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Original Article

Lipopolysaccharide binding protein in cirrhotic patients with severe sepsis

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Abstract

Background: Lipopolysaccharide binding protein (LBP) is an acute-phase protein produced by the liver. It has been shown that LBP plays an important role in the inflammatory response to sepsis. LBP has also been shown to protect animals from endotoxin challenge by facilitating the removal of endotoxin from the blood circulation. Cirrhotic patients are susceptible to bacterial infection. It is unknown whether pre-existing liver dysfunction impacts the LBP levels and thus the prognosis in severe sepsis.

Methods: We evaluated the serum LBP, inflammatory cytokines, and the relationship between LBP concentrations, functional liver reserve and outcomes in 58 critically ill cirrhotic patients with severe sepsis.

Results: The serum LBP levels were significantly higher in 28-day survivors, while the interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels were significantly higher in non-survivors. We analyzed the receiver operating characteristic (ROC) curve to determine the cut-off point for LBP to predict 28-day mortality. The cumulative rates at 28 days were 58.3% versus 16.7% for the high LBP group (>46 ng/mL) and low LBP group (<46 ng/mL) ($p < 0.001$). The high-LBP group had significantly lower INR, Child-Pugh, Model for End-stage Liver Disease (MELD) scores and TNF-α level. Meanwhile, the LBP levels were inversely correlated with INR, and Child-Pugh, MELD and sequential organ failure assessment (SOFA) scores.

Conclusion: The concentration of LBP is associated inversely with disease severity scores and outcomes in critically ill cirrhotic patients with severe sepsis.

Keywords: lipopolysaccharide binding protein; liver cirrhosis; severe sepsis

1. Introduction

Cirrhotic patients are susceptible to bacterial infection, which can lead to multiple organ dysfunction and decreased survival.[1–4] Lipopolysaccharide (LPS), a component of the cell membrane of Gram-negative bacteria, plays a central role in the pathophysiology of sepsis.[5,6] In response to endotoxin challenge, cirrhotic patients show an augmented capacity to produce pro-inflammatory cytokines, [7–10] which may be linked to multiple organ dysfunction in severe sepsis. Despite advances in intensive care, the prognosis for severe sepsis in liver cirrhosis is still poor.[11,12] Recognition of bacterial components by the innate immune system is an important event for triggering the inflammatory response, which is necessary to eliminate the invading microorganisms. Lipopolysaccharide-binding protein (LBP) is a

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58-kDa protein that potently enhances the sensitivity of monocytes and granulocytes to LPS by facilitating binding of LPS to the CD14 cell membrane molecule and Toll-like receptor 4, activating the innate immune system by releasing inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6).[5,6] In several clinical settings, serum LBP seems to better reflect the long-term exposure to bacteria and their endotoxins than endotoxin itself.[13–15] In fact, in cirrhotic patients without bacterial infection, increased levels of LBP can identify a subset of ascitic cirrhotic patients with increased levels of cytokines and a more pronounced vasodilatation.[15] However, LBP mediates LPS transfer to high-density lipoprotein (HDL) particles, leading to neutralization of LPS.[16,17] Taken together, LBP may have dual effects in terms of modulation of the innate immune response.[18] Indeed, it has been shown that LBP plays a concentration-dependent dual role in the pathogenesis of sepsis. Low levels of LBP enhance the LPS-induced activation of mononuclear cells (MNC), whereas the acute-phase rise in LBP concentrations inhibits LPS-induced cellular stimulation.[18,19] After the pathophysiological role of LBP was unraveled, the diagnostic and prognostic values of LBP in patients with systemic inflammatory response syndrome (SIRS) and sepsis have been evaluated. Although the levels of LBP have been consistently high in patients with SIRS and sepsis,[13,20] the diagnostic and prognostic values of LBP in different clinical settings have been conflicting.[13,20,21] The reason for these discrepancies is unclear and probably due to heterogeneity of patient groups.

Like many other acute phase proteins, LBP is mainly synthesized in the liver.[22] Accordingly, upregulation of LBP may be compromised in case of impaired synthetic capacity of the liver, making the interpretation difficult. Severe sepsis can precipitate acute or chronic liver failure in cirrhotic patients and induce a wide array of metabolic and immunological abnormalities.[23,24] Although the clinical relevance of LBP has been shown in stable cirrhotic patients without bacterial infection,[15] the prognostic significance of LBP has never been evaluated in cirrhotic patients with severe sepsis. Considering the impaired biosynthesis of LBP in cirrhotic liver and the bipolar role that LBP plays in innate immunity, the prognostic values of LBP in cirrhotic patients with severe sepsis may be quite different from those of other clinical entities. Therefore, we conducted this prospective observational study to investigate whether the levels of LBP are associated with poor outcomes in patients with liver cirrhosis and severe sepsis. Other potential indicators of inflammation such as c-reactive protein (CRP), TNF-α, and IL-6 were also measured.

2. Methods

2.1. Patient information, data collection, and definitions

This study was conducted with the approval of the institutional review board of Chang Gung Memorial Hospital, Taiwan. Formal consent was obtained from the next of kin. The study enrolled 58 consecutive cirrhotic patients with severe sepsis requiring intensive monitoring and/or treatment. Severe sepsis was defined by the criteria of the American College of Chest Physicians/Society of Critical Care Medicine,[25] namely sepsis associated with organ dysfunction, hypoperfusion abnormality, or sepsis-induced hypotension. Liver cirrhosis was defined histologically or based on clinical, image, and laboratory findings. All patients were treated with a standard treatment protocol for management of severe sepsis and septic shock.[26] Management included early targeted resuscitation, broad empiric antibiotic coverage, infection source control, and effective shock evaluation and treatment. The empiric antibiotic therapy was as previously described.[12,27] The empiric antibiotic regimen was modified on the basis of microbiological data. The major outcome analyzed was 28-day mortality.

The severity of liver disease on the day of blood sampling was graded by the Child-Pugh and Model for End-stage Liver Disease (MELD) scores.[28,29] Meanwhile, multiple organ dysfunction also was assessed by sequential organ failure assessment (SOFA) scores.[30–32] For these scoring systems and physiological evaluations, the most abnormal value for each organ system on the day of blood sampling was recorded.

Bacteremia was defined as the presence of viable bacteria in the blood,[25] as evidenced by a positive blood culture. Spontaneous bacteremia was defined as bacteremia without identified infection focus. Culture-negative sepsis was defined as the presence of systemic inflammatory response syndrome (SIRS)[25] and negative cultures after exclusion of the possibility of non-infection inflammatory conditions as the causes of SIRS.

2.2. Laboratory investigations

Blood cultures and appropriate cultures from the infection focus were obtained. Hematological and biochemical data were also collected systemically within 24 hours of admission to ICU.

Fasting blood samples were obtained in the morning. The blood samples were allowed to clot and were spun immediately in a refrigerated centrifuge. The serum was obtained and frozen at −80°C. LBP was measured by an enzyme-linked immunosorbent assay (Cell Sciences, Inc, Canton, MA). The concentrations of TNF-α and IL-6 were measured by an enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, MN). C-reactive protein was measured by a latex-enhanced immunoturbidimetric method (Daiichi Pure Chemical, Ibaraki, Japan).

2.3. Statistical analysis

Descriptive statistics are expressed as mean ± SD. All variables were tested for normal distribution using the Kolmogorov-Smirnov test. Student's *t* test was used to compare the means of continuous variables and the normal distribution data. Otherwise, the Mann-Whitney U test was used. Categorical data were tested using the Chi-square (χ^2) test. The correlation between LBP levels and disease severity scores was analyzed with linear regression using the Pearson method. Discrimination was tested using the area under a receiver operating characteristic (ROC) curve[33] to assess the ability of LBP to predict 28-day mortality. ROC analysis was also performed to calculate the cut-off values, sensitivity, specificity, overall correctness, and positive

Table 1
Patients' demographic data and clinical characteristics grouped according to 28-day mortality.

	All patients (n = 58)	28 days survival (n = 22)	28 days non-survival (n = 36)	p
Age (y)	55.3 ± 13.1	56.7 ± 16.8	54.4 ± 10.4	NS (0.532)
Sex (M/F)	46/12	18/6	30/6	NS (0.333)
BUN (mg/dL)	56.0 ± 46.7	36.1 ± 24.2	68.1 ± 52.9	0.010
Serum creatinine (mg/dL)	3.43 ± 2.96	2.00 ± 1.94	4.32 ± 3.14	0.003
Na (mEq/L)	140 ± 7.8	140 ± 6.1	139 ± 8.7	NS (0.652)
K (mEq/L)	3.6 ± 1.0	3.5 ± 0.6	3.8 ± 1.2	NS (0.281)
Bilirubin (mg/dL)	15.5 ± 12.3	7.9 ± 9.8	20.1 ± 11.4	<0.001
PT prolongation (s)	15.2 ± 14.3	7.4 ± 5.4	20.0 ± 15.9	0.001
Albumin (g/dL)	2.67 ± 0.50	2.64 ± 0.52	2.69 ± 0.50	NS (0.698)
MAP (mmHg)	68.3 ± 13.8	75 ± 8	64 ± 15	0.002
IL-6 (pg/mL)	220 (72–660)	71.5 (35–179)	327 (196–717)	<0.001
TNF-α (pg/mL)	23.0 (11.2–40.1)	9 (4.9–19.3)	33.5 (24–76.5)	<0.001
CRP (mg/L)	35.1 (19.7–73.6)	39 (15.8–69)	32 (20.8–83)	NS (0.960)
LBP (ng/mL)	42.2 ± 21.5	55.5 ± 15.8	34.0 ± 19.0	<0.001
SOFA score	12.6 ± 5.4	8.2 ± 3.2	15.3 ± 4.6	<0.001
MELD score	31.9 ± 13.7	21.2 ± 8.8	38.6 ± 11.8	<0.001
Child-Pugh score	11.4 ± 2.4	9.7 ± 2.2	12.4 ± 1.8	<0.001

BUN = blood urea nitrogen; CRP = C reactive protein; F = female; LBP = lipopolysaccharide-binding protein; M = male; MAP = mean arterial pressure; MELD = Model for End-stage Liver Disease; NS = not significant; OSF = organ system failure; SOFA = sequential organ failure assessment.

and negative predictive values. The best Youden index (sensitivity + specificity − 1)[34] was also used to determine the best cut-off point of LBP to predict 28-day mortality. All statistical tests were two-tailed, and the significance level was set at p = 0.05 or less. Data were analyzed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Patients' characteristics

Fifty-eight critically ill cirrhotic patients were enrolled in this investigation. The cause of liver cirrhosis was hepatitis B virus (HBV) in 26 patients, alcohol in 17, hepatitis C virus (HCV) in nine, HBV plus alcohol in two, HBV plus HCV in three, and an unknown cause in one. Overall, the ICU and 28-day mortality rates for the entire group were 58.6% and 62.1%, respectively. Table 1 lists the patients' demographic data and clinical characteristics. Compared to survivors, the non-survivors had higher disease severity and poorer liver reserve as evidenced by higher SOFA, MELD, and Child-Pugh scores. Microbiological information was available for all patients. Fifty-two patients had at least one positive microbiological culture. Positive cultures were obtained from the blood in 27 (46.5%) patients, from urine in 16 (27.5%), from sputum in 18 (31.0%), from ascites in 16 (27.5%) patients, and from a CVP catheter tip in two (3.4%).

3.2. Concentrations of LBP and cytokines

The levels of LBP were significantly higher in those who survived (Table 1), while the levels of TNF-α and IL-6 were

Table 2
Patients' demographic data and clinical characteristics grouped according to LBP.

	All patients (n = 58)	High LBP(>46 ng/mL) (n = 22)	Low LBP (≤46 ng/mL) (n = 36)	p
Age (y)	55.3 ± 13.1	56.6 ± 14.2	54.5 ± 12.5	NS (0.559)
Sex (M/F)	46/12	17/5	29/7	NS (0.765)
ICU mortality rate	34 (58.6%)	7 (31.8%)	27 (75.0%)	0.001
28-d mortality rate	36 (62.1%)	6 (27.3%)	30 (83.3%)	<0.001
BUN (mg/dL)	56.0 ± 46.7	49.3 ± 42.0	60.1 ± 49.5	NS (0.379)
Serum creatinine (mg/dL)	3.43 ± 2.96	2.56 ± 2.16	3.96 ± 3.28	NS (0.057)
Na (mEq/L)	140 ± 7.8	143 ± 6	138 ± 8	0.005
K (mEq/L)	3.6 ± 1.0	3.5 ± 0.7	3.7 ± 1.1	NS (0.531)
Bilirubin (mg/dL)	15.5 ± 12.3	12.0 ± 12.6	17.6 ± 11.7	NS (0.095)
PT prolongation (s)	15.2 ± 14.3	9.6 ± 6.8	18.6 ± 16.5	0.018
Albumin (g/dL)	2.67 ± 0.50	2.66 ± 0.39	2.68 ± 0.57	NS (0.861)
MAP (mmHg)	68.3 ± 13.8	71 ± 13	67 ± 14	NS (0.305)
IL-6 (pg/mL)	220 (72–660)	129 (35–655.8)	231 (114–660)	NS (0.167)
TNF-α (pg/mL)	23.0 (11.2–40.1)	11.5 (5.4–28.9)	29.5 (17.8–50.5)	0.011
CRP (mg/L)	35.1 (19.7–73.6)	50.3 (29.7–98)	26.6 (17.2–68.1)	NS (0.091)
SOFA score	12.6 ± 5.4	10.23 ± 5.11	14.00 ± 5.08	0.008
MELD score	31.9 ± 13.7	26.3 ± 12.0	35.4 ± 13.6	0.011
Child-Pugh score	11.4 ± 2.4	10.6 ± 2.5	11.9 ± 2.2	NS (0.058)

BUN = blood urea nitrogen; F = female; LBP = lipopolysaccharide-binding protein; M = male; MAP = mean arterial pressure; MELD = Model for End-stage Liver Disease; NS = not significant; OSF = organ system failure; SOFA = sequential organ failure assessment.

higher in those who died. Low levels of LBP were associated with higher levels of TNF-α (Table 2). The levels of LBP were inversely correlated with Child-Pugh, MELD, and SOFA scores (Fig. 1).

The discriminating power of LBP to predict 28-day mortality was tested using the area under a receiver operating characteristic (ROC) curve. The area under ROC curve for LBP was 0.809 [95% CI: 0.691–0.927] (Fig. 2). By analyzing the ROC curve, the cut-off point for LBP to best predict 28-day mortality was obtained (46 ng/mL; sensitivity: 72.7%; specificity: 83.3%).

The clinical characteristics and outcomes in patient subgroups stratified by LBP level are listed in Table 2. The ICU and 28-day mortality rates for the patients who had a lower

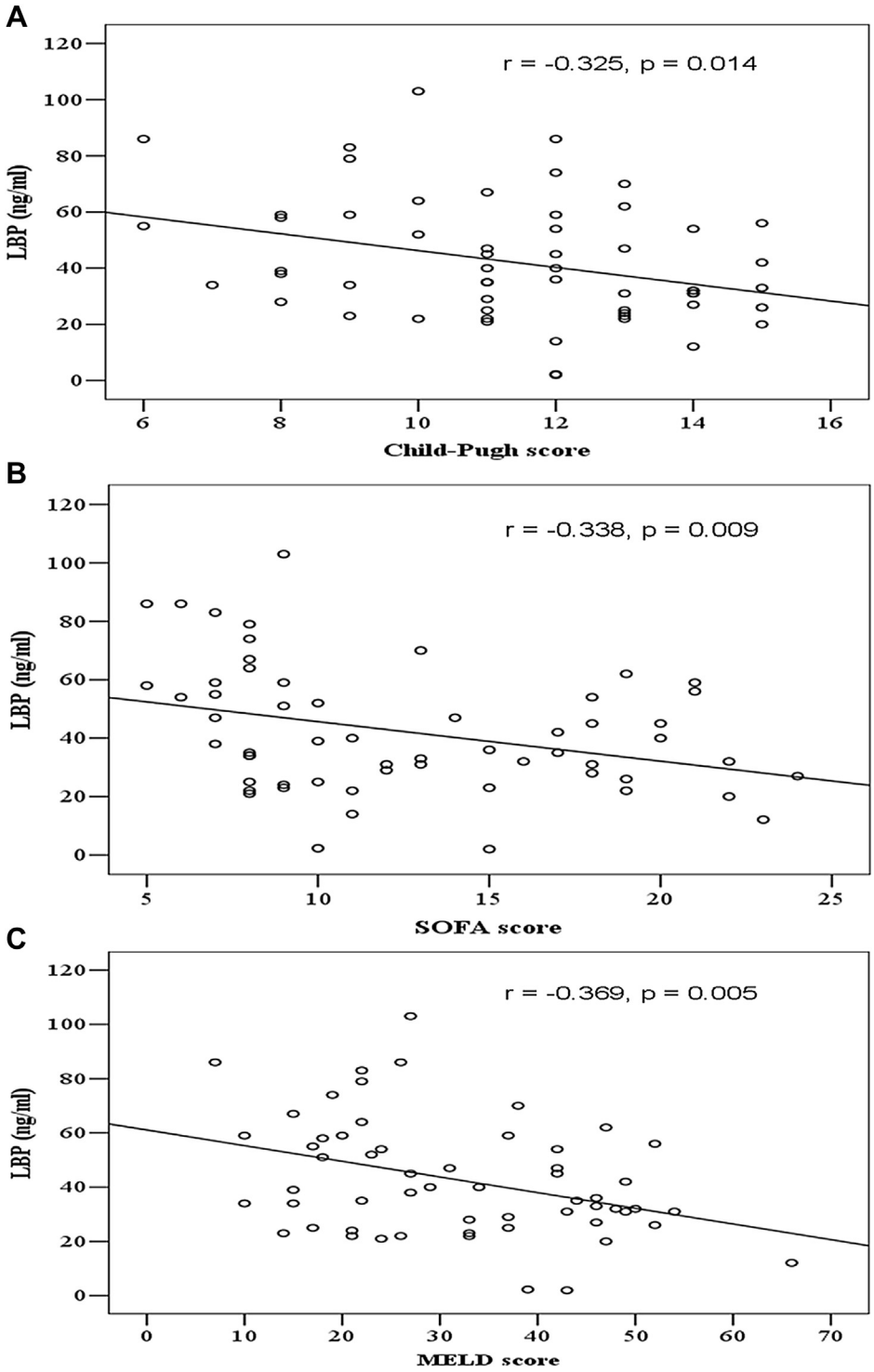

Fig. 1. Linear regression using the Pearson method to assess the correlation between the LBP levels and the disease severity scores.

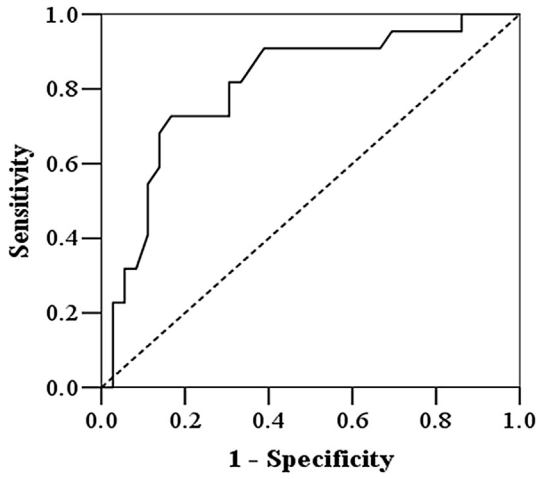

Fig. 2. Receiver operating characteristic (ROC) curve to test the discriminating power of LBP to predict 28-day mortality. The area under ROC curve for LBP is 0.809 (95% CI: 0.691–0.927).

LBP were significantly higher than for those with a higher LBP. Follow-up to 28 days or the time of death was complete for the entire groups. The cumulative rates of survival at 28 days were 16.7% and 72.7% for the low-LBP group and high-LBP group, respectively ($p < 0.001$) (Fig. 3).

To clarify further whether the association between low levels of LBP and mortality was confounded by liver dysfunction and the severity of multiple organ dysfunction, we tried to compare the concentrations of LBP between the 28-day survivors and non-survivors with comparable Child-Pugh, MELD, and SOFA scores. We used the median values as cut-off values to stratify the patients into high and low groups of different prognostic scores. The differences in the levels of LBP between survivors and non-survivors remained, while the differences in Child-Pugh scores between survivors and non-survivors had been eliminated (Table 3), suggesting

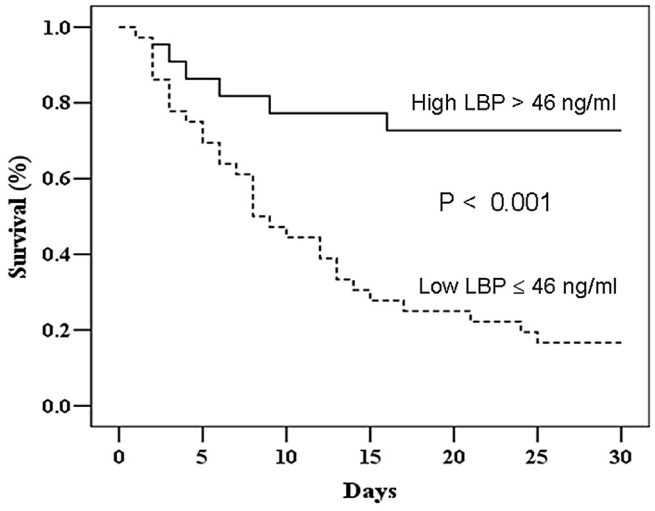

Fig. 3. Cumulative survival in patients with high ($n = 22$) and low ($n = 36$) LBP after admission to intensive care unit. Day 0 indicates the 1st day of admission to intensive care unit.

that LBP level was associated with mortality independent of liver dysfunction. However, the differences in the levels of LBP between survivors and non-survivors did not consistently remain when we stratified patients using MELD and SOFA scores (Supplementary Tables 1 and 2).

4. Discussion

This study is the first to evaluate the relationship between levels of serum LBP and outcomes in critically ill cirrhotic patients with severe sepsis. This investigation showed that low levels of LBP at admission to ICU are associated with impaired liver reserve, multiple organ dysfunction and increased mortality in this clinical setting.

Bacterial translocation and episodic endotoxemia are common phenomena in cirrhotic patients.[35,36] Although LBP has served as a surrogate marker of bacterial translocation in liver cirrhosis,[2,15] its clinical value in the setting of sepsis remains inconclusive. Data about the association between LBP concentrations at admission to the ICU and outcomes have been conflicting. In this study, we found that 28-day mortality rates were significantly higher in patients with lower levels of LBP at admission to ICU. This finding is in agreement with previous studies,[13,37] but in contrast to others, in which high levels of LBP were either associated with adverse outcomes[38,39] or not related to outcomes.[21,40] Despite the conflicting data about the prognostic significance of LBP in the literature, the dynamics of LBP levels during sepsis were consistent. The higher levels were observed at the initial tests and decreased thereafter, with the lowest at the last tests.[21,37,40] The reasons for this dynamic trend are not clear. Although Prucha et al reported that liver failure was not likely to be responsible for this phenomenon in a study of small sample size,[21] their conclusion should not readily be extrapolated to cirrhotic patients, in whom acute or chronic liver failure may occur in the setting of severe sepsis and may impact the biosynthesis of LBP.

Investigators have reported dual biological activity of LBP, namely both pro- and anti-inflammatory properties.[18] In contrast to the upregulation of the LPS-induced cytokine release by LBP, an LPS-neutralizing effect has also been observed at high concentrations of LBP. LBP could suppress endotoxin-triggered cytokine secretion and prevent liver failure, leading to a significantly increased survival rate in endotoxin-challenged mice as well as in a murine model of bacteremia.[19] However, Zweigner et al investigated whether high levels of acute-phase concentrations of LBP in patients with severe sepsis could modulate the LPS-induced TNF-α secretion from monocytes.[41] They found that serum containing high concentrations of LBP from septic patients could reduce TNF-α overproduction, an effect that was reversed by LBP depletion. In agreement of this contention, our results showed that TNF-α levels were significantly lower in the high LBP group.

Interestingly, in patients with sequential episodes of sepsis, LBP response seems to be of lesser magnitude following each consecutive episode of sepsis.[37] This phenomenon may

Table 3
Lipopolysaccharide-binding protein and outcome data in patient subgroups stratified by Child-Pugh scores.

	Low Child-Pugh group[a]			High Child-Pugh group[b]		
	28-d survivors ($n = 18$)	28-d non-survivors ($n = 10$)	p (Mann-Whitney)	28-d survivors ($n = 4$)	28-d non-survivors ($n = 26$)	p (Mann-Whitney)
LBP (ng/mL)	53.0 ± 19.4	38.1 ± 24.1	0.031	67.5 ± 15.3	32.4 ± 16.9	0.005
IL-6 (pg/mL)	138 ± 216	423 ± 412	0.029	243 ± 321	609 ± 715	NS (0.143)
TNF-α (pg/mL)	10.8 ± 9.7	28.7 ± 18.7	0.003	38.8 ± 48.9	77.2 ± 83.0	NS (0.065)
Child-Pugh score	9.00 ± 1.73	10.1 ± 1.10	NS (0.102)	12.75 ± 1.50	13.31 ± 1.05	NS (0.268)

LBP = lipopolysaccharide-binding protein; NS = not significant.
[a] Child-Pugh score ≤ 11.
[b] Child-Pugh score > 11; the value used as cut-off was the median value.

substantiate the prognostic significance of LBP in cirrhotic patients with severe sepsis, in whom low levels of LBP may reflect both impaired hepatic synthesis and accumulating adverse effects of sequential sepsis episodes in susceptible individuals.

The mechanisms behind how LBP prevents endotoxin-induced toxicity remain unclear. It has been shown that LBP *in vivo* is associated with HDL.[17] Moreover, LBP facilitates transfer of LPS into HDL, resulting in a detoxification process.[17,42,43] In this regard, our group has shown that serum levels of HDL are inversely correlated with liver reserve, disease severity, and levels of inflammatory cytokines in cirrhotic patients with severe sepsis.[12] Taken together, impaired synthesis of HDL and LBP from diseased livers may make cirrhotic patients even more susceptible to the toxicity of bacterial products. High levels of endotoxin during sepsis may further overwhelm the already impaired neutralization ability provided by low levels of HDL and LBP and subsequently become even more unopposed, thus perpetuating the overproduction of inflammatory cytokines. A vicious cycle ensues, with further failure of multiple organ functions.

In contrast to subgroup analysis stratified by the Child-Pugh score, the differences in the levels of LBP between survivors and non-survivors did not consistently remain in the sub-group analysis stratified by MELD and SOFA scores (Supplementary Tables 1 and 2). Because both MELD and SOFA scores evaluate extra-hepatic organ function in addition to hepatic function, our findings suggested the significant confounding effects of extra-hepatic dysfunction on the association between LBP and outcomes. In this regard, LBP may impact survival through its pathophysiological link with multiple organ failure. Further studies are needed to clarify this issue.

There are limitations in our study. First, we only measured LBP levels at admission to ICU. As previously discussed, the concentrations of LBP levels may vary significantly over the course of a given septic episode.[21,37,40] Secondly, our study suffers from absence of a non-cirrhotic control group. Therefore, the impact of pre-existing chronic liver failure on LBP levels cannot be elucidated. Finally, the case number is small. We need a bigger cohort to allow better sub-group analyses to evaluate the confounding effects of disease scores on the association between LBP levels and mortality.

In conclusion, low serum levels of LBP are associated with increased concentrations of TNF-α and adverse outcomes of cirrhotic patients with severe sepsis. These findings may shed light on the pathophysiology in cirrhosis with severe sepsis. Whether the levels of LBP can enable clinicians to identify those patients who are at risk for deterioration and in need of timely intervention is unknown. It is also unknown whether recombinant LBP can serve as an adjuvant therapeutic strategy in cirrhosis with endotoxemia.[44] For potential clinical application of LBP, further investigations on the best timing of the testing and dosing regimen in order to achieve a protective effect of LBP are needed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcma.2013.10.006.

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