Comparative Study of Plasma Endotoxin with Procalcitonin Levels in Diagnosis of Bacteremia in Intensive Care Unit Patients

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Abstract

Background: Both procalcitonin (PCT) and plasma endotoxin levels cannot be solely used for a definite diagnosis of bacteremia or sepsis, and there has been few study comparing the values of the two biomarkers for the diagnosis of bacteremia. The aim of this study was to identify bacteria causing bacteremia and evaluate the role of the two biomarkers in the diagnosis of bacteremia in Intensive Care Unit (ICU).
Methods: The medical records of 420 patients in ICU were retrospectively reviewed. Patients ($n = 241$) who met the inclusion criteria were subjected to blood culture (BC) for the analysis of the endotoxin or PCT levels. The exclusion criteria included the presence of infection with human immunodeficiency virus and/or AIDS, neutropenia without sepsis, pregnancy, treatment with immunosuppressive therapies, or blood diseases such as hematological tumors. Patients' BC episodes were divided into BC negative, Gram-negative (GN) bacteria, Gram-positive bacteria, and fungi groups. The PCT and plasma endotoxin levels were compared in the different groups.
Results: A total of 241 patients with 505 episodes of BC were analyzed. The GN bacteria group showed higher levels of PCT and endotoxin than the BC negative, Gram-positive bacteria, and fungi groups. GN bacteremia was more prevalent than Gram-positive bacteremia. The GN bacteremia caused by non-*Enterobacteriaceae* infection presented higher endotoxin level than that by *Enterobacteriaceae*, but no significant difference in PCT levels was observed between the two groups. The plasma endotoxin significantly differed among different groups and was bacterial species dependent.
Conclusions: Plasma endotoxin was more related to GN than to Gram-positive bacteremia, and that endotoxin level was species dependent, but PCT level remained relatively more stable within the GN bacteria caused bacteremia. Both GN and positive bacteria caused bacteremia in the ICU patients in different regions of China. And PCT is a more valuable biomarker than endotoxin in the diagnosis of bacteremia.

Key words: Bacteremia; Endotoxin; Intensive Care Unit; Procalcitonin; Sepsis

Introduction

Procalcitonin (PCT) is widely used as a marker before final blood culture (BC) confirmation in clinical diagnosis of bacteremia and sepsis, with diagnostic sensitivity 74.8–100.0%, specificity 70.0–100.0%, positive predictive value 55.0–100.0%, and negative predictive value 56.3–100.0%, and PCT in combination with careful clinical parameters can discriminate infection caused bacteremia from inflammatory sepsis in 77% of cases.[1] However, PCT alone still has some limitations, especially lack of definitive cut-off in the indeterminate zone.[1] For example, PCT cannot reliably differentiate sepsis from other noninfectious causes of systemic inflammatory response syndrome in critically ill patients[2] and is of no use in determining new fever caused by bacteremia in the Intensive Care Unit (ICU) patients.[3]

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Meanwhile, although the prognostic value of endotoxin level for bacteremia and sepsis remains disparate, less than two-thirds of patients with Gram-negative (GN) bacteremia are detected with endotoxemia and vice versa.[4] Endotoxemia broadly parallels the frequency and importance of GN patient sepsis[5] and is only not detected in >20% GN bacteraemic patients among the 58 studies overall or >20% studies of patients with sepsis syndrome.[6] While the association of endotoxemia with bacteremia is bacterial type dependent, endotoxemia is more commonly detected in patients with bacteremia caused by non-*Enterobacteriaceae* than a commensal member of *Enterobacteriaceae*.[7]

Therefore, both of the markers have some advantages and disadvantages and cannot be solely used for a definite diagnosis of bacteremia or sepsis. Furthermore, there has been few study comparing the values of the two biomarkers for the diagnosis of bacteremia within the same patient cohort. The aim of this retrospective study was to compare the role of the two biomarkers in the diagnosis of bacteremia in ICUs of a Chinese Hospital.

METHODS

Patient cohort

The study data were retrospectively collected from 420 patients with consecutive admissions to the emergency and ICUs of Changzheng Hospital in Shanghai, China from January 1, 2010 to December 31, 2012. The Ethics Committee of Changzheng Hospital in Shanghai, China approved this study and patient consents were waived.

Trained research assistants screened the patients using the hospital's electronic medical record system. All patients subjected to BC tests for the analysis of the endotoxin or PCT levels during ICU stay were enrolled in this study. The exclusion criteria included the presence of infection with human immunodeficiency virus and/or AIDS, neutropenia without sepsis, pregnancy, treatment with immunosuppressive therapies, or blood diseases such as hematological tumors.

Data collection

The relevant patient demographics including age, gender, comorbidities, infection sites, microbial isolates, and major laboratory test results were recorded at baseline. Sepsis, severe sepsis, and septic shock were defined according to the internationally accepted criteria.[8,9] The disease severity in each patient was assessed upon admission using two different scores: The Acute Physiology and Chronic Health Evaluation (APACHE) II Score[10] and the Sequential Organ Failure Assessments (SOFAs) Score.[11] The comorbidities were measured using the Charlson Comorbidity Index (CCI).[12]

Definition

The blood samples were collected through venous puncture using the BACTEC system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) based on both standard aerobic and anaerobic media coupled with the 9240 automated BC system (Becton Dickinson Diagnostic Instrument System, Paramus, NJ, USA). The bacterial identification was based on standard methods as described instructions by manufactures. One episode of bacteraemia was defined as the recovery of any bacterial species in one or more BCs. Patients from whom *Staphylococcus* non-*aureus* was isolated in BCs were not eligible, except when at least two consecutive samples were grown for the same species harboring the same antibiotic resistance patterns. Mixed cultures were considered significant when organisms other than the contaminants were isolated.

Thus, all episodes of BC can be divided into four groups according to BC: BC⁻ (no isolates), G⁻ (GN bacteria), G⁺ (Gram-positive bacteria), and Fungi (fungi). Notably, the PCT and endotoxin levels of the mixed cultures with one or more isolates were not compared with the four groups described above. Furthermore, G⁻ was separated into *Enterobacteriaceae* and non-*Enterobacteriaceae* groups according to the previously reported standards.[7,13] The group of commensal *Enterobacteriaceae*, predominantly comprise *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Proteus mirabilis,* and *Providencia rettgeri*. The group of non-*Enterobacteriaceae* in the present analysis included *Acinetobacter baumannii, Burkholderia cepacia,* and *Pseudomonas aeruginosa*.

Procalcitonin and plasma endotoxin assay

The PCT levels were measured using an immunoluminometric assay (LUMItest PCT kit, BRAHMS Diagnostica, Hennigsdorf bei Berlin, Germany). The chemiluminescence was measured using a luminometer (Lumat, LB 9507, Berthold, Wildbad, Germany).

The endotoxin concentration was assayed using a *Limulus* test involving a turbidimetric time assay (Zhanjiang A & C Biological Ltd., China) at 450 nm with Toxinometer BET-16 (Tianda Tianfa Technology Co., Ltd., Tianjin, China) at 37.8°C.[14] To measure the endotoxin levels in plasma, 1 ml whole blood in 1 ml of anticoagulants and eluent was centrifuged at 1500 r/min for 10 min; Plasma (0.2 ml) was added to 0.8 ml diluent and heated at 75°C for 10 min. An aliquot (0.1 ml) of stock solution processed above was added to 0.1 ml *Limulus* amebocyte lysate (LAL) reagent, and the kinetic turbidity of the mixture was measured using a tube reader (Zhanjiang A and C Biological Ltd., China).

Statistical analysis

Data analysis was conducted using SPSS 18 software (SPSS Inc., IL, USA). The data are presented as the mean ± standard deviation, median and interquartile ranges (25th and 75th percentiles) or numbers and percentages. The categorical variables were compared using Chi-square or Fisher's exact tests, where appropriate. The differences in the parametric data between different strata were calculated using Student's *t*-test and analysis of variance (ANOVA) with *post-hoc* LSD test for the

two groups. To compare the nonparametric data, the Mann–Whitney *U*-test was used for two groups, and the Kruskal–Wallis test with *post-hoc* Mann–Whitney *U*-test was performed for multiple comparisons. Spearman's rank correlation or Pearson's tests were performed to evaluate the association between the two groups, where appropriate. The data were analyzed using the receiver operating characteristic (ROC) curve for the plasma endotoxin and PCT levels for the prediction of GN bacteremia. *P* < 0.05 was set for significant difference.

RESULTS

Patient characteristics

A total of 420 patients admitted to the ICU were screened for the study; 179 patients did not meet the inclusion criteria and were excluded, and 241 patients met the inclusion criteria (male, 68.0%), of which 71 (29.5%) patients had bacteremia. The primary reasons for infections were pneumonia and abdominal infections by predominant GN bacteria conformed from BC. The mortality rate for all patients was 24.5%, with a higher mortality rate for patients with severe sepsis (29.2%) or septic shock (62.2%) than sepsis (9.8%) or nonsepsis (12.6%). The mean age, APACHE II, SOFA, and CCI scores of the nonsurviving patients were significantly higher than those of the surviving patients. Sex, infection sites, and the presence of *Enterobacteriaceae* infection did not affect the mortality of the patients. A summary of the patient demographics and clinical parameters of the study population are listed in Table 1.

Blood culture results

Among 505 isolated samples, 92 (18.2%) isolates were positive for BC. Of the 92 isolates, a total of 69 (75.0%) isolates were GN microorganisms, including 13 *B. cepacia*, 11 K. *pneumoniae*, 10 *Acinetobacter baumannii*, six *E. coli*, and five *Enterobacter cloacae*. Whereas, only 13 (14.1%) isolates were Gram-positive microorganisms, of which, a total of 6 (6.5%) isolates were fungi, with 4 isolates being *Candida albicans*. More than one microorganism was found in 4 (4.3%) episodes after the BC, with *B. cepacia* being present in two episodes of BC. The isolated microorganisms are presented in Table 2.

Association of endotoxin or procalcitonin level with different microorganisms

The PCT concentration and endotoxin level significantly differed among the four groups G⁻, G⁺, fungi, and BC⁻ (*P* < 0.000, PCT; and *P* = 0.0244, endotoxin). The PCT level was significantly higher in the G⁻ group than in the BC⁻ (*P* < 0.0001) and G⁺ (*P* = 0.0484) groups; patients with fungi isolates also had a higher level of PCT than patients with BC⁻ (*P* = 0.0244). The plasma endotoxin level in the G⁻ group was significantly higher than in the BC⁻ group (*P* = 0.0025), and no significant difference was found between G⁻ group and the BC⁻ (or G⁺) group [Figure 1].

Table 1: Patient characteristics based on the diagnosis at admission

Characteristics	Surviving	Nonsurviving	P
n (%)	182 (75.5)	59 (24.5)	
Age (years), mean ± SD	49.6 ± 17.9	56.3 ± 17.8	0.017
Female/male, n	56/126	21/38	0.490
APACHE II at admission, mean ± SD	12.2 ± 6.1	19.2 ± 7.3	0.000
SOFA at admission, mean ± SD	4.9 ± 3.2	8.8 ± 4.4	0.000
PCT* (ng/ml), mean ± SD	3.3 ± 9.1	7.9 ± 31.5	0.353
Endotoxin* (EU/ml), mean ± SD	0.4 ± 3.2	0.2 ± 0.2	0.717
Infection sites, n (%)			
Lung	114 (74.0)	40 (26.0)	0.346
Abdomen	35 (74.5)	12 (25.5)	0.810
Neurology	12 (66.7)	6 (33.3)	0.345
Soft tissue	11 (84.6)	2 (15.4)	0.439
Others	17 (89.5)	2 (10.5)	0.145
Severity of disease, n (%)			
Nonsepsis	76 (87.4)	11 (12.6)	0.002
Sepsis	55 (90.2)	6 (9.8)	0.003
Severe sepsis	34 (70.8)	14 (29.2)	0.450
Septic shock	17 (37.8)	28 (62.2)	0.000
CCI, n (%)			
0	115 (82.1)	25 (17.9)	0.008
≥1	67 (66.3)	34 (33.7)	0.054
Patients with isolates of BC, n (%)			
G⁻	32 (66.7)	16 (33.3)	0.160
G⁺	8 (61.5)	5 (38.5)	0.382
Fungi	3 (50.0)	3 (50.0)	0.064
Mixed	2 (50.0)	2 (50.0)	0.039
No isolates	137 (80.6)	33 (19.4)	0.008
Patients with *Enterobacteriaceae* or non-*Enterobacteriaceae*, n (%)			
Enterobacteriaceae	21 (72.4)	8 (27.6)	0.701
Non-*Enterobacteriaceae*	18 (64.3)	10 (35.7)	0.739
Both	4 (66.7)	2 (33.3)	0.709

*PCT and endotoxin levels from first-time BC. APACHE II: The Acute Physiology and Chronic Health Evaluation II Score; SOFAs: The Sequential Organ Failure Assessments Score; CCI: The Charlson Comorbidity Index; BC: Blood culture; G⁻: Gram-negative bacteria; G⁺: Gram-positive bacteria; PCT: Procalcitonin; SD: Standard deviation.

Patients with non-*Enterobacteriaceae* isolates showed a significantly higher plasma endotoxin level than patients with *Enterobacteriaceae* (*P* = 0.0276); the PCT level did not differ significantly between the above two patient groups (*P* = 0.2964) [Figure 2].

It was significantly different among all the different groups in the species level for endotoxin (*P* = 0.0446), not PCT (*P* = 0.5529) [Figure 1]. The endotoxin level of the patients with *B. cepacia* infection was significantly higher than that with *S. marcescens* (*P* = 0.0236), K. *pneumoniae* (*P* = 0.0048), and *E. cloacae* (*P* = 0.0180); the difference between *E. cloacae* and *P. aeruginosa* in the endotoxin level was also nearly significantly different (*P* = 0.0519). The PCT level in the patients with *E. cloacae* infection was significantly (*P* = 0.0319) and almost (*P* = 0.0893) higher than those with *S. marcescens* and *A. baumannii*, respectively [Figure 3].

Table 2: Microorganisms isolated from blood culture (n = 92)	
Microorganisms	***n***
Gram-negative bacilli	69
Enterobacteriaceae	35
K. pneumonia	11
S. marcescens	10
E. coli	6
E. cloacae	5
K. oxytoca	2
S. enteritidis	1
Non-*Enterobacteriaceae*	34
B. cepacia	13
A. baumannii	10
P. Aeruginosa	4
A. junii	1
P. paucimobilis	1
B. diminuta	1
S. paucimobilis	1
C. meningosepticum	1
A. xylosoxidans subsp. *denitrificans*	1
H. influenzae	1
Gram-positive cocci	13
Micrococcaceae	10
S. capitis	2
S. hemolyticus	2
S. hominis	1
S. epidermidis	1
S. aureus	1
MRSA	1
Kocuria roseus	2
Streptococcaceae	3
E. faecalis	2
G. bergeri	1
Fungi	6
C. albicans	4
C. glabrata	1
C. haemulonii	1
Mixed	4
A. baumannii + S. lentus	1
C. glabrata + E. coli	1
B. cepacia + A. baumannii	1
B. cepacia + S. marcescens	1

K. pneumonia: *Klebsiella pneumonia*; *S. marcescens*: *Serratia marcescens*; *E. coli*: *Escherichia coli*; *E. cloacae*: *Enterobacter cloacae*; *K. oxytoca*: *Klebsiella oxytoca*; *S. enteritidis*: *Salmonella enteritidis*; *B. cepacia*: *Burkholderia cepacia*; *A. baumannii*: *Acinetobacter baumannii*; *P. Aeruginosa*: *Pseudomonas Aeruginosa*; *A. junii*: *Acinetobacter junii*; *P. paucimobilis*: *Pseudomonas paucimobilis*; *B. diminuta*: *Brevundimonas diminuta*; *S. paucimobilis*: *Sphingomonas paucimobilis*; *C. meningosepticum*: *Chryseobacterium meningosepticum*; *A. xylosoxidans*: *Achromobacter xylosoxidans*; *H. influenza*: *Haemophilus influenza*; *S. capitis*: *Staphylococcus capitis*; *S. hemolyticus*: *Staphylococcus hemolyticus*; *S. huminis*: *Staphylococcus huminis*; *S. epidermidis*: *Staphylococcus epidermidis*; *S. aureus*: *Staphylococcus aureus*; *K. roseus*: *Kocuria roseus*; *E. faecalis*: *Enterococcus faecalis*; *G. bergeri*: *Gemella bergeri*; *C. albicans*: *Candida albicans*; *C. glabrata*: *Candida glabrata*; *C. haemulonii*: *Candida haemulonii*; *S. lentus*: *Staphylococcus lentus*; MRSA: Methicillin-resistant *S. aureus*.
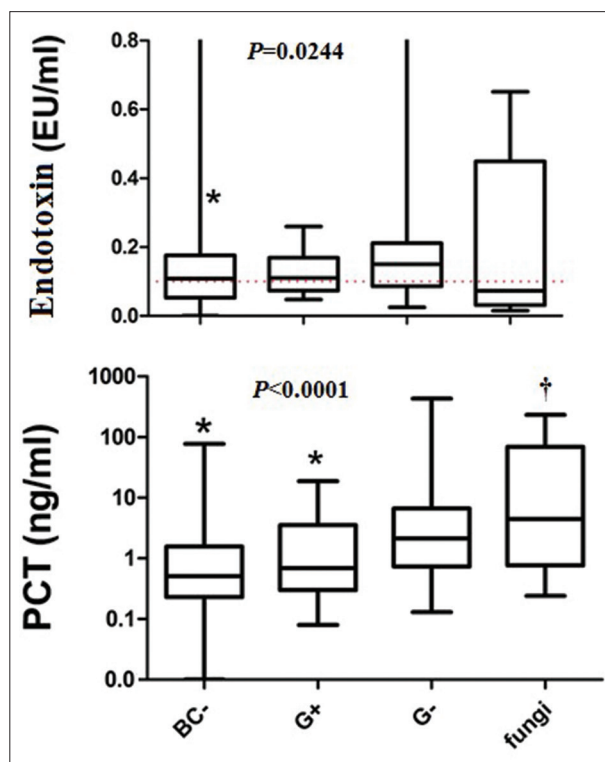
Figure 1: Comparison of the procalcitonin and plasma endotoxin levels in different groups according to the blood culture results. $P < 0.05$: *compared with G⁻; †compared with BC⁻. BC⁻: Blood culture negative group; G⁻: Gram-negative bacteria; G⁺: Gram-positive bacteria.

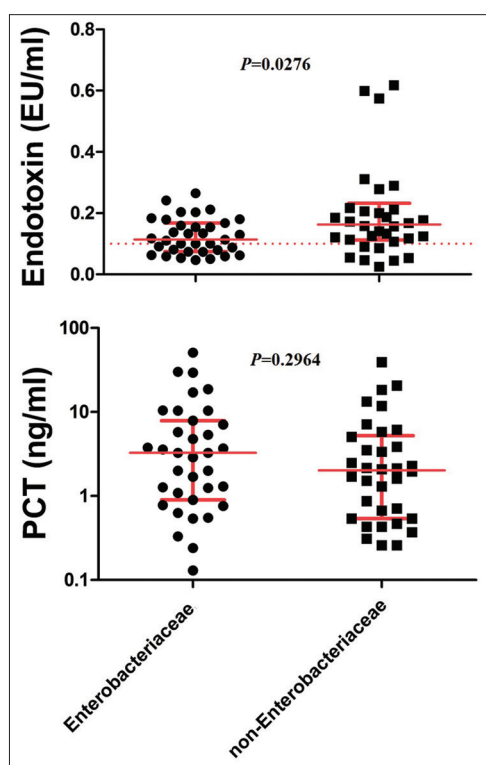## Procalcitonin and endotoxin levels in prediction of bacteremia

The area under the ROC curves for the PCT and endotoxin levels used to predict GN isolates of BC were 0.741 (95% confidence interval [*CI*]: 0.683–0.779, $P < 0.001$) and 0.614 (95% *CI*: 0.550–0.678, $P = 0.002$), respectively.

Discussion

In this retrospective study, to investigate whether PCT or endotoxin level in the blood is more valuable for diagnosis of bacteremia, we evaluated the association of the PCT or plasma endotoxin level of different types of bacteria in the ICU patients with sepsis, severe sepsis, or septic shock. We found that: (1) GN bacteria were predominant within the microorganisms found in the BCs; (2) the level of PCT is more closely associated with GN bacteremia than that of endotoxin; (3) GN bacteremia exhibited a higher level of endotoxin than nonbacteremia; and (4) bacteremia with non-*Enterobacteriaceae* had a significantly higher level of endotoxin than bacteremia with *Enterobacteriaceae*.

In this study, we observed that age, comorbidities, severities at admission and status of bacteremia differ significantly between survivors and nonsurvivors, and sex difference or infection sites not affect mortality. GN bacteria were predominant within the microorganisms found in the
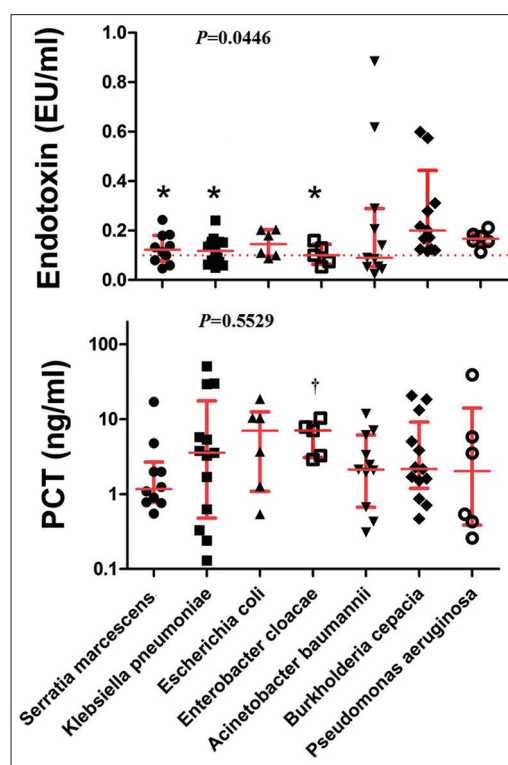
Figure 2: Comparison of the procalcitonin and plasma endotoxin levels between *Enterobacteriaceae* and non-*Enterobacteriaceae*. The *P* values are shown in the figure.

Figure 3: Comparison of the procalcitonin and plasma endotoxin levels in different groups according to bacterial species. *P* < 0.05: *Compared with *Burkholderia cepacia*; †compared with *Serratia marcescens*.

BCs, and the major Gram-positive bacteria were from coagulase-negative staphylococci. In some countries, Gram-positive bacteria may have a high percentage of microorganisms found in the BCs.[15-17] The difference in the predominant bacterial type may result from geographic variation, case mix, and antibiotic prescription habits. Of note, in this study, *B. cepacia* was found to have the highest positive rate among all the microorganism species. However, *B. cepacia* was not found in patients with severe sepsis or septic shock in 22 ICUs across the mainland of China, and the reason was unknown.[18] It was consistent with previous reports that patients with bacteremia have a high mortality rate than patients without isolates from BCs and especially high for patients with fungi isolates.[18,19] There was no significant difference between the mortality rates caused by GN bacteremia with *Enterobacteriaceae* and non-*Enterobacteriaceae*. However, a previous study had shown that mortality was higher in *A. baumannii* (one of non-*Enterobacteriaceae*) bacteremia, particularly compared with *K. pneumoniae* bacteremia (one of *Enterobacteriaceae*).[20]

We found that endotoxin level in GN bacteremia was higher than that in Gram-positive bacteremia, and in the GN bacteremia, non-*Enterobacteriaceae* had a significantly higher level of endotoxin than *Enterobacteriaceae*. These results were consistent with the previous reports.[7,21] It was not surprised that lipopolysaccharide (LPS) is the major component of GN bacteria outer membrane, and LAL reacts with bacterial endotoxin or LPS. Gram-positive bacteria and

fungi in some patients were also found to present higher endotoxin level than normal. This was also found in previous studies where a positive LAL assay was observed with peptidoglycan derived from the cell walls of Gram-positive organisms or (1-3)-b-D-glucans from fungi.[22,23] In addition, LPS from the gut or other infection sites might enter the blood without bacterial translocation.[24,25] Moreover, endotoxin level in some patients was found to be below detection limit possibly because that endotoxin can bind to monocytes, red cells, and platelets.[26-28]

The different endotoxin levels found in bacteremia caused by non-*Enterobacteriaceae* and *Enterobacteriaceae* were similar to the findings as in the aforementioned studies[6,7] and might result from different LPS structures between the two types of bacteria. According to lipid A structure, LPS predominantly has hexa-acyl or nonhexa-acyl lipid A. *Enterobacteriaceae* typically produces hexa-acyl lipid A structure, and non-*Enterobacteriaceae* produces nonhexa-acyl lipid A structure.[13] Furthermore, different bacterial species or different genomovars within the same species *B. cepacia* differ in their ability to cause life-threatening pneumonia and possess different lipid A structures;[5,13] lipid A even from the same species can be penta-, hexa-, or hepta-acylated, and depending on the temperature, some *Enterobacteriaceae* such as *Yersinia pestis* can make tetra-, penta-, or hexa-acyl lipid A.[29-31]

We found that PCT was more closely associated with GN bacteremia than endotoxin. In fact, PCT has been widely

used as a sepsis biomarker for discriminating bacterial and nonbacterial infections and to predict bacteremia with different statuses.[2,32-34] The endotoxin levels did not reflect GN bacteremia, particularly the bacteremia caused by *Enterobacteriaceae*. This might be because the following limitations: The LAL is not specific to hexa-acyl LPS;[13,35,36] LPS recognition by the horseshoe crab likely reflects a defense against aquatic bacteria; the assay widely recognizes diverse lipid A structure to enhance the detection of bacteria in biological fluids, leading to some of the problems in detection of endotoxin in patients.[37] In addition, in this study *E. coli* LPS was used as a standard to test endotoxin, but LPS from each bacterium found by BC should be used as standard LPS to test the corresponding endotoxin.[14,22]

In conclusion, this is the comparative study of the PCT and endotoxin as a predictive indicator for bacteremia and sepsis. We found that GN was predominant within the microorganisms found in the BCs of the ICU patients in our hospital, and the level of PCT is more closely associated with GN bacteremia than that of endotoxin, with the plasma endotoxin level of GN bacteremia being species dependent. Our findings demonstrated that diversified types of bacteria caused bacteremia in ICUs in different regions of China and that PCT is a more valuable biomarker than endotoxin in the diagnosis of GN bacteremia.

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Conflicts of interest

There are no conflicts of interest.

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