C, Pericas R, Torras X, et al. Bacterial DNA in the diagnosis of spontaneous bacterial peritonitis. *Aliment Pharmacol Ther*. 2011;33:275–84
- 13-Mortensen C, Jensen JS, Hobolth L, Dam-Larsen S, Madsen BS, Andersen O, et al. Association of markers of bacterial translocation with immune activation in decompensated cirrhosis. *Eur J Gastroenterol Hepatol*. 2014;26:1360–6.
- 14-Abdel-Razik A, Mousa N, Elhammady D, Elhelaly R, Elzebery R, et al. Ascitic fluid calprotectin and serum procalcitonin as accurate diagnostic markers for spontaneous bacterial peritonitis. *Gut Liver* 2016;10: 624-631.

- 15-Lesińska M, Hartleb M, Gutkowski K, Nowakowska-Dulawa E.** Procalcitonin and macrophage inflammatory protein-1 beta (MIP-1 $\beta$ ) in serum and peritoneal fluid of patients with decompensated cirrhosis and spontaneous bacterial peritonitis. *Adv Med Sci* 2014;59: 52-56.
- 16-Bode-Jänisch S, Schütz S, Schmidt A, Tschernig T, Debertin AS, et al.** Serum procalcitonin levels in the postmortem diagnosis of sepsis. *Forensic Sci Int* 2013;226: 266-272.
- 17-Popoiag RE, Fierbinteanu-Braticevici C.** Spontaneous bacterial peritonitis: update on diagnosis and treatment. *Rom J Intern Med.* 2021; 20;59(4):345-350.
- 18-Wu J, Jiang F, Zeng T, Xu H, Lei Y, et al.** Role of serum procalcitonin assay for diagnosis of spontaneous bacterial peritonitis in end-stage liver diseases. *Acta Academiae Medicinae Sinicae* 2014;36: 37-41.
- 19-Thévenot T, Briot C, Macé V, Lison H, Elkrif L, et al.** The periscreen strip is highly efficient for the exclusion of spontaneous bacterial peritonitis in cirrhotic outpatients. *Am J Gastroenterol* 2016;111: 1402-1409.
- 20-Oey RC, Kuiper JJ, van Buuren HR, de Man RA.** Reagent strips are efficient to rule out spontaneous bacterial peritonitis in cirrhotics. *Neth J Med* 2016;74: 257-261.
- 21-Honar N, Geramizadeh B, Dehghani SM, Kalvandi G, Shahramian I, et al.** Evaluation of leukocyte esterase reagent strips test in the diagnosis of spontaneous bacterial peritonitis in children with cirrhosis. *Arq Gastroenterol* 2015;52: 195-199.
- 22-Kayazawa M, Saitoh O, Kojima K, et al.** Lactoferrin in whole gut lavage fluid as a marker for disease activity in inflammatory bowel disease: comparison with other neutrophil-derived proteins. *Am J Gastroenterol* 2002; 97:360– 369.
- 23-Khalifa NA, Abdel-Azzez HA, Hassaneen AM, El-Shami EA, Hassanin HM.** Ascitic Fluid Lactoferrin as a Diagnostic Marker for Spontaneous Bacterial Peritonitis. *Afro-Egypt J Infect Endem Dis* 2013; 3: 49-55.
- 24-Makhlouf, N.A., Morsy, K.H., Mahmoud, A.A., and Hassaballa, A.E.** Diagnostic Value of Ascitic Fluid Lactoferrin, Calprotectin, and Calprotectin to Albumin Ratio in Spontaneous Bacterial Peritonitis. *Int. J. Curr. Microbiol. App. Sci.* 2018;7(2): 2618-2631.
- 25-Rodríguez-Ramos C, Galan F, Díaz F, Elvira J, Martín-Herrera L, Girón-González JA.** Expression of proinflammatory cytokines and their inhibitors during the course of spontaneous bacterial peritonitis. *Dig Dis Sci.* 2001;46:1668–76.
- 26-Martínez-Brú C, Gómez C, Cortés M, Soriano G, Guarner C, Planella T, et al.** Ascitic fluid interleukin-8 to distinguish spontaneous bacterial peritonitis and sterile ascites in cirrhotic patients. *Clin Chem.* 1999;45:2027–8
- 27-Kim JK, Chon CY, Kim JH, Kim YJ, Cho JH, Bang SM, et al.** Changes in serum and ascitic monocyte chemotactic protein-1 (MCP-1) and IL-10 levels in cirrhotic patients with spontaneous bacterial peritonitis. *J Interferon Cytokine Res Off J Int Soc Interferon Cytokine Res.* 2007;27:227–30
- 28-Abdel-Razik A, Mousa N, Elbaz S, Eissa M, Elhelaly R, Eldars W.** Diagnostic utility of interferon gamma-induced protein 10 kDa in spontaneous bacterial peritonitis: a single-center study. *Eur J Gastroenterol Hepatol.* 2015;27:1087–93.
- 29-Andus T, Gross V, Holstege A, Ott M, Weber M, David M, et al..** High concentrations of soluble tumor

- necrosis factor receptors in ascites. *Hepatology* 1992;16:749–55.
- 30-Spahr L, Morard I, Hadengue A, Vadas L, Pugin J (2001)** Procalcitonin is not an accurate marker of spontaneous bacterial peritonitis in patients with cirrhosis. *Hepatology* 48: 502-505.
- 31-Pruimboom WM, Bac DJ, van Dijk AP, Garrelds IM, Tak CJ, Bonta IL, et al.** Levels of soluble intercellular adhesion molecule 1, eicosanoids and cytokines in ascites of patients with liver cirrhosis, peritoneal cancer, and spontaneous bacterial peritonitis. *Int J Immunopharmacol.* 1995;17:375–84.
- 32-Guler K, Vatanserver S, Kayacan SM, Salmayenli N, Akkaya V, et al.** High sensitivity C-reactive protein in spontaneous bacterial peritonitis with nonneutrocytic ascites. *Hepatology* 2009;56: 452-455.
- 33- Dale I, Fagerhol MK, Naesgaard I.** Purification and partial characterization of highly immunogenic human leukocyte protein, the L1 antigen. *Eur J Biochem* 1983;134: 1-6.
- 34-Sampson B, Fagerhol MK, Sunderkotter C, Golden BE, Richmond P, KLEIN N, Kovar IZ, Beattie JH, Wolska-Kusnierz B, Saito Y, Roth J.** Hyperzincemia and hypercalprotectinaemia: a new disorder of zinc metabolism. *Lancet* 2002;360: 1742-1745.
- 35-Burri E, Schulte F, Muser J, Meier R, Beglinger C.** Measurement of calprotectin in the ascitic fluid to identify elevated polymorphonuclear cell count. *World J Gastroenterol* 2013;19: 2028-2036.
- 36-Abdel-Razik A, Mousa N, Elhammady D, et al.** Ascitic Fluid Calprotectin and Serum Procalcitonin as Accurate Diagnostic Markers for Spontaneous Bacterial Peritonitis. *Gut and Liver*; 2015.
- 37-Lutz P, Pfarr K, Nischalke HD, Krämer B, Goeser F, et al.** The ratio of calprotectin to total protein as a diagnostic and prognostic marker for spontaneous bacterial peritonitis in patients with liver cirrhosis and ascites. *Clin Chem Lab Med* 2015;53: 2031-2039.
- 38-Weil D, Heurgue-Berlot A, Monnet E, Chassagne S, Cervoni J-P, Feron T et al.** Accuracy of calprotectin using the Quantum Blue Reader for the diagnosis of spontaneous bacterial peritonitis in liver cirrhosis. *Hepatology Res* 2019;49(1):72–81
- 39-Abdel Rahman, E.M., Attia, F.A., Alsebaey, A. et al.** Ascitic calprotectin as a useful marker in the diagnosis of spontaneous bacterial peritonitis in adults. *Egypt Liver Journal* 2020;10, 14.
- 40-Ahmed, A., Morsy, K., Yousef, L., Izzaldin, M., Mohammad, A.** Diagnostic Value of Ascitic Fluid Homocysteine and Calprotectin in Cirrhotic Patients with Spontaneous Bacterial Peritonitis. *The Egyptian Journal of Hospital Medicine*, 2022; 86(1): 548-554.
- 41-Mirković D, Majkić-Singh N, Ignjatović S.** Homocysteine: chemistry, metabolism, and role in pathophysiological processes. *Jugoslav Med Biochem* 2003;22:127–140.
- 42-Ćulafić MD, Marković LM, Obrenovi ZR, Mijač DD.** Plasma homocysteine levels in patients with liver cirrhosis. *Vojnosanit Pregl* 2013;70:57–60.
- 43-Abdel-Razik A, Eldars W, Elhelaly R, Eldeeb AA, Abdelsalam M, El-Wakeel N, Aboulmagd A.** Homocysteine: a new diagnostic marker in spontaneous bacterial peritonitis. *Eur J Gastroenterol Hepatol.* Jul 2018;30(7):779-785.
- 44-Abd Ellatif M, Kashmoola M, Hussein A.** Serum Homocysteine as an Early Diagnostic Marker of

Spontaneous Bacterial Peritonitis in Patients with Hepatic Cirrhosis. *The Egyptian Journal of Hospital Medicine* 2022;86: 266-271.

- 45-Samir M. K., Hany R., Sara M. A., Andrew R. R.** Homocysteine as a Diagnostic Marker in Spontaneous Bacterial Peritonitis. *BMFJ* 2022;39(1):164-175.