MAJOR ARTICLE

Lipopolysaccharide-Binding Protein Serum Levels in Patients with Severe Sepsis Due to Gram-Positive and Fungal Infections

Laurent Blairon,[1] Xavier Wittebole,[2] and Pierre-François Laterre[2]

Departments of [1]Clinical Biology and [2]Intensive Care and Emergency Medicine, St.-Luc University Hospital, Brussels, Belgium

Lipopolysaccharide-binding protein (LBP) is increased in patients with severe gram-negative infections, but LBP serum levels have not been reported for in patients with gram-positive and fungal infections. LBP serum levels were determined in patients with severe sepsis secondary to gram-positive or fungal infections and were compared with LBP serum levels obtained from patients with gram-negative mixed infections and from healthy volunteers. Thirty-seven episodes of severe sepsis were analyzed among 24 patients. LBP serum levels were significantly increased in patients with severe sepsis (46.4 ± 28.3 μg/mL), compared with that of healthy volunteers (5.7 ± 1.9 μg/mL; $P < .0001$). On the other hand, LBP serum levels obtained from patients with gram-negative infections (40.80 ± 34.79 μg/mL) did not differ from those obtained from patients with gram-positive (35.55 ± 23.95 μg/mL) or fungal (39.90 ± 22.19 μg/mL) infections. These data suggest that LBP is an aspecific marker of sepsis, and the response was not clearly correlated with severity. Furthermore, in patients with multiple episodes of sepsis, LBP response seems to be of lesser magnitude after each subsequent episode of severe sepsis.

Endotoxin (lipopolysaccharide [LPS]) plays a major role in the genesis of severe sepsis and septic shock [1]. LPS-binding protein (LBP) has been characterized as an acute-phase protein produced by the liver that possesses a binding site for the lipid A moiety of LPS [2, 3]. Interaction between LBP-LPS complex and CD14 macrophage receptor stimulates the production of cytokines responsible for the chain reaction leading to septic shock [4]. Also, interactions between the LBP-LPS complex and CD14 induce the synthesis of soluble CD14 to bind to endothelial cells, polymorphonuclear cells, and lymphocytes [1]. Moreover, endotoxemia is associated with elevated LBP serum levels [5, 6].

It has been shown that LBP plays an important role in the inflammatory response that is secondary to gram-negative bacterial infections [7]. LBP serum levels significantly increase in gram-negative bacteremia in baboons [7] and humans [8] and in patients with systemic inflammatory response syndrome (SIRS) [9].

Several studies have reported increased LBP serum levels in adults and in neonates with gram-positive infections [6, 10–13]. In one study performed in patients with chronic renal failure and peritonitis secondary to gram-positive agents, increased LBP serum levels have been observed in peritoneal fluid samples, but there were no data for concomitant serum levels [14]. Kaden et al. [10] have reported an increase in LBP serum levels in 2 patients with *Pneumocystis carinii* and mycotic superinfection, who had undergone transplantation, but no detail is available for LBP serum levels in pure my-

Received 17 June 2002; revised 25 September 2002; electronically published 19 December 2002

The study protocol was approved by the ethics committee of St.-Luc University Hospital. Informed consent was obtained from the patient or from the patient's relatives.

Reprints or correspondence: Dr. Pierre-François Laterre, Département des Soins Intensifs et Urgences, Cliniques Universitaires St.-Luc, 10 Avenue Hippocrate, B-1200 Brussels, Belgium (laterre@rean.ucl.ac.be).

The Journal of Infectious Diseases 2003; 187:287–91

0022-1899/2003/18702-0013$15.00

Table 1. Characteristics of infected population (n = 24).

Characteristic	Value
Age, median ± SD, years	57.7 ± 15.2
Sex ratio, M:F	14:10
APACHE II score, median ± SD	16.1 ± 6.8
MODS score, median ± SD	6.5 ± 3.4
Focus (n = 37), no. of episodes	
Pneumonia	15
Peritonitis	8
Isolated positive blood culture	5
Empyema	3
Endocarditis	2
Mediastinitis	1
Meningococcemia	1
Osteomyelitis	1
Pyelonephritis	1
Causative microorganisms, no. of episodes	
Gram negative	13
Gram positive	17
Fungus	5
Mixed gram positive and fungus	2
Mixed with gram negative	0
Positive blood cultures, no. (%)	13 (33.3)

NOTE. MODS, multiple organ dysfunction syndrome.

cotic infections. Nevertheless, none of these previous studies have clearly compared LBP serum levels in gram-negative, gram-positive, and fungal infections.

In the present study, we compared LBP serum levels in patient with sepsis due to gram-negative and gram-positive bacteria and fungi. LBP serum levels also were determined and compared with those of patients with multiple episodes of severe sepsis.

PATIENTS AND METHODS

Setting and study design. This prospective study was performed in a 7-bed unit in medical-surgical intensive care unit (ICU) of a 900-bed teaching hospital. All patients admitted with or developing severe sepsis or a septic shock admitted between 24 February 2001 and 5 July 2001 to the ICU were included in the study. A control group was comprised of 18 healthy volunteers from the Department of Clinical Biology (St.-Luc University Hospital, Brussels, Belgium). Severe sepsis and septic shock were defined according to the American College of Chest Physician/Society of Critical Care Medicine Consensus Conference [15]. Proven infection was defined by a positive culture in blood, in bronchoalveolar or bronchial aspirate samples associated with a new chest infiltrate, or in a normally sterile fluid sample. APACHE II and multiple organ dysfunction syndrome (MODS) scores were measured for each patient [16, 17].

Laboratory analysis. Blood samples were collected daily from each patient to determine LBP and interleukin (IL)–6 levels. We analyzed levels at baseline, which was defined as the first 24 h in which proven infection and severe sepsis criteria were met. We then compared those levels in survivors and nonsurvivors, healthy volunteers, and patients with gram-negative, gram-positive, and/or fungal infections. We looked for a correlation among age, hepatic dysfunction, and presence of a positive blood culture. LBP and IL-6 serum levels were determined daily as follow-up until day 5 or death, to compare levels between survivors and nonsurvivors. In the control group, only 1 sample was collected. Samples were centrifuged at 700 *g* for 10 min, decanted, aliquoted, and then frozen at −20°C. LBP and IL-6 serum levels were determined in duplicate by an immunoluminometric assay, using the LBP and IL-6 kits on an Immulite One automate (Diagnostic Product Corporation) [18]. LBP assessment on Immulite shows a good correlation with a previously used ELISA, however, with a slight drift (1.9 μg/mL). The Immulite method requires 10 μL serum diluted in 100 μL LBP of sample diluent LLBZ4 (dilution 1:101). The prediluted sample is pipetted into a test unit containing a bead coated with monoclonal murine anti–LBP antibody. The LBP sample then was incubated under intermittent agitation with an alkaline phosphatase–labeled polyclonal rabbit anti–LBP antibody over a 30-min cycle at 37°C. Unbound serum was removed by centrifugal wash. Sustained light emission was detected after injection of a phosphate ester of adamantyl dioxetane (chemiluminescent substrate) and was proportional to LBP serum levels. LBP minimal threshold of detection was 0.2 μg/mL. No decrease in the relative light unit curve (RLU)

Table 2. Microorganisms found in samples.

Microorganisms	No. of samples
Pseudomonas aeruginosa	5
Escherichia coli	4
Stenotrophomonas maltophilia	2
Legionella	1
Klebsiella species	1
Enterobacter species	1
Neisseria meningitidis	1
Enterococcus species	10
Staphylococcus aureus	7
Coagulase-negative *Staphylococcus* species	2
Corynebacterium species	1
Candida species	7

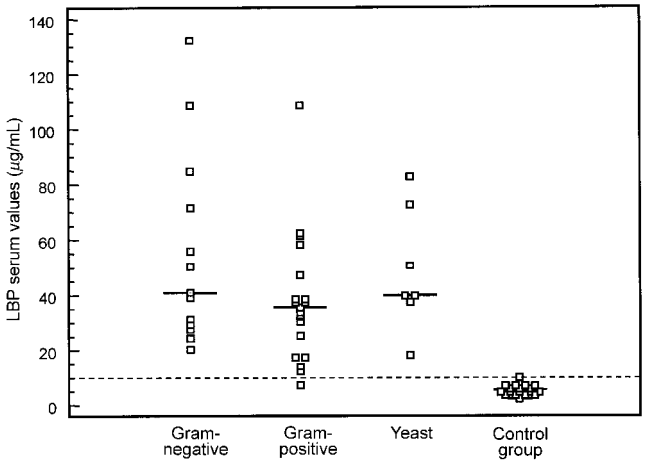

Figure 1. Lipopolysaccharide-binding protein (LBP) distribution at baseline, according to the causative agent of infection and in control group. Solid bars indicate median. Dashed line indicates LBP cutoff (10 μg/mL) suggested by the manufacturer.

has been observed for antigen levels up to 934 μg/mL. IL-6 measurement on Immulite was assessed according to the same mechanism as LBP, with the exception that the IL-6 sample underwent a 2-cycle incubation. The serum volume for measurement in duplicate was at least 450 μL, and no predilution was required. The lower limit of detection was 5 pg/mL. No Hook effect was known for very high levels.

Statistics. Statistical analysis was done by use of MedCalc Software version 6.00.012. Results are expressed as median ± SD. Data have been compared with a Mann-Whitney *U* test. $P < .05$ was considered to be significant.

RESULTS

Thirty-seven episodes of severe sepsis occurred in 24 patients. Characteristics of the population are listed in table 1. The observed mortality was 37.5% (9/24 patients). The cause of death was refractory septic shock and MODS in 8 patients and brain herniation in 1 patient. In 1 patient with pneumonia due to *Legionella pneumophila,* the diagnosis was established by a research of urinary antigens using an EIA method (Biotest). Pneumonia was the leading cause of severe sepsis (40.5%), followed by peritonitis (21.6%). In 5 (13.5%) episodes, microorganisms were cultured only from blood samples. In 13 episodes, severe sepsis was associated with a positive blood culture. The different microorganisms found in our patients are summarized in table 2; gram-positive microorganisms and fungus were the only involved pathogens in 17 (46%) and 5 (13.5%) episodes, respectively. In 2 patients, we reported a coinfection with *Enterococcus* species and fungus.

LBP serum levels were significantly higher in infected patients (46.4 ± 28.3 μg/mL), compared with those in the control group (5.50 ± 1.91 μg/mL; $P < .0001$). Serum LBP and IL-6 levels in control group did not exceed the cutoff levels provided by the manufacturer (LBP, <10 μg/mL; IL-6, <5 pg/mL) [19]. There was no significant difference between LBP serum levels at baseline in patients with gram-negative infections (40.80 ± 34.79 μg/mL) and those in patients with gram-positive (35.55 ± 23.95 μg/mL; $P = .063$) and fungal (39.90 ± 22.19 μg/mL; $P = .139$) microorganisms (figure 1; table 3). Slightly lower LBP serum levels were encountered in nonsurvivors than in survivors at baseline, as described elsewhere [6], but the evolution was similar in the 2 groups (figure 2). On the other hand, IL-6 serum levels at baseline (figure 3) were higher in nonsurvivors than in survivors ($P < .05$). LBP serum levels at baseline did not differ in patients with positive blood culture (38.5 ± 31.0 μg/mL) from that in patients without positive blood culture (39.1 ± 27.7 μg/mL; $P = .74$). We did not encounter any influence of age on LBP levels (Spearman's coefficient, $r = 0.002$; $P = .993$; aged <65 years, 35.55 ± 38.44 μg/mL; aged >65 years, 39.55 ± 15.68 μg/mL). We also failed to find a correlation with hepatic dysfunction, as defined by bilirubinemia (Spearman's coefficient $r = -0.146$; $P = .38$), International Normalized Ratio (INR; Spearman's coefficient $r = 0.048$; $P = .774$), and levels in cirrhotic and noncirrhotic patients (42.98 ± 25.95 μg/mL vs. 38.50 ± 29.21 μg/mL; $P = .71$). No correlation was observed between LBP serum levels and severity, as defined by APACHE II (Spearman's coefficient, $r = -0.222$; $P = .298$) and MODS scores (Spearman's coefficient, $r = -0.024$; $P = .910$).

Seven patients had ⩾2 episodes of severe sepsis during the same hospital stay that were separated by a period of recovery. With the exception of 1 patient, subsequent severe sepsis epi-

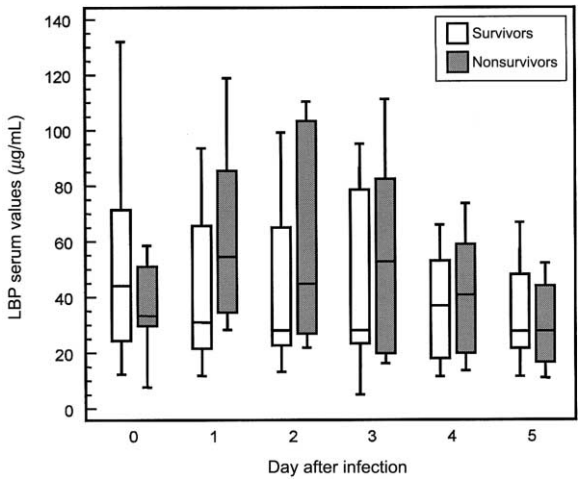

Figure 2. Baseline and follow-up lipopolysaccharide-binding protein (LBP) serum levels in survivors and nonsurvivors. Data are median ± SD.

Table 3. Lipopolysaccharide-binding protein (LBP) and interleukin (IL)–6 serum levels at baseline.

Infection	LBP, μg/mL	IL-6, pg/mL
Gram negative	40.80 ± 34.79 (20.30–132.00)	360.00 ± 347.95 (31.80–1000)
Gram positive	35.55 ± 23.95 (7.30–108.50)	84.40 ± 222.71 (5.00–1000)
Fungus	39.90 ± 22.19 (18.15–82.70)	190.00 ± 274.07 (84.40–870.00)
Control group	5.50 ± 1.91 (2.30–10.00)	<5

NOTE. Data are median ± SD (minimum–maximum).

sodes were associated with an LBP serum level lower than the previous one (figure 4).

DISCUSSION

This study showed that increased LBP serum levels in patients with gram-positive and fungal infections had the same magnitude as patients with gram-negative infections. Subsequent episodes of severe sepsis in the same patient were associated with a lower response in LBP at baseline.

Until now, there have been limited data on LBP serum levels in gram-positive and fungal infections [6, 10–13]. Opal et al. [6] described increased LBP serum levels in patients with gram-positive and fungal infections, but LBP serum levels in patients with gram-negative bacteremia were significantly higher. Furthermore, data for LBP in patients with gram-positive and fungal bloodstream infections were not clearly separated. In our study, we found no difference in LBP serum levels regarding the type of the causative microorganism. The same team reported significantly lower LBP serum levels in nonsurvivors, compared with that in survivors, within 24 h of sepsis onset and at day 28. In our study, the same observation was made at baseline, but the difference was not significant during the follow-up period. Zweigner et al. [11] and Froon et al. [12] found no difference in peak LBP concentration in patients with gram-positive or gram-negative infections, but gave no data regarding LBP levels in patients with mycotic infections. Froon et al. [12] observed no difference in LBP levels between survivors and nonsurvivors, but they only considered peak levels and not baseline levels. This observation was compatible with the fact that we observed an attenuation of the difference in LBP levels between survivors and nonsurvivors after the first 24 h of sepsis onset. Zweigner et al. [11] did not analyze LBP levels in regards to survival. One study performed in patients with chronic ambulatory peritoneal dialysis has described elevated LBP serum levels in peritoneal efflux of patients suffering from gram-negative and gram-positive peritonitis [14], but no corresponding LBP serum levels were provided. Kaden et al. [10] described elevated LBP serum levels in 2 patients, who had undergone kidney transplantation, with *Pneumocystis carinii* pneumonia complicated by mycotic superinfection. However, no information on pure or mixed mycotic infections due to common fungus, such as *Candida* species, were given. These cases did not permit to distinguish the respective roles of *P. carinii* and of the mycotic agents in LBP increase. Furthermore, no information was available on the type of fungi involved. Our study, despite its statistical limitation due to the number of cases, showed that LBP serum levels are increased with the same magnitude in infections caused by fungi as that in infections caused by other microorganisms.

There was no clear association between LBP and advanced age. These results are in accordance with those of Zweigner et al. [11]. On the other hand, Opal et al. [6] found more elevated levels in people aged <63 years, despite a similar APACHE II score. Because of the hepatic synthesis of LBP, we found it pertinent to examine the relationships between LBP and biological markers of liver function as INR and bilirubin, but we failed to find an association between these parameters. No data regarding this subject were found in the literature.

LBP previously has been recognized as a marker of overall inflammation increasing in SIRS [9] or MODS [20]. LBP also has been shown to increase in other nonspecific inflammation syndromes, such as side effects of antiparasitic drugs [21], hemorrhagic colitis, and hemolytic uremic syndrome [22] or after

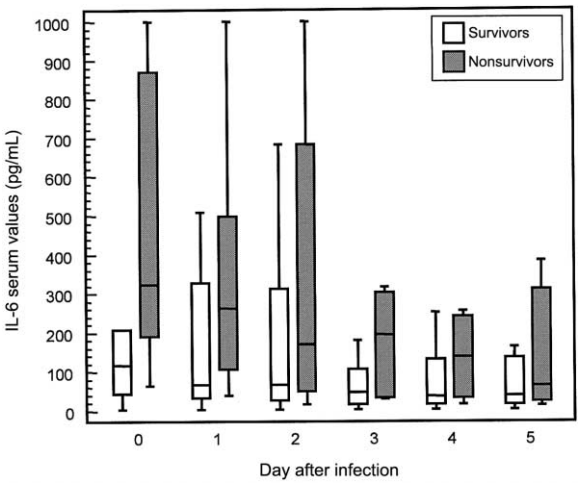

Figure 3. Baseline and follow-up interleukin (IL)–6 serum levels in survivors and nonsurvivors (median ± SD).

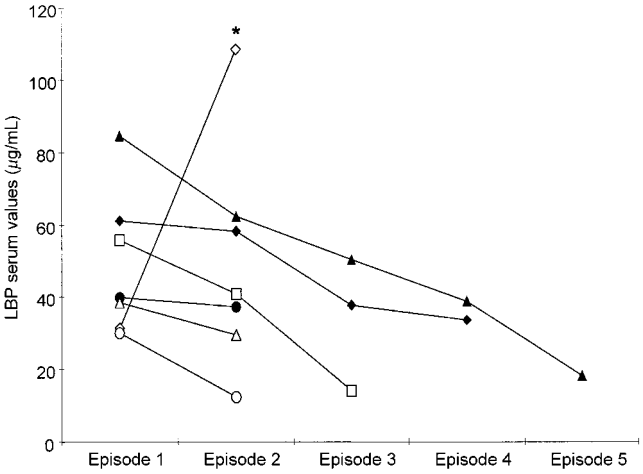

Figure 4. Baseline lipopolysaccharide-binding protein (LBP) serum levels in 7 patients with >1 episode of sepsis. Episodes are separated by a period of recovery. *, The only nonsurvivor patient.

cardiopulmonary bypass surgery [23]. Because of these various causes of elevated LBP serum levels, it appears logical that increases in LBP levels in patients with severe sepsis does not depend on the causative microorganism, as confirmed by the present and previous works. In our study, LBP serum levels were not correlated with severity scores, as opposed to IL-6 serum levels. The additional observation made in our study is that the LBP response was attenuated in patients with multiple episodes of sepsis during the same hospital stay. With the exception of a patient dying of brain herniation after recurrent septic shock, the LBP response appears to be attenuated after each new subsequent episode of severe sepsis. This phenomenon could be viewed as a desensibilization due to a possible decreased synthesis of receptors, but more work is needed to confirm these data.

In conclusion, LBP serum levels are similar in severe sepsis due to gram-negative and gram-positive bacteria, as well as fungi. We confirm that LBP should be viewed as a nonspecific marker of the acute-phase response and should not be used as a diagnostic tool of the type of microorganism responsible of the infection. Finally, LBP response seems attenuated after repeated episodes of severe sepsis.

References

1 Glauser MP, Zanetti G, Baumgartner JD, Cohen J. Septic shock: pathogenesis. Lancet **1991**; 338:732–6.

2 Schumann RR, Leong SR, Flaggs GW, et al. Structure and function of lipopolysaccharide-binding protein. Science **1990**; 249:1429–31.

3 Tobias PS, Soldau K, Ulevitch R. Isolation of a lipopolysaccharide–binding acute-phase reactant from rabbit serum. J Exp Med **1986**; 164: 777–93.

4 Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS-binding protein. Science **1990**; 249:1431–5.

5 Gallay P, Heumann D, Le Roy D, Barras C, Glauser MP. Lipopolysaccharide-binding protein as a major plasma protein for endotoxemic shock. Proc Natl Acad Sci USA **1993**; 90:9935–8.

6 Opal SM, Scannon PJ, Vincent JL, et al. Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock. J Infect Dis **1999**; 180:1584–9.

7 Haudek SB, Natmessnig BE, Redl H, Schlag G, Hatlen LE, Tobias PE. Isolation, partial characterization, and concentration in experimental sepsis of baboon lipopolysaccharide binding protein. J Lab Clin Med **2000**; 136:363–70.

8 Geller DA, Kispert PH, Su GL et al. Induction of hepatocyte lipopolysaccharide binding protein in models of sepsis and the acute phase response. Arch Surg **1993**; 128:22–8.

9 Myc A, Buck J, Gonin J, Reynolds B, Hammerling U, Emanuel D. The level of lipopolysaccharide-binding protein is significantly increased in patients with the systemic inflammatory response syndrome. Clin Diagn Lab Immunol **1997**; 4:113–6.

10 Kaden J, Zwerenz P, Lambrecht HG, Dostatni R. Lipopolysaccharide-binding protein as a new and reliable infection marker after kidney transplantation. Transpl Int **2002**; 15:163–72.

11 Zweigner J, Gramm HJ, Singer OC, Wegscheider K, Schumann RR. High concentrations of lipopolysaccharide-binding protein in serum of patients with severe sepsis or septic shock inhibit the lipopolysaccharide response in human monocytes. Blood **2001**; 98:3800–8.

12 Froon AHM, Dentener Ma, Greve JWM, Ramsay G, Buurman WA. Lipopolysaccharide toxicity-regulating proteins in bacteremia. J Infect Dis **1995**; 171:1250–7.

13 Berner R, Furll B, Stelter F, Drose J, Muller HP, Schutt C. Elevated levels of lipopolysaccharide-binding protein and soluble CD14 in plasma in neonatal early-onset sepsis. Clin Diagn Lab Immunol **2002**;9: 440–5.

14 Schäfer K, Schumann RR, Stöteknuel S, Schollmeyer P, Dobos GJ. Lipopolysaccharide-binding protein is present in effluents of patients with gram-negative and gram-positive CAPD peritonitis. Nephrol Dial Transplant **1998**; 13:969–74.

15 American College of Chest Physician/Society of Critical Care Medicine Consensus Conference. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med **1992**; 20:864–74.

16 Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med **1985**; 13:818–29.

17 Marshall JC, Cook DJ, Christou NV, Bernard GR, Sprung CL, Sibbald WJ. Multiple Organ Dysfunction Score: a reliable descriptor of a complex clinical outcome. Crit Care Med **1995**; 23:1638–52.

18 Olson DR, Palmieri T, Ross AF, Becker DM, Mulqueen PJ. The Immulite assay tube: a new approach to heterogenous ligand assay. Clin Chem **1991**; 37:1521–2.

19 Meszaros K, Aberle S, White M, Parent B. Immunoreactivity and bioactivity of lipopolysaccharide-binding protein in normal and heat-inactivated sera. Infect Immun **1995**; 63:363–5.

20 Sablotski A, Borgermann J, Baulig W, et al. Lipopolysaccharide-binding protein (LBP) and markers of acute-phase response in patients with multiple organ dysfunction syndrome (MODS) following open heart surgery. Thorac Cardiovasc Surg **2001**; 49:273–8.

21 Haarbrink M, Abadi GK, Buurman WA, Dentener MA, Terhell AJ, Yazdanbakhsh M. Strong association of interleukin-6 and lipopolysaccharide-binding protein with severity of adverse reaction after diethylcarbamazine treatment of microfilaremic patients. J Infect Dis **2000**; 182:564–9.

22 Proulx F, Seidman E, Mariscalco MM, Lee K, Caroll S. Increased circulating levels of lipopolysaccharide binding protein in children with *Escherichia coli* O157:H7 hemorrhagic colitis and hemolytic uremic syndrome. Clin Diagn Lab Immunol **1999**; 6:773.

23 Bouma M, Maessen J, Weerwind P, et al. Release of lipopolysaccharide toxicity-modulating proteins in patients undergoing cardiopulmonary bypass using noncoated and heparin-coated extracorporeal circuits. Chest **1997**; 111:557–83.