Role of Lipopolysaccharide Binding Protein in Diagnosis of Spontaneous Bacterial Peritonitis in Chronic Liver Cirrhosis

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Abstract

Background: Chronic liver disease (CLD) is a continuous process of inflammation, destruction, and regeneration of liver parenchyma, which leads to fibrosis and cirrhosis associated with progressive deterioration of liver functions for more than six months, which includes synthesis of clotting factors, other proteins, detoxification of harmful products of metabolism, and excretion of bile. Chronic hepatitis B, C, and D infections are the most common causes of chronic liver disease worldwide especially in Egypt, East Asia and Sub Saharan Africa. There are various genotypes of hepatitis C. A molecular epidemiological study revealed a high prevalence of HCV genotype 4, subtype 4a among Egyptian patients living in Sharkia governorate, Egypt. In Europe and North America, genotype 1a and 1b are more prevalent, while in Southeast Asia, genotype 3 is more common. Chronic hepatitis C, if not treated, may lead to hepatocellular carcinoma. Spontaneous bacterial peritonitis (SBP), an infection of ascitic fluid without a definitive intra-abdominal source that can be surgically treated, is a common complication in patients with cirrhosis and ascites. Patients with ascites who have been followed prospectively for one year have a 10% to 25% incidence of having at least one episode of SBP during that time period. When patients with ascites underwent routine paracentesis, the incidence of active SBP ranged from 10% to 27% at the time of hospital admission. Lipopolysaccharide (LPS)-binding protein (LBP) is a critical component of innate immunity, implicated in the initiation of host defences against Gram-negative bacteria. LBP alerts the host to the presence of minute amounts of LPS. LPS released from Gram-negative bacteria is present as aggregates, because of the amphiphilic structure of the molecule. LPS aggregates are transformed to monomers by the action of LBP, which has been described as a lipid-transfer molecule catalyzing movement of phospholipids including LPS. When LPS/LBP monomers are transferred to lipoproteins, LPS is inactivated; when LPS/LBP complexes are transferred to cells harboring CD14 at their surface, cells are activated. Thus, the relative contribution of these two pathways will determine the response of the host to LPS.

Keywords: Lipopolysaccharide Binding Protein, Spontaneous Bacterial Peritonitis, Chronic Liver Cirrhosis

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Introduction

Chronic liver disease (CLD) is a continuous process of inflammation, destruction, and regeneration of liver parenchyma, which leads to fibrosis and cirrhosis associated with progressive deterioration of liver functions for more than six months, which includes synthesis of clotting factors, other proteins, detoxification of harmful products of metabolism, and excretion of bile. The causes of chronic liver disease include toxins, alcohol abuse for a prolonged time, infection, autoimmune diseases, genetic and metabolic disorders. Cirrhosis is a final stage of chronic liver disease that results in destruction of liver architecture, the formation of widespread nodules, vascular reorganization, neo-angiogenesis, and deposition of an extracellular matrix. The underlying mechanism of fibrosis and cirrhosis at a cellular level is the recruitment of stellate cells and fibroblasts, resulting in fibrosis **(1)**.

Spontaneous bacterial peritonitis (SBP), an infection of ascitic fluid without a definitive intra-abdominal source that can be surgically treated, is a common complication in patients with cirrhosis and ascites. Patients with ascites who have been followed prospectively for one year have a 10% to 25% incidence of having at least one episode of SBP during that time period. When patients with ascites underwent routine paracentesis, the incidence of active SBP ranged from 10% to 27% at the time of hospital admission**.** The prognosis is generally improved if antibiotics are begun before the onset of shock and renal failure. However, because of the severe underlying liver disease that is usually a progenitor to the development of SBP, inpatient non–infection-related mortality rates have still been quite high at 20% to 40%. If the patient survives that hospitalization, one-year and two-year mortality rates for those with SBP are approximately 70% and 80%, respectively **(2).**

Further adding to the inherent morbidity of SBP is its proclivity for recurrence. After an episode of SBP has been successfully cleared with antibiotic therapy, recurrence rates range from 40% to 70% within the first year**.** In view of these data bearing a rather grim prognosis for those with SBP, further research and experience in the diagnosis and management of this disease have continued to progress. These new findings, together with ongoing education for health care providers, may bring hope of an improved prognosis to patients **(3)**.

Pathogenesis:

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**.** 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. ⁻ Frequent and prolonged bacteremia are potential consequences of these defects in host defenses**.** In terms of important predictors for identifying cirrhotic patients at greatest risk for SBP, both a high serum bilirubin (above 2.5 mg/dL) and a low ascitic fluid protein concentration (less than 1.0 g/dL) have been shown to be independent factors for both initial episodes of SBP as well as for recurrence **(4).**

As to why a higher serum bilirubin might be linked to a greater risk of acquiring SBP, the association is probably indirect. Elevated serum bilirubin levels usually coincide with a more severe or advanced stage of liver disease. Serum bilirubin is one of five markers used to stage the severity of liver disease according to Child–Pugh rankings. The higher the number in these rankings, the greater the risk of SBP. This helps to explain why 70% of cases of SBP are seen in patients with Child–Pugh class C cirrhosis**.** Patients with low protein levels are at higher risk for SBP. Conversely, patients with ascitic fluid of typically high protein content, such as those with malignant ascites or congestive heart failure, are

relatively resistant to SBP. Additional studies have confirmed the validity of the ascitic fluid protein concentration as the best predictor of the first episode of SBP**.** In summary, the development of SBP probably involves a relatively prolonged case of bacteremia translocating to an opsonin-deficient site in the body. In the case of SBP, that site is ascitic fluid **(5).**

Clinical Presentation and Diagnosis:

The clinical presentation of SBP is highly variable. SBP may be manifested as a relatively insidious asymptomatic colonization (bacterascites), or it can quickly emerge as a sepsis syndrome with a high fatality rate. Presenting signs and symptoms can include fever, changes in mental status, abdominal tenderness, gastrointestinal (GI) bleeding, chills, nausea, or vomiting. In one study, fever (68%), mental status alterations (61%), and abdominal tenderness (46%) were the most frequent observations in patients with SBP. Yet some authors report that as many as 30% of patients with paracentesis-proven SBP may be completely asymptomatic**.** Because of the tremendous variability in presentations, and also because such presentations may overlap with other conditions often seen in cirrhosis (e.g., encephalopathy), a proper assessment, as described next, is essential in diagnosis **(6).**

- **Microbiologic Testing:**

With SBP, blood cultures may be positive up to one-third of the time. Routine urine cultures are also recommended in this situation; even if the patient lacks classic symptoms of a urinary tract infection, organisms colonizing in the urine have the potential to travel to the ascitic fluid. In fact, asymptomatic bacteriuria is an independent risk factor for SBP **(7).**

- **Ascitic Fluid Analysis via Diagnostic Paracentesis:**

Even after pan-culturing is properly completed, some series show that 30% to 40% of all patients with SBP have negative cultures of both blood and ascitic fluid. Furthermore, these culture methods take at least 24 to 48 hours to produce the desired results. Because of these shortcomings, paracentesis, when used to obtain an ascitic fluid cytologic analysis, remains the single most important test for identifying and assessing a course of SBP. Unlike the microbiologic cultures already mentioned, the paracentesis fluid analysis can be performed safely and can produce valuable results in just one to four hours **(2).**

Of all the information gleaned from the ascitic fluid cytologic "tap," the neutrophil count remains the best test for making a presumptive diagnosis of SBP. Polymorphonuclearneutrophilic leukocyte (PMN) counts, starting at 250 or total WBC cound more than 500 cells/mL (depending upon the culture results and the patient's clinical presentation), are considered valid markers for SBP **(2).**

- **Primary Spontaneous Bacterial Peritonitis versus Secondary Peritonitis: Differential Diagnosis and Pathogenicity:**

Another important consideration is differentiating SBP from secondary peritonitis. Depending upon the series of patients studied, approximately 5% to 15% of patients with infected ascites have an intra-abdominal source, such as a perforated bowel. This differentiation is of paramount importance, because the mortality rate of SBP approaches 100% if treatment includes antibiotics without surgical intervention. However, the mortality rate is about 80% if a patient with SBP receives an unnecessary exploratory laparotomy**.** Using the results of retrospective studies, Akriviadis and Runyon developed an algorithm for identifying patients with infection secondary to perforation and for distinguishing SBP from nonperforation secondary peritonitis depending on the patient's response to antibiotic therapy **(8).**

An initial pretreatment ascitic fluid cytologic analysis can be helpful in distinguishing SBP from secondary peritonitis. Both types are

characterized by PMN counts greater than 250 cells/mm, but secondary peritonitis often shows total protein concentrations above 1 g/dL, glucose concentrations lower than 50 mg/dL, and serum lactate dehydrogenase (LDH) levels above the upper limit of normal (ULN) **(9).**

Unlike secondary peritonitis, SBP tends to be monomicrobic about 92% of the time. The most commonly occurring organisms are enteric gram-negative rods such as *Escherichia coli* and *Klebsiella* spp., which cause more than half of all infections. Gram-positive organisms cause about 25% of infections. Although some series show *Streptococcus pneumoniae* as the most common streptococcal organism, others find the *viridans* group as the predominant gram-positive pathogen. *Enterococcus* spp. have been documented in 6% to 10% of cases. *S. aureus* is noted infrequently, representing only about only 2% to 4% of all SBP infections. In one series quite representative of the literature, the most frequently isolated organisms included *E. coli,* streptococci, and *Klebsiella* spp.; these accounted for more than 80% of all cases of SBP **(3)**.

There is another significant difference between SBP and secondary peritonitis. Although facultative anaerobic organisms such as Enterobacteriaceae and streptococci are common SBP pathogens, obligate anaerobes, such as *Bacteroides* spp., are rarely implicated as a cause of SBP. This observation is attributed to the relatively high oxygen content of ascitic fluid, an environment in which facultative anaerobes cannot proliferate as long as needed to attain pathogenicity**.** Lipopolysaccharide (LPS)-binding protein (LBP) is a critical component of innate immunity, implicated in the initiation of host defences against Gram-negative bacteria. LBP alerts the host to the presence of minute amounts of LPS. LPS released from Gram-negative bacteria is present as aggregates, because of the amphiphilic structure of the molecule. LPS aggregates are transformed to monomers by the action of LBP, which has been described as a lipid-transfer molecule catalyzing movement of phospholipids including LPS. When LPS/LBP monomers are transferred to lipoproteins, LPS is inactivated; when LPS/LBP complexes are transferred to cells harboring CD14 at their surface, cells are activated. Thus, the relative contribution of these two pathways will determine the response of the host to LPS **(10).**

Whereas LBP may have a protective role in alarming the host to minute doses of LPS, upon exposure to larger quantities of LPS, the amplification of LPS effects mediated by LBP may be detrimental to the host. Indeed, LBP has been shown to play a toxic role in experimental endotoxemia. Blockade of LBP activity with polyclonal and monoclonal antibodies was found to protect mice from lethal endotoxemia. Similarly, the disruption of the LBP gene has been associated with resistance to LPS. If the role of LBP appears straightforward in endotoxemia, its contribution in fighting or helping infection is still largely unknown, inasmuch as LBP has been shown to protect mice from *Salmonella* infections **(11).**

Function of Lipopolysaccharide Binding Protein:

LBP is produced in hepatocytes as a 50-kDa protein and is constitutively secreted into the bloodstream at a concentration of approximately 5–15 µg/ml. The protein concentration rises in the “acute phase” to 50 µg/ml, and LBP, which does not have activity by itself, binds to LPS with high affinity. Upon binding to LPS, LBP does not suppress or block the effects of LPS, but enhances endotoxin effects and activates cellular responses at subthreshold LPS levels. LPS-induced Tumor Necrosis Factor (TNF) production and TNF-mRNA expression in peritoneal macrophages is enhanced strongly when LPS is complexed to LBP **(12).**

Peritoneal macrophages that are rendered unresponsive to LPS stimulation by a process called adaptation can have their ability to produce TNF restored by the addition of LBP. Also,

macrophages detect and bind LPS much faster when it is complexed with LBP; it thus acts as an opsonin for Gram-negative bacteria. Furthermore, the LPS-induced response in neutrophilic granulocytes can be enhanced by the addition of LBP to the system **(13).**

The biological effects of LBP can thus be summarized as complexing to LPS and subsequently enabling the organism to detect small amounts of LPS better and to trigger the defence cascade in a stronger way as compared to LPS alone. During the "acute phase", synthesis of LBP-RNA and protein rises dramatically within 24 h; thus far, no clear interpretation of this phenomenon has been found. The rise in LBP synthesis could occur as a reaction to depletion of circulating LBP during shock; however, the (relatively late) rise in LBP does not explain the development of shock, since the first shock symptoms are usually seen much earlier (after 6-8 h). As far as we know, the LPS effects on B cells cannot be augmented by the addition of LBP; moreover, in experimental setups in which high levels of LPS were used, no LPS-enhancing LBP effect could be seen either **(13)**.

Blocking experiments revealed that pretreatment of serum with antibodies against LBP and subsequent depletion of LBP from the serum resulted in a much weaker response to LPS challenge compared to LBP containing serum **(14).**

CD14, a Receptor for Complexes of Lipopolysaccharide (LPS) and LPS Binding Protein (LBP):

Leukocytes respond to lipopolysaccharide (LPS) at nanogram per milliliter concentrations with secretion of cytokines such as tumor necrosis factor-u (TNF-a). Excess secretion of TNF-ac causes endotoxic shock, an often fatal complication of infection. LPS in the bloodstream rapidly binds to the serum protein, lipopolysaccharide binding protein (LBP), and cellular responses to physiological levels of LPS are dependent on LBP. CD14, a differentiation antigen of monocytes, was found to bind complexes of LPS and LBP, and blockade of CD14 with monoclonal antibodies prevented synthesis of TNF-a by whole blood incubatedwith LPS. Thus, LPS may induce responses by interacting with a soluble binding protein in serum that then binds the cell surface protein CD14 **(15).**

Lipopolysaccharide binding protein is a 60-kD serum glycoprotein that forms high-affinity complexes with bacterial endotoxin (LPS). LBP also functions as an opsonin. It binds to the surface of bacteria or to LPS-coated erythrocytes and mediates the adhesion of these coated particles to macrophages. Interaction of LPS-LBP complexes with macrophages helps not only this adhesive function but also induces the synthesis of TNF by the macrophages. The molecule on the cell surface that mediates binding of LBP-coated particles is restricted to monocytes and macrophages, is mobile in the plane of the membrane, and is distinct from receptors for other known opsonins such as immunoglobulin and complement (Fc-yRI, Fc-yRII, Fc-yRIII, CR1, and CR3). In order to determine the role of various surface structures in the binding of erythrocytes coated with LPS-LBP complexes (ELBPs), macrophages were allowed to spread on surfaces coated with monoclonal antibodies (MAbs) to macrophage surface proteins **(12).**

Mobile membrane proteins diffuse to the substrate attached portion of a spread macrophage and are trapped by interaction with the specific MAb, thus causing the apical surface to be specifically depleted of a single protein. Depletion of proteins with MAbs to CD16 (Fc-yRIII), CD1 lb/CD18 (CR3), CD18, or HLA caused no diminution of the binding of ELBPs. However, three separate MAbs to CD14 caused strong down-modulation of the binding of ELBP, suggesting that expression of CD14 on the apical surface of the macrophage is necessary for recognition of ELBPs **(16).**

An I8-kDa protein in the membrane of 702/3 cells and two proteins of 95 and 31 kDa in the

membranes of RAW 263.1 cells have also recently been identified as binding to LPS. A lectin like monocyte membrane molecule was shown to interact with the sugar part of LPS and recently in the granules of neutrophilic granulocytes, a protein was found that binds to LPS, is bactericida1 and has 44% sequence homology with LBP. In addition, hybrophobic interactions, mediated by the lipid A moiety of LPS with the lipid bilayer of the cell membrane, have been postulated and must be taken into consideration**.** One explanation for this relatively large group of candidate and/or proven LPS receptors distinct from the CD14 receptor is that different LPS-recognizing molecules may be able to detect various subtypes of LPS (from different bacterial strains) or that distinct cellular LPS recognition and reaction pathways could be responsible for distinct cellular reactions **(17).**

One method currently under clinical investigation for interfering with processes that lead to endotoxic shock involves the application of anti-LPS mAb to patients in shock. In addition to this direct means of therapy by elimination of the primary cause of the disease, an understanding of the complex mechanisms of the host reaction may lead to additional therapeutic measures for interfering with the cascade of events involved in endotoxinaemia-mediated shock **(18).**

Lipopolysaccharide Binding Protein as a Potential Marker of Infection in

Spontaneous Bacterial Peritonitis

Bacterial translocation (BT) is considered to be the underlying mechanism associated with the development of infection in cirrhosis. In addition, the ensuing increased inflammatory response to bacteria and/or bacterial products has been implicated to other complications of cirrhosis such as hepatic encephalopathy, hepatorenal syndrome and aggravation of portal hypertension leading to high fatality rates **(19).**

Lipopolysaccharide-binding-protein is a soluble acute phase protein with a long half-life, produced by hepatocytes, that enhances the binding of bacterial lipopolysacharide (LPS) to CD14 cell membrane molecule and Toll-like receptor 4, activating a cascade that leads to cytokine production and inflammatory response. LBP levels are considered to reflect the long-term exposure to bacteria and endotoxins. Peripheral blood LBP levels have been used as surrogate marker of bacterial translocation **(19).**

In a study about high serum lipopolysaccharide binding protein and its association with increased mortality in patients with decompensated cirrhosis showed; Episodes of transient bacteraemia induced by bacterial passage from the intestine to the systemic circulation occur in patients with decompensated cirrhosis. Many of these episodes may remain undiagnosed while other may cause infection or trigger complications of cirrhosis. LPS is not a reliable marker of endotoxinaemia because of its short half-life. LBP is an endotoxin binding protein which can modulate the biological activity of circulating endotoxins and its serum or plasma levels have been shown to rise in response to LPS **(20).**

In addition, LBP has a long half-life and can be reliably assessed by immunoassays. Increased serum LBP identifies a subpopulation of patients with decompensated cirrhosis and frequent episodes of subclinical exposure to endotoxins. It may therefore serve as an indirect marker of the grade of endotoxaemia. Increased CD14 and cytokine levels, such as TNFa and IL-6 and a hyperdynamic circulatory state, were observed in cirrhotic patients with high LBP levels. Moreover, elevated LBP was associated with increased susceptibility of the host to bacterial infections and its concentration was reduced with norfloxacin prophylaxis. However, its performance on predicting mortality in decompensated cirrhosis has not been sufficiently evaluated **(21).**

According to previous investigators, LBP is both a bacterial translocation biomarker and an acute phase protein identifying the presence of systemic infection. Studies in humans have shown that LBP concentration increased in the presence of bacterial infection. In a cohort study, LBP was found to be highly correlated with other markers of infection and/or inflammation such as CRP, leukocytes and neutrophils. They also found that LBP displayed a very good negative predictive value to rule out infection. As bacterial infection in cirrhosis may be clinically silent, it is difficult to diagnose and empiric antibiotics are usually prescribed with the suspicion of an infection. This strategy carries the long-term risk of potentially increasing rates of resistance in antibiotics. According to the present data, low LBP values and no definitive evidence of infection could help clinician to stop empiric antibiotics **(13).**

Previous investigators have demonstrated that LBP levels were increasing with severity of cirrhosis and portal hypertension. High levels were also determined in cirrhotics with ascites compared to those without. In our cohort, it was demonstrated that serum LBP concentrations were higher in patients with ascites and/or history of ascites compared to those without. Moreover, serum LBP values were higher in those with current or past episode of variceal bleeding compared to those without, probably demonstrating elevated levels of LBP in patients with increased portal hypertension. Failure of paracentesis in our cohort was attributed to good patient compliance both to diuretics and unsalted diet resulting in small or no ascitic fluid collection **(22).**

Previous research has shown that proteins such as calprotectin and procalcitonin (PCT) can be determined in ascitic fluid. Ascitic fluid calprotectin allowed fast diagnosis and better prognosis of SBP. PCT may differentiate distinct cirrhotic subgroups. In the present study, it is the first time that LBP was investigated in ascites. Ascitic fluid LBP levels were associated with other surrogate markers of inflammation in ascites, such as leukocyte and neutrophil counts and lactate dehydrogenase. In addition, ascitic fluid LBP displayed a very good negative predictive value to exclude spontaneous bacterial peritonitis. However, They did not find any prognostic significance of ascitic fluid LBP and its levels were highly correlated with those in the serum indicating a probable interplay between serum and ascites**.** Serum and ascitic fluid LBP concentrations showed a very good negative predictive value to rule out infection and spontaneous bacterial peritonitis, respectively. In addition, high LBP levels were identified in patients with decompensated cirrhosis with no evidence of infection who died during the 90-day follow-up period, a finding probably related to mechanisms of abnormal intestinal permeability and bacterial translocation. Elevated serum LBP is a satisfactory marker of short-term mortality **(23).**

In a study about Value of Different Diagnostic Markers in Spontaneous Bacterial Peritonitis in HCV Egyptian Cirrhotic Patients showed; Spontaneous bacterial peritonitis (SBP) is the development of peritonitis despite the absence of an obvious primary source of infection. Bacterial endotoxins promote the synthesis of lipopolysaccharide (LPS) binding protein (LBP), and forms a LPS-LBP complex which may increase in serum of patients with SBP. Ascitic Fluid (AF) Complement 3 (C3) level is the most important factor to provide local protection against bacterial peritonitis and its level is markedly reduced in AF of patients with SBP **(24).**

The serum level of LBP, AF C3, culture and PMNLs count were studied and correlated with other serum and AF parameters in cases with SBP. In SBP patients, the most frequently occurring symptoms are fever and abdominal pain (occurred in 68.5% and 75.5% of the patients, respectively) with $p < 0.05$. In our study , 88.6% of patients with SBP (group B) were in Child class C and the rest of the patients (11.4%) were in Child class B which denoted that SBP developed only in patients with Child classes B and C, this was in agreement with

many studies concluded that SBP developed with more advanced liver disease **(24).**

Gram-negative bacteria infection in the intra-abdominal cavity causes serum and body fluid levels of LBP to increase significantly. Patients with cirrhosis complicated with SBP have significantly elevated levels of serum LBP. The serum and ascites LBP levels are significantly elevated in SBP patients with suspected clinical diagnosis. Measurements of both the serum LBP and ascites LBP may have diagnostic value for SBP **(24).**

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