MEDICAL SCIENCE MONITOR

Variation of Circulating Inflammatory Mediators in *Staphylococcus aureus* and *Escherichia coli* Bloodstream Infection

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF 1 **Jinyan Duan***
BCDEF 1,2 **Yinjing Xie***
D 3 **Jiyong Yang**
DF 3 **Yanping Luo**
C 1 **Yuni Guo**
AG 1 **Chengbin Wang**

1 Department of Clinical Laboratory Medicine, Chinese PLA General Hospital, Beijing, P.R. China
2 Department of Laboratory Medicine, Shenzhen People's Hospital, Shenzhen, Guangdong. P.R. China
3 Department of Microbiology, Chinese PLA General Hospital, Beijing, P.R. China

Corresponding Author:
Source of support:

* These authors contributed equally to this work
Chengbin Wang, e-mail: wangcb301@126.com
This work was supported by a grant from the National Key Technology Support Program (2012BA119B05)

Background:
The aim of this study was to examine the behavior of circulating inflammatory mediators and to exclude gram-positive from gram-negative bloodstream infections. Results may be helpful in selection of optimal specific antibiotic therapies.

Material/Methods:
Mice (25–27 g) were randomized to 3 groups infected with *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Escherichia coli* (*E. coli*) ATCC 25922, or phosphate-buffered saline (PBS). The white blood cell count (WBC) and the concentrations of serum C-reactive protein (CRP), procalcitonin (PCT), interleukin (IL)-1α, IL-1β, IL-6, IL-10, monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1α (MIP-1α) were detected in blood samples at different time intervals after intravenous tail injection.

Results:
The results showed that compared to the control mice, infected animals exhibited significantly higher levels of all mediators after bacterial infection. Moreover, compared to the mice that received *S. aureus*, animals with *E. coli* infection showed significantly greater increases in serum IL-1α, IL-1β, IL-6, MCP-1, and MIP-1α levels.

Conclusions:
These results suggest that the use of the analyzed serum markers at an early stage of bloodstream infection may give useful information for the clinician to distinguish gram-negative from gram-positive infections.

MeSH Keywords:
Bacterial Infections • *Escherichia coli* Infections • *Staphylococcus aureus*

Full-text PDF:
http://www.medscimonit.com/abstract/index/idArt/896576

📄 2945   ▦ —   📊 6   📚 48

Background

Bloodstream infections are characterized by high mortality and morbidity [1]. Bacterial infections may spread via the bloodstream with the consequent development of systemic inflammatory response syndrome (SIRS), and even sepsis, which is a widespread and uncontrolled inflammatory response and a major cause of morbidity and mortality [2]. Currently, the most important bloodstream infections are caused by gram-negative and gram-positive bacteria. The clinical signs and symptoms are almost indistinguishable between these 2 types of infection [3]. Hence, in the majority of these bloodstream-infected patients, the causative infectious agents cannot be identified due to the nonspecific signs and symptoms [4]. Therefore, it is customary to start empirical broad-spectrum antimicrobial therapy [5], but this contributes to the emergence of drug-resistant strains of bacteria and leads to increased medical costs. Several studies showed that early use of selective antibiotics can effectively reduce the mortality of bloodstream infection [6–8]; therefore, the differential diagnosis of bloodstream infections as early as possible to assist the selective use of antibiotics is particularly important during clinical trials [9,10].

Early diagnosis of bloodstream infections remains problematic. Although the "gold standard" of bacterial detection is blood culture, in nearly half of neutropenic fever episodes in children with cancer, no microorganisms were isolated [11]. Moreover, lower positive rate, higher false-negative and false-positive rates, and longer detection period limit the diagnostic value of blood culture [12,13]. Traditional diagnostic indicators of blood infection – white blood cell count (WBC), erythrocyte sedimentation rate, and body temperature – are not sufficiently sensitive and specific to meet clinical needs [14,15]. Molecular biology is a modern diagnostic approach to bloodstream infections that warrants increased research attention [16]. Overtreatment of these patients without a confirmed bacterial infection results in increased risk of bacterial resistance. The specificity and sensitivity of tests for bloodstream infection are limited. In an attempt to improve diagnosis of bloodstream infections, biomarkers such as pro-inflammatory cytokines and acute-phase proteins have become additional diagnostic tools to optimize and expedite the clinical diagnosis.

For the correct diagnosis of bloodstream infections, use of inflammatory mediators provides another means to assist in the diagnosis of infection and predict disease outcome. Thus, more targeted selection of antibiotic treatment can reduce the incidence of drug-resistant pathogens and prevent deterioration of the patient's condition. Circulating inflammatory mediators, especially C-reaction protein (CRP), procalcitonin (PCT), and interleukin (IL)-6, have been suggested to be predictors of systemic microbial bloodstream infection [17–20]. However, the

early cellular response to gram-negative and gram-positive bacteria is still unclear, and little is known about the differentiation between gram-positive and gram-negative bacterial infections using circulating inflammatory mediators.

Clinical data on the detection of pathogens in patients with bloodstream infections show that the highest detection rates of gram-positive and gram-negative bacteria were in *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), respectively [21,22]. More than 50% of sepsis was caused by *S. aureus* and *Staphylococcus epidermidis* [23]. *S. aureus* is widely present in air and water, as well as in human and animal excrement. *S. aureus* is present on more than 50% of healthy human skin [24], and it is also one of the most common pathogens causing hospital-acquired and community-acquired infections. It is the cause of a variety of human diseases, including sepsis, endocarditis, food poisoning, and toxic shock syndrome [25]. With the widespread use of antibiotics, resistant strains of *S. aureus* have been rapidly increasing, and the problem of methicillin-resistant *Staphylococcus aureus* (MRSA) has caused worldwide concern [26,27]. *E. coli* is the most important human intestinal flora of parasites. It is also the most common gram-negative bacilli causing infectious diseases, it is the main pathogen in a variety of common bacterial infections in humans, and is among the top 3 pathogens causing bloodstream infections, abdominal bowel tract infections, neonatal meningitis, and bacterial diarrhea in travelers [28,29]. Drug resistance of *E. coli* continues to increase, has become the human body's largest repository of resistant genes, and is the subject of worldwide research [30].

The present study was designed to focus on *S. aureus* and *E. coli* bloodstream infections, and to compare the induction of PCT, CRP, and IL-6 to discriminate between gram-negative and gram-positive infections. We also compared the induction of inflammatory mediators in infected mice after injection of *S. aureus* ATCC 25923 or *E. coli* ATCC 25922 as models to discriminate between gram-negative and gram-positive bacterial blood stream infections.

Material and Methods

Animals

Specific-pathogen-free male ICR mice purchased from the Beijing Sibeifu Experimental Animal Center, weighing between 25 and 28 g, were used to establish bloodstream infection models. They were kept in a specific-pathogen-free facility. Each mouse was only used for 1 experiment. All mice were acclimated for 1 week before the experiment. Mice were maintained with humane care in accordance with the "Guide for the Care and Use of Laboratory Animals" approved by the Institute of

Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Bacterial strains

Staphylococcus aureus ATCC 25923 and *Escherichia coli* ATCC 25922 were used in these studies. These 2 strains were maintained at –0°C in trypticase soy broth (HBA; Oxoid Ltd., Hampshire, UK) before use in the experiment.

Experimental procedures

To keep the circadian rhythms of the mice identical across the studies, all experiments were conducted at the same time. Mice were weighed and randomly assigned to body weight-matched experimental groups before the experiment. On the experiment day, the mice were separated into 3 treated groups: *S. aureus*, *E. coli*, and PBS (Cambrex, Walkersville, MD, USA). For hematological studies and quantitative determination of inflammatory mediators, separate groups of normal and infected mice were used in a time-course experiment (n=10 mouse/group/time point).

Mouse bloodstream infection model

S. aureus and *E. coli* from frozen stocks were plated on blood agar plates (HBA; Oxoid Ltd., Hampshire, UK). After 24 h of incubation of the agar plates, 1 isolated colony of each strain was transferred to 10 mL of brain-heart infusion broth (HBA; Oxoid Ltd., Hampshire, UK). The broth culture was incubated for 10-12 h at 37°C with continuous shaking. The challenge doses of *S. aureus* and *E. coli* were based on preliminary experiments (data not shown). According to the challenge dose, the broth cultured with *S. aureus* and *E. coli* were diluted to 1×10^8 cfu/mL and 2×10^9 cfu/mL in PBS (Cambrex, Walkersville, MD, USA), respectively, to establish a consistent systemic infection mouse model. Male ICR mice weighing between 25 and 27 g were used in these studies. Mice were inoculated with a strain of *S. aureus* or *E. coli* in a final volume of 0.1 mL/10g of PBS by intravenous tail injection. The time of challenge was designated as time 0 of the experiment. Body weight of each mouse used in this study was measured every day at the same time.

Colony-forming units (CFU) analysis

To confirm that the infection model in mice was successfully established, the CFU analysis was carried out at 1, 3, 6, and 12 h, as well as 1, 2, 3, 5 and 7 days after *S. aureus* or *E. coli* administration.

Histology studies

To assess the host inflammation response of mice to bacterial infection, the liver of each mouse was fixed in 4% neutral formalin solution after being washed with 0.9% NaCl for coronal section with hematoxylin and eosin (H&E) stain.

Mouse WBC counts

At harvest, whole blood of mice was collected into sterile tubes containing K_2EDTA for hematological studies. Total peripheral leukocytes were counted by using an automatic hematological analyzer equipped with veterinary software (BC-2800 Vet; Mindray, Shenzhen, China). WBC was determined at 1, 3, 6, and 12 h, as well as at 1, 2, 3, 5, and 7 days after *S. aureus*, *E. coli*, or PBS administration in mice.

Mouse inflammatory mediators

To measure production of inflammatory mediators in serum, whole blood was collected into sterile tubes and these samples were immediately centrifuged at 2000×g for 20 min at 4°C. The serum was then collected into several small tubes, which were stored at –80°C until the day of analysis (within 2 months). Quantitative determination of mouse CRP and PCT in serum was performed by enzyme-linked immunosorbent assays (ELISA), using specific kits for CRP and PCT (USCN, Wuhan, China). We used multi-analyte suspension arrays (MASA; Bio-Plex, Bio-Rad Laboratories, California, USA) for measurement of IL-1α, IL-1β, IL-6, IL-10, monocyte chemotactic protein-1 (MCP-1), and macrophage inflammatory protein-1α (MIP-1α). They utilize color-coded microspheres or beads, laser technology, and high-speed digital signal processing to determine the concentration of cytokines. All samples were analyzed according to the manufacturer's instructions.

Data analysis

Data were analyzed with SPSS 17.0 (SPSS, Chicago, IL, USA). The normality test of data was performed first; if its distribution was normal, the mean value ± standard deviation (S.D.) was calculated for each treatment group. Data were analyzed by one-way analysis of variance (ANOVA). Student-Newman-Keuls (SNK) post hoc analyses was performed on ANOVA-significant results to analyze the differences in the effect of each treatment. The median, interquartile, and median spacing was determined for abnormal distribution of data for each treatment group. Data are analyzed by non-parametric statistics rank sum test. Integrated areas under the curve (AUC) were calculated by trapezoid analysis and were used to compare the differences in the responses of each mouse to pyrogen and PBS injections [36]. A *p*-value ≤0.05 was considered statistically significant.
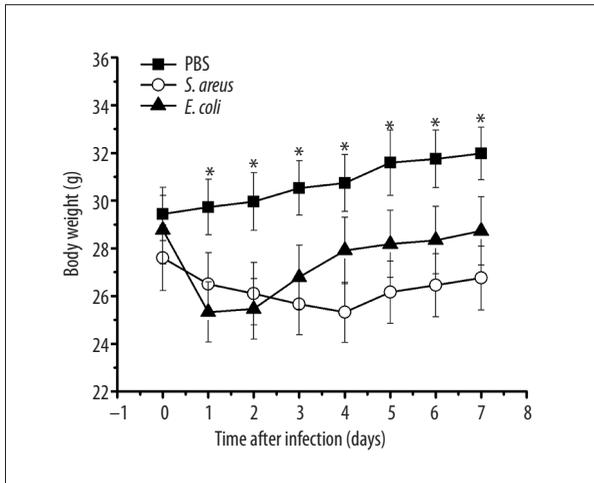
Figure 1. Body weight of mice. The values presented are the means ± standard deviation (S.D.) for 10 per group. Body weight of mice was determined at the same time of day after i.v. injection of either PBS (■), *S. aureus* (○), or *E. coli* (▲). *, $p<0.05$ (significantly different from the control values by one-way analysis of variance).

Results

Effects of infection on body weight of mice

To test the infection in mice, body weight of mice was measured every day at the same time (Figure 1). The mean body weight in the PBS-treated control groups increased throughout the study and was significantly higher than that of the 2 infected groups ($p<0.05$), and it did not differ between the 2 infected groups.

The CFU analysis

The number of CFUs in *S. aureus*-infected mouse blood reached a peak in the first few hours after infection, and decreased rapidly after 12 h. The same trend occurred in the *E. coli*-infected mice (Figure 2).

Liver histology

Pathological changes in mouse livers were observed visually at 24 h after injection with either *S. aureus* or *E. coli* (Figure 3).
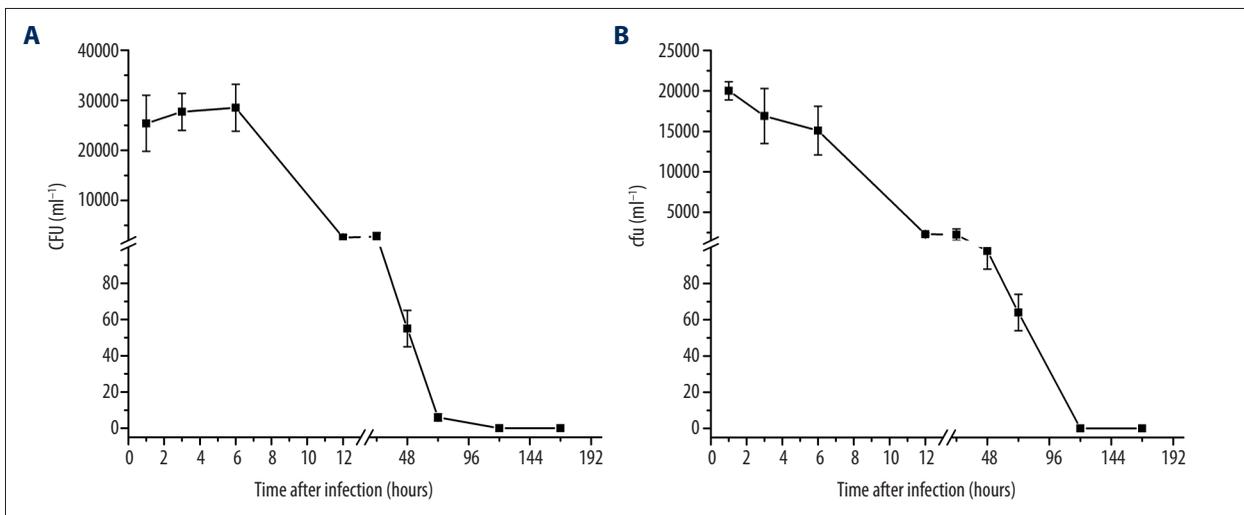
Figure 2. The CFU analysis in blood of infected mice. (**A**) The CFU analysis of mice infected with *S. aureus*; (**B**) The CFU analysis of mice infected with *E. coli*.
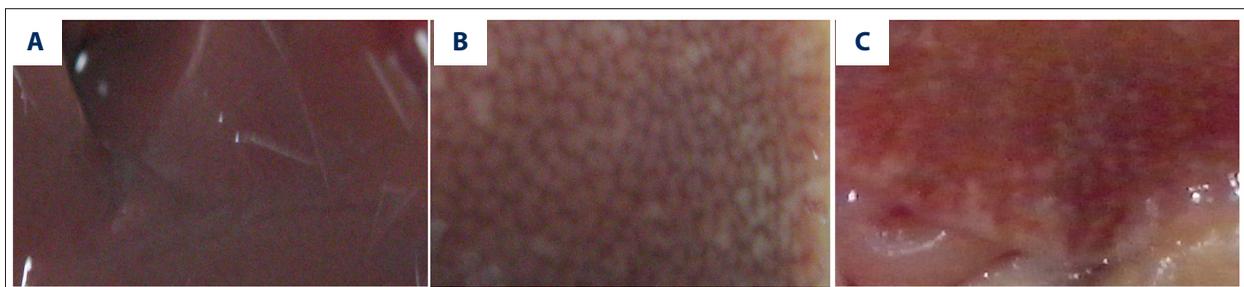
Figure 3. The gross changes of liver. (**A**) Liver of normal mice; (**B**) Liver of mice at 24 h after administration of *E. coli*; (**C**) Liver of mice at 24 h after administration of *S. aureus*. There was much necrosis in the livers of mice at 24 h after administration of *S. aureus* or *E. coli,* as the arrows indicated.
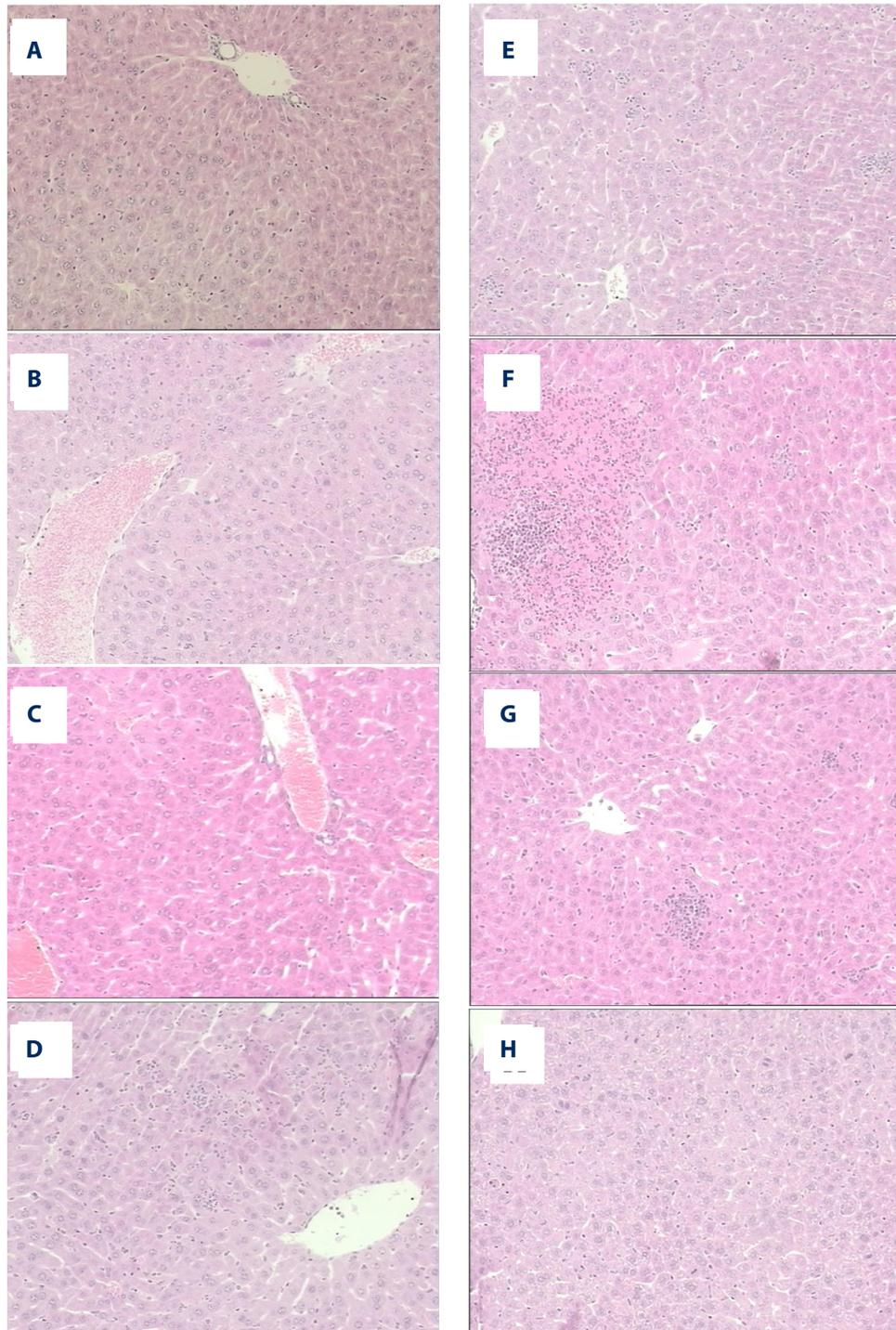
Figure 4A–H. Histological alterations of livers of mice (hematoxylin and eosin stain, ×10). (**A–H**) Livers of mice at 0, 3, 6, 12 h and 1, 2, 3, 5, 7 days after administration of *S. aureus*.
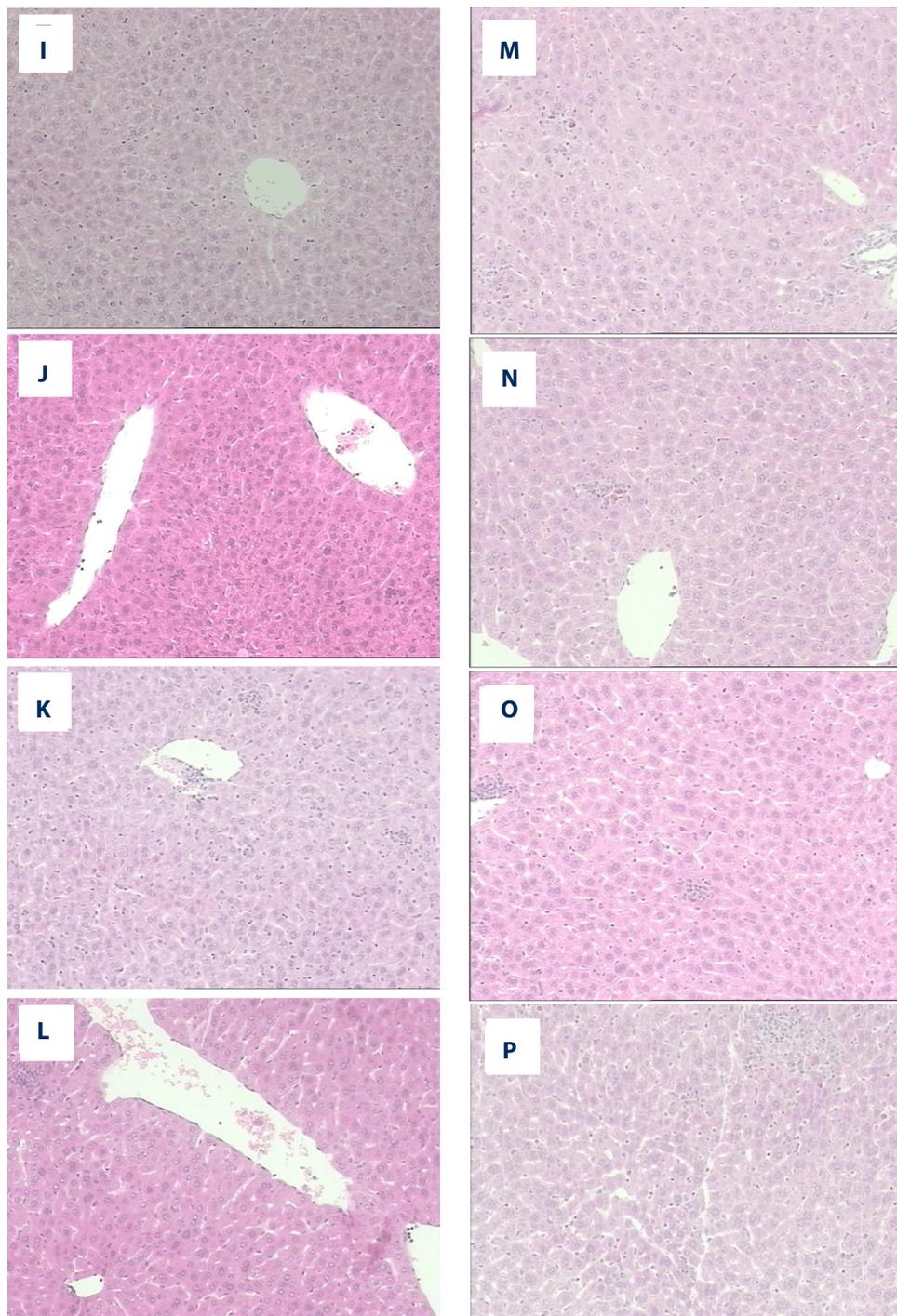
Figure 4I–P. Histological alterations of livers of mice (hematoxylin and eosin stain, ×10). Livers of mice at 0, 3, 6, 12 h and 1, 2, 3, 5, 7 days after administration of *E. coli*.
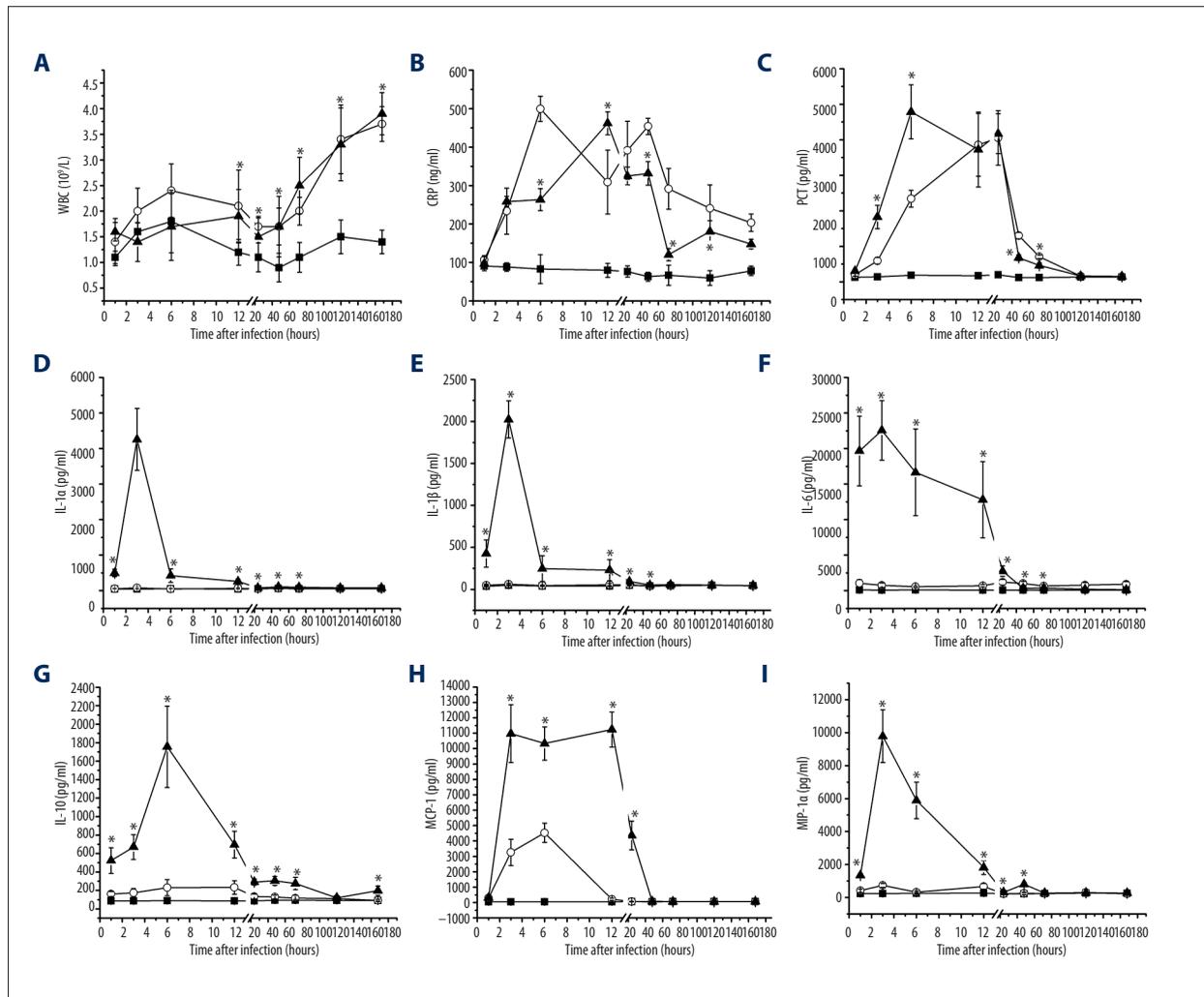
Figure 5. (**A–I**) Effects of *S. aureus* and *E. coli* on WBC and serum CRP, PCT, IL-1α, IL-1β, IL-6, IL-10, MIP-1α, and MCP-1 levels in mice. Serum levels of these mediators in separate groups of mice were determined at 0, 1, 3, 6, 12 h and 1, 2, 3, 5, 7 days after i.v. injection of either PBS (■), *S. aureus* (○), or *E. coli* (▲). Each experiment involved 10 mice per time point. At all of the time points tested after injection of PBS (control), mice showed no significant elevation in serum levels of these cytokines (*p*>0.05). Symbols represent means; bars indicate standard deviation (S.D.). *, *p*<0.05 between *S. aureus*- and *E. coli*- injected mice (ANOVA, Student-Newman-Keuls post hoc test) for the same time point.

Intravenous infection with either *S. aureus* or *E. coli* for 3 h caused leukocyte margination at the vascular side of the liver (Figure 4B, 4J). At 6 h after infection, a small number of leukocytes were observed in the liver tissue (Figure 4C, 4K), and infiltration of many inflammatory cells was observed at 12 h after injection (Figure 4D, 4L). Small inflammatory lesions appeared in the liver at 1 day after infection with either *S. aureus* or *E. coli* (Figure 4E, 4M), and more and larger lesions appeared at 2, 3, and 5 days (Figure 4F–4H, 4N–4P). Seven days after injection, the liver tissues of *S. aureus* or *E. coli* mice were the same as in the normal group.

Levels of WBCs and inflammatory mediators

The kinetics of WBC, CRP, PCT, IL-1α, IL-1β, IL-6, IL-10, MCP-1, and MIP-1α in response to *S. aureus* or *E. coli* infection were studied. Significant differences were found between the production of these mediators in normal and infected mice (*p*<0.05). Figure 5 A shows the effects of i.v. administration of both bacterial strains on the total counts of white blood cells in mice. The absolute leukocyte count was significantly increased at 12 h after challenge with either *S. aureus* or *E. coli* (*p*<0.05), and remained elevated until 5 days after injection. High-level secretion of CRP and PCT was observed in sera of both *S. aureus*- and *E. coli*-infected mice (Figure 5B, 5C). As shown in Figure 5B, both *S. aureus* and *E. coli* evoked a significantly
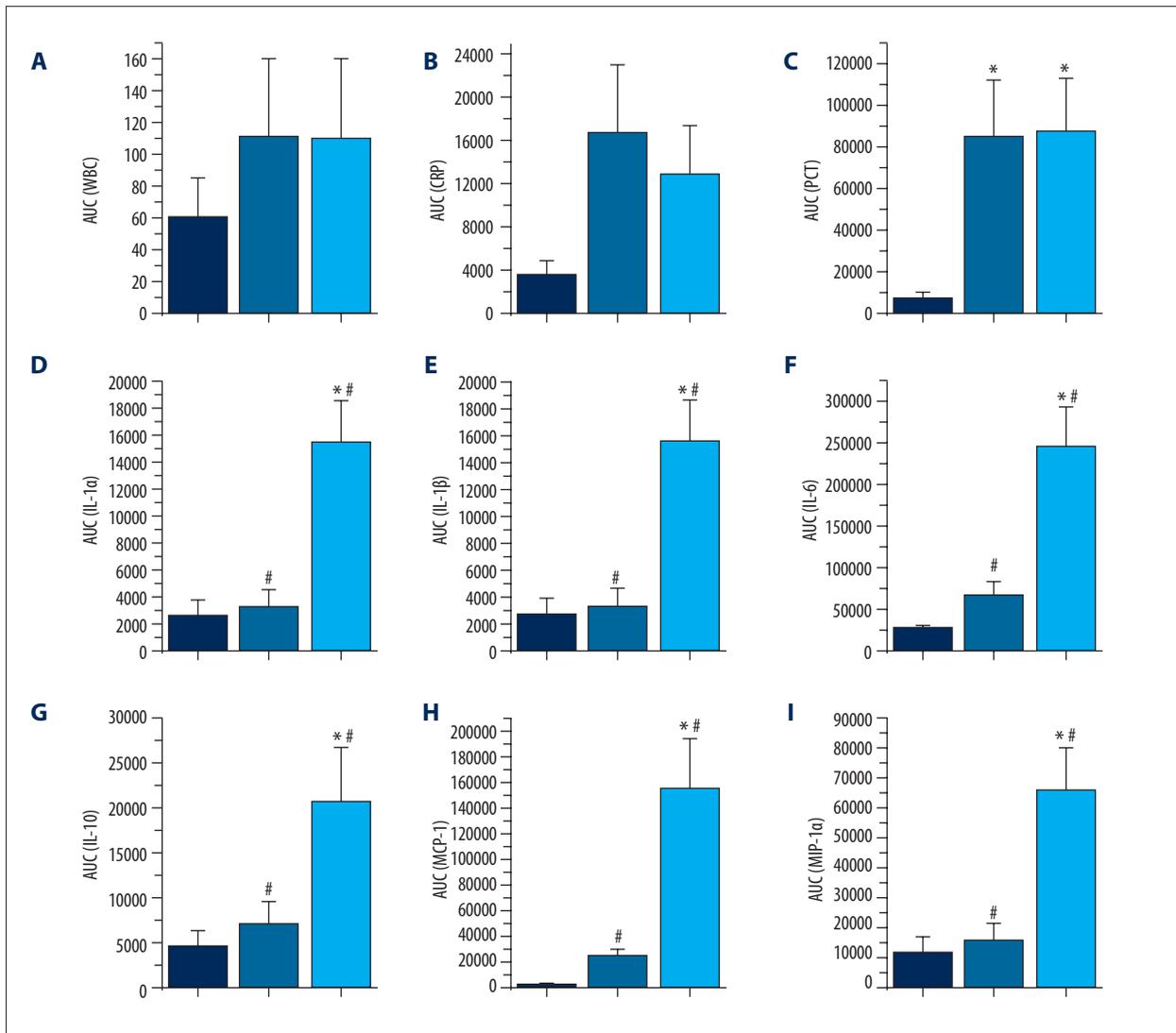
Figure 6. Mean integrated WBC (**A**), CRP (**B**), PCT (**C**), IL-1α (**D**), IL-1β (**E**), IL-6 (**F**), IL-10 (**G**), MIP-1α (**H**), and MCP-1 (**I**) responses of mice after PBS (hatched bars), *S. aureus* (black bars), or *E. coli* (white bars) challenge. Statistical analysis was performed on AUC data from 0 to 168 h after the time of injection and was calculated from the data shown in Figure 4. Bars represent means ± standard deviation (S.D.) for each group of mice (nd, not detected). *, statistically significant differences (*p<0.05*) between *S. aureus*- and *E. coli*- treated mice and their respective PBS-treated groups, respectively. #, significantly different (*p<0.05*) from corresponding values between *S. aureus*- and *E. coli*-treated mice (ANOVA, Student-Newman-Keuls post hoc test).

higher production of CRP in serum of mice at 3 h (*p<0.05*), and reached a peak at 6 to 12 h after injection. Concentrations of PCT (Figure 5C) in serum were found to rise at 1 h (*p<0.05*), reached a peak at 6 to 12 h after injection or either *S. aureus* or *E. coli*, and returned to the pre-injection values at 5 days after challenge. However, unlike WBC, CRP and PCT, the concentrations of IL-1α (Figure 5 D), IL-1β (Figure 5E), IL-6 (Figure 5F), IL-10 (Figure 5G), MCP-1 (Figure 5H), and MIP-1α (Figure 5I) in serum were observed to rise at 1 h (*p<0.05*), and reached a peak at 3 to 6 h after *E. coli* challenge, in contrast to the PBS controls. However, no difference was found between *S.*

aureus-treated groups and *E. coli*-treated groups of mice in these inflammatory mediators (*p>0.05*).

Furthermore, AUC was used to compare the production of inflammatory mediators between *S. aureus*-treated groups and *E. coli*-treated control groups of mice (Figure 6). The results showed that compared to *S. aureus*, injection of *E. coli* evoked a significant increase in IL-1α (Figure 6D), IL-1β (Figure 6E), IL-6 (Figure 6F), IL-10 (Figure 6G), MCP-1 (Figure 6H), and MIP-1α (Figure 6I) levels in mice. In contrast, levels of WBC (Figure 6A), CRP (Figure 6B), and PCT (Figure 6C) failed to discriminate between *S. aureus* infection and *E. coli* infection (*p>0.05*).

Discussion

Microbial pathogenic infections have played a devastating role in human history. There is always a need for proper diagnosis of microbial infection and its administration in the field of medical science. The isolation and identification of pathogens are considered as essential procedures for diagnosing bloodstream infection, which is an important step towards limiting morbidity and mortality [31]. However, blood cultures require at least 24 to 48 h and must be interpreted carefully by experts due to loss of sensitivity and specificity [32–34]. Circulating inflammatory mediators such as PCT, CRP, and IL-6 have been repeatedly evaluated for the accurate and prompt diagnosis of bloodstream infections [31,35].

The present study was specifically designed to find inflammatory mediators that differentiate gram-positive from gram-negative infections in order to administer the correct antibiotic therapies. The *S. aureus* and *E. coli* bloodstream infection mouse models were established and confirmed by CFU analysis. The pathologic histology check showed that liver inflammation occurred in the infected mice, and the situation progressively worsened in the first few days, which caused the inflammatory lesions appearing on the liver. The symptom of inflammation was further evidence substantiating the successful establishment of the bloodstream infection mouse models.

The results of the animal experiments demonstrate that an advantage of the serum IL-1α, IL-1β, IL-6, IL-10, MIP-1α, MCP-1, CRP, and PCT detection over blood culture is its rapid performance; it takes only a few hours to perform inflammatory mediator assays, which is significantly quicker than blood culture incubation. Moreover, we found that increased serum IL-1α, IL-1β, IL-6, IL-10, MIP-1α, and MCP-1 can differentiate *E. coli* from *S. aureus* stimuli in mice, but CRP and PCT are not specific mediators to discriminate between *E. coli* and *S. aureus* infections. This suggests that IL-1α, IL-1β, IL-6, IL-10, MIP-1α, and MCP-1 may be able to differentiate gram-positive from gram-negative infections. Although this is unique and of considerable importance, further studies on the contribution of these markers in discrimination between gram-positive and gram-negative infections are necessary to verify these findings. The present knowledge regarding inflammatory mediators in infectious patients is still limited but indicates that the host response to microbial infection is mediated by the release of inflammatory mediators into the bloodstream [36–38]. However, little is known about the mechanism to exclude gram-positive from gram-negative infections by using these circulating inflammatory mediators. In most relevant studies, infections were induced by the major constituent of cell walls of bacteria, like the lipopolysaccharide (LPS, endotoxin) of gram-negative bacteria, and the peptidoglycan or lipoteichoic acid of gram-positive bacteria. The pathogenesis of gram-positive and gram-negative infections induced by living bacteria has not yet been discovered [3,39,40]. Thus, in this study, we used experimental models induced by *S. aureus* and *E. coli* to mimic the pathophysiologic events that possibly occur in patients with bloodstream infection.

To confirm the bloodstream infections, models were successfully established, and we monitored the body weight and performed the histological evaluation of the livers of infected mice. The present results show that the increase in body weight of infected mice was significantly slower than that of the control group. Furthermore, significant indications of inflammatory cells and focus of infection were obvious on the livers of the infected mice. Both the body weight results and the histological evaluation indicate that the bloodstream infection mouse model was successfully established.

The present results indicate that cytokines such as IL-1α, IL-1β, IL-6, IL-10, MIP-1α, and MCP-1 are able to discriminate between *S. aureus* and *E. coli* infection, which confirms previous experimental and clinical studies showing that the production of inflammatory mediators was different between gram-positive and gram-negative infections [41–44]. Several reports showed that the differences in the cytokine pattern responses to gram-positive and gram-negative bacteria may correspond to the different cell surface receptors stimulated by different bacteria [45]. There is evidence to support that LPS in the gram-positive bacterial cell walls in complex with LPS-binding protein interacts with CD14 and the tri-molecular complex binds to Toll-like receptor 4 (TLR-4), while LAT and peptidoglycan in gram-negative bacteria interact with TLR-2 [46–48].

Conclusions

The present findings illustrate the potential of IL-1α, IL-1β, IL-6, IL-10, MIP-1α, and MCP-1 in distinguishing gram-positive from gram-negative infections. Although further studies using a variety of gram-positive and gram-negative bacteria to verify this hypothesis are still necessary, this study successfully established experimental methods for use in further studies. We will collect more clinical specimens to provide additional evidence to support the diagnostic use of these circulating inflammatory mediators in clinical practice. Our ultimate goal is to provide evidence to guide the choice of specific antibiotic therapies before culture results are available.

References:

1. Rathinam VA, Vanaja SK, Waggoner L et al: TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by gram-negative bacteria. Cell, 2012; 150: 606–19

2. Giamarellos-Bourboulis EJ, Scaglione F: Presentations from the 4th European Conference on Bloodstream Infections Introduction. Int J Antimicrob Agents, 2010; 36(Suppl.2): S1

3. Draing C, Sigel S, Deininger S et al: Cytokine induction by Gram-positive bacteria. Immunobiology, 2008; 213: 285–96

4. McCann CD, Moore MS, May LS et al: Evaluation of real-time PCR and pyrosequencing for screening incubating blood culture bottles from adults with suspected bloodstream infection. Diagn Microbiol Infect Dis, 2015; 81: 158–62

5. Yoshida J, Harada Y, Kikuchi T et al: Does antimicrobial use density at the ward level influence monthly central line-associated bloodstream infection rates? Infect Drug Resist, 2014; 7: 331–35

6. Kara A, Devrim I, Bayram N et al: Risk of vancomycin-resistant enterococci bloodstream infection among patients colonized with vancomycin-resistant enterococci. Braz J Infect Dis, 2015; 19: 58–61

7. Wareham DW, Bean DC, Khanna P et al: Bloodstream infection due to Acinetobacter spp: epidemiology, risk factors and impact of multi-drug resistance. Eur J Clin Microbiol Infect Dis, 2008; 27: 607–12

8. Vardakas KZ, Apiranthiti KN, Falagas ME: Antistaphylococcal penicillins versus cephalosporins for definitive treatment of meticillin-susceptible *Staphylococcus aureus* bacteraemia: A systematic review and meta-analysis. Int J Antimicrob Agents, 2014; 44: 486–92

9. Chiu CW, Li MC, Ko WC et al: Clinical impact of Gram-negative nonfermenters on adults with community-onset bacteremia in the emergency department. J Microbiol Immunol Infect, 2015; 48: 92–100

10. Henriquez-Camacho C, Losa J: Biomarkers for sepsis. Biomed Res Int, 2014; 2014: 547818

11. Pacheco-Rosas DO, Huelgas-Plaza AC, Miranda-Novales MG: [Serum lactate as a biomarker of severe sepsis in children with cancer, neutropenia and fever]. Rev Med Inst Mex Seguro Soc, 2014; 52(Suppl.2): S24–29

12. Wlodarczyk M, Rudnicka W, Janiszewska-Drobinska B et al: Interferon-gamma assay in combination with tuberculin skin test are insufficient for the diagnosis of culture-negative pulmonary tuberculosis. PLoS One, 2014; 9: e107208

13. Kocoglu ME, Bayram A, Balci I: Evaluation of negative results of BacT/Alert 3D automated blood culture system. J Microbiol, 2005; 43: 257–59

14. Loonen AJ, de Jager CP, Tosserams J et al: Biomarkers and molecular analysis to improve bloodstream infection diagnostics in an emergency care unit. PLoS One, 2014; 9: e87315

15. Zareifar S, Farahmand Far MR, Golfeshan F, Cohan N: Changes in platelet count and mean platelet volume during infectious and inflammatory disease and their correlation with ESR and CRP. J Clin Lab Anal, 2014; 28: 245–48

16. Muller B, Schuetz P, Trampuz A: Circulating biomarkers as surrogates for bloodstream infections. Int J Antimicrob Agents, 2007; 30(Suppl.1): S16–23

17. Minano FJ, Tavares E, Maldonado R: Role of endogenous macrophage inflammatory protein-2 in regulating fever induced by bacterial endotoxin in normal and immunosuppressed rats. Clin Exp Pharmacol Physiol, 2004; 31: 723–31

18. Reichsoellner M, Raggam RB, Wagner J et al: Clinical evaluation of multiple inflammation biomarkers for diagnosis and prognosis for patients with systemic inflammatory response syndrome. J Clin Microbiol, 2014; 52: 4063–66

19. Talebi-Taher M, Babazadeh S, Barati M, Latifnia M: Serum inflammatory markers in the elderly: are they useful in differentiating sepsis from SIRS? Acta Med Iran, 2014; 52: 438–42

20. Wakabayashi G, Gelfand JA, Jung WK et al: *Staphylococcus* epidermidis induces complement activation, tumor necrosis factor and interleukin-1, a shock-like state and tissue injury in rabbits without endotoxemia. Comparison to *Escherichia coli*. J Clin Invest, 1991; 87: 1925–35

21. Huttunen R, Attman E, Aittoniemi J et al: Nosocomial bloodstream infections in a Finnish tertiary care hospital: A retrospective cohort study of 2175 episodes during the years 1999–2001 and 2005–2010. Infect Dis (Lond), 2015; 47: 20–26

22. Kung CT, Hsiao SY, Su CM et al: Serum adhesion molecules as predictors of bacteremia in adult severe sepsis patients at the emergency department. Clin Chim Acta, 2013; 421: 116–20

23. Van Amersfoort ES, Van Berkel TJ, Kuiper J: Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock. Clin Microbiol Rev, 2003; 16: 379–414

24. Li DZ, Chen YS, Yang JP et al: Preliminary molecular epidemiology of the *Staphylococcus aureus* in lower respiratory tract infections: A multicenter study in China. Chin Med J (Engl), 2011; 124: 687–92

25. Koreen L, Ramaswamy SV, Graviss EA et al: Spa typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol, 2004; 42: 792–99

26. Okuma K, Iwakawa K, Turnidge JD et al: Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J Clin Microbiol, 2002; 40: 4289–94

27. Giacometti A, Cirioni O, Ghiselli R et al: RNAIII-inhibiting peptide improves efficacy of clinically used antibiotics in a murine model of staphylococcal sepsis. Peptides, 2005; 26: 169–75

28. Al-Hasan MN, Juhn YJ, Bang DW et al: External validation of bloodstream infection mortality risk score in a population-based cohort. Clin Microbiol Infect, 2014; 20: 886–91

29. Gordon KA, Jones RN: Susceptibility patterns of orally administered antimicrobials among urinary tract infection pathogens from hospitalized patients in North America: comparison report to Europe and Latin America. Results from the SENTRY Antimicrobial Surveillance Program (2000). Diagn Microbiol Infect Dis, 2003; 45: 295–301

30. Paterson DL, Rossi F, Baquero F et al: *In vitro* susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2003 Study for Monitoring Antimicrobial Resistance Trends (SMART). J Antimicrob Chemother, 2005; 55: 965–73

31. Luzzani A, Polati E, Dorizzi R et al: Comparison of procalcitonin and C-reactive protein as markers of sepsis. Crit Care Med, 2003; 31: 1737–41

32. Buchan BW, Allen S, Burnham CA et al: Comparison of the next-generation Xpert MRSA/SA BC assay and the GeneOhm StaphSR assay to routine culture for identification of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in positive-blood-culture broths. J Clin Microbiol, 2015; 53: 804–9

33. Hall KK, Lyman JA: Updated review of blood culture contamination. Clin Microbiol Rev, 2006; 19: 788–802

34. Jeong S, Park Y, Cho Y, Kim HS: Diagnostic utilities of procalcitonin and C-reactive protein for the prediction of bacteremia determined by blood culture. Clin Chim Acta, 2012; 413: 1731–36

35. Fioretto JR, Martin JG, Kurokawa CS et al: Interleukin-6 and procalcitonin in children with sepsis and septic shock. Cytokine, 2008; 43: 160–64

36. Su H, Chang SS, Han CM et al: Inflammatory markers in cord blood or maternal serum for early detection of neonatal sepsis-a systemic review and meta-analysis. J Perinatol, 2014; 34: 268–74

37. Sugitharini V, Prema A, Berla Thangam E: Inflammatory mediators of systemic inflammation in neonatal sepsis. Inflamm Res, 2013; 62: 1025–34

38. Karlsson H, Larsson P, Wold AE, Rudin A: Pattern of cytokine responses to gram-positive and gram-negative commensal bacteria is profoundly changed when monocytes differentiate into dendritic cells. Infect Immun, 2004; 72: 2671–78

39. Dugo L, Collin M, Cuzzocrea S, Thiemermann C: 15d-prostaglandin J2 reduces multiple organ failure caused by wall-fragment of Gram-positive and Gram-negative bacteria. Eur J Pharmacol, 2004; 498: 295–301

40. Ryu YH, Baik JE, Yang JS et al: Differential immunostimulatory effects of Gram-positive bacteria due to their lipoteichoic acids. Int Immunopharmacol, 2009; 9: 127–33

41. Tavares E, Maldonado R, Ojeda ML, Minano FJ: Circulating inflammatory mediators during start of fever in differential diagnosis of gram-negative and gram-positive infections in leukopenic rats. Clin Diagn Lab Immunol, 2005; 12: 1085–93

42. Nuutila J, Jalava-Karvinen P, Hohenthal U et al: CRP/CD11b ratio: A novel parameter for detecting gram-positive sepsis. Hum Immunol, 2009; 70: 237–43

43. Vanska M, Koivula I, Jantunen E et al: IL-10 combined with procalcitonin improves early prediction of complications of febrile neutropenia in hematological patients. Cytokine, 2012; 60: 787–92

44. Feezor RJ, Oberholzer C, Baker HV et al: Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. Infect Immun, 2003; 71: 5803–13

45. Rockel C, Hartung T: Systematic review of membrane components of gram-positive bacteria responsible as pyrogens for inducing human monocyte/macrophage cytokine release. Front Pharmacol, 2012; 3: 56

46. Schmidt RR, Pedersen CM, Qiao Y, Zahringer U: Chemical synthesis of bacterial lipoteichoic acids: an insight on its biological significance. Org Biomol Chem, 2011; 9: 2040–52

47. Hadley JS, Wang JE, Foster SJ et al: Peptidoglycan of *Staphylococcus aureus* upregulates monocyte expression of CD14, Toll-like receptor 2 (TLR2), and TLR4 in human blood: Possible implications for priming of lipopolysaccharide signaling. Infect Immun, 2005; 73: 7613–19

48. Power CP, Wang JH, Manning B et al: Bacterial lipoprotein delays apoptosis in human neutrophils through inhibition of caspase-3 activity: regulatory roles for CD14 and TLR-2. J Immunol, 2004; 173: 5229–37