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Original Article

Diagnostic Accuracy of Procalcitonin for Differentiating Bacteraemic Gram-Negative Sepsis from Gram-Positive Sepsis

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Abstract

Objective: The identification of bacteraemia in patients with suspected sepsis is crucial for survival. A cheap, fast and reliable biomarker, which can predict the causative pathogen group, may be useful to confirm or exclude the presence of bacteraemia. This study aimed to evaluate the relationship between procalcitonin (PCT) and the causative pathogen in intensive care patients with sepsis and bacteraemia.

Methods: Patients with diagnosed sepsis, a positive blood culture and measured serum procalcitonin levels during their intensive care unit stay were included in the study. Demographic data, PCT level, leukocyte count, C-reactive protein level, creatinine level, lymphocyte count, leukocyte/lymphocyte ratio and the group of the pathogen that detected in the blood culture were retrospectively recorded.

Results: Overall, 136 sepsis patients who were diagnosed with bacteraemia were included in the study. The PCT level was 7.31 ng mL^{-1} in the gram-negative group and 0.46 ng mL^{-1} in the gram-positive group. For PCT, the sensitivity was 70.83% and the specificity was 84.21%, with the cut-off value being ≤1.3. The area under the receiver operating characteristics curve for PCT was 0.80.

Conclusion: Patients with gram-negative sepsis had higher PCT values than those with gram-positive sepsis. Our results suggest that PCT value may be a useful tool for distinguishing between gram-negative and gram-positive bacteraemia.

Keywords: Sepsis, procalcitonin, bacteraemia

Introduction

Bacteraemia is a condition with high mortality and morbidity rates and is severe in sepsis-causing infections especially in intensive care patients. The recognition of bacteraemia and early initiation of appropriate empirical antimicrobial therapy in patients with suspected sepsis are important factors for survival (1). Inappropriate empirical antimicrobial therapy is a risk factor for increased mortality (2). In addition, it leads to antibiotic resistance and secondary infections that develop with multidrug-resistant pathogens (3). Blood culture is the gold standard for definitive diagnosis of the pathogen that causes bacteraemia, but there are limitations in the use of blood culture for the diagnosis of sepsis. It provides a result within 24–48 h, and it is found negative in 30% of patients (4). Currently, even polymerase chain reaction (PCR)-based diagnostic systems which recognise pathogen DNA give results within 6 h, and these systems are quite expensive and require special equipment (5). A cheap, fast and reliable biomarker which supports or excludes the presence of bacteraemia and can give an idea about the active pathogen group can be useful in directing antibiotic treatment.

Procalcitonin (PCT) is the precursor protein of the hormone calcitonin. Calcitonin is comprises 116 amino acids and is released from the C cells of the thyroid or the neuroendocrine cells of the lung and intestines. Under normal conditions, it is present in a very small amount in circulation, whereas it rapidly increases in the serum in cases of infection (6). It is a valuable biomarker to distinguish infectious and non-infectious inflammatory conditions in critical patients and has a prognostic value for sepsis in this patient group (4, 7, 8). It is used in the determination of the duration of antibiotic treatment and in making the decision of antibiotic cessation in patients with sepsis (9). Thus, both the cost is decreased and measures are taken against the development of resistant pathogen by avoiding unnecessary and long-term

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use of antibiotics. For these reasons, PCT has become the most commonly used biomarker in directing the diagnosis and treatment of sepsis.

PCT has high sensitivity and specificity in predicting systemic bacterial infections and can distinguish bacterial infections from viral infections (10). The prediction of possible pathogens in bacteraemia may have a positive effect on patient outcome with a rapid and proper regulation of empirical antimicrobial therapy. Some studies emphasised that the PCT levels can be used to differentiate between bacteraemia originating from Gram-negative and Gram-positive bacteria, but they also emphasised that more studies are needed in this regard (10-12).

The aim of the present study was to evaluate the relationship between the PCT level and the causative pathogen in patients with sepsis in whom bacteraemia was found.

Methods

The local ethics committee (09.2016.0243/70737436-050.06.04) of Marmara University Medical Faculty approved our study. The present study was retrospectively planned in accordance with the Declaration of Helsinki by investigating the records of the patients who were hospitalised in our intensive care unit (ICU) for a period of 24 months. During ICU stay, patients who were diagnosed with sepsis (13), whose blood culture and PCT serum levels were simultaneously examined and in whom bacteraemia was consequently detected owing to the reproduction observed in blood culture were included in the study. In our unit, the PCT serum levels are examined in all patients who are suspected to have sepsis. Patients who had non-infectious causes such as severe trauma, major surgery, burn, severe kidney and liver failure, which could affect the PCT levels and patients with multiple pathogenesis in blood culture were excluded from the study. Demographic data, Sepsis-related Organ Failure Assessment (SOFA) scores, PCT, leucocytes, C-reactive protein (CRP), creatinine, lymphocytes, polymorphonuclear leucocyte (PML)/lymphocytes ratio and the pathogen group reproducing in blood culture were recorded in patients. When more than one bacteraemia attack was detected in the same patient, only the data at the time of the first attack were included in the study.

Under appropriate conditions for blood culture, 10 mL of whole blood was simultaneously withdrawn from two different regions and inoculated into BACTEC aerobes and anareobic bottles (Becton Dickinson, Sparks, MD, USA). A total of two sets of blood cultures were extracted from the patients. By collecting samples from positive signalling bottles for Gram staining and culture, microorganism identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. The PCT levels were measured from the serum with automated system (VIDAS B.R.A.H.M.S assay, bioMerieux, Marcy L'Etoile, France). The lower limit of the test positivity was 0.05 ng mL^{-1} as determined by the manufacturer.

Bacteraemia was defined as microbial growth in one or more flasks. Contamination was considered when coagulase-negative staphylococcus, *Corynebacterium* spp. and other skin flora bacteria reproduced in a single bottle. The pathogens of skin flora are considered to be the causative pathogens when they reproduced in blood cultures collected from two different regions. Patients were grouped as Gram-positive (GP)-induced bacteraemia and Gram-negative (GN)-induced bacteraemia according to the pathogen that reproduced in blood culture and that was considered as causative.

Statistical analysis

While the study data were evaluated, in addition to the descriptive statistical methods (mean, standard deviation, median, Q_1, Q_3, frequency, ratio, minimum and maximum), normal distribution consistency of the quantitative data was assessed by Shapiro–Wilk's test and graphical examination. Student's t-test was used for two-group comparisons of normally distributed variables, and Mann–Whitney U test was used for two-group comparisons of non-normally distributed variables. The relationship between qualitative data was assessed by Pearson's chi-square test. Spearman correlation analysis was used to evaluate the relationships between quantitative variables. Receiver operating characteristic (ROC) curve analysis was performed to determine the threshold value for PCT level in the differentiation of Gram (+) and Gram (–) cases, and the threshold value was determined as the PCT level with the highest Youden Index value. A p-value <0.05 was considered statistically significant.

Results

A total of 410 patients who were diagnosed with sepsis and whose blood culture tests were performed were hospitalised in our ICU within the 24-month period during which the study was conducted. Proliferation was detected in blood cultures of 136 patients. Owing to multiple microorganisms in 5 blood cultures and fungus proliferation in 7 blood cultures, 12 patients were excluded from the study, and a total of 124 patients were included in the study. Of the patients, 50.8% (n=63) were women and 49.2% (n=61) were men, and the mean age was 56.31±19.69 years. GN bacteria proliferation occurred in the blood culture of 76 (61.3%) patients, and GP bacteria proliferation occurred in the blood culture of 48 (38.7%) patients. The PCT levels were significantly higher in the GN group (7.31 ng mL^{-1}) than those in the GP group (0.46 ng mL^{-1}) (p<0.001). Similar to the PCT levels, the CRP values were significantly higher in the GN group (p=0.03). Both groups were similar in terms of severity of the disease when SOFA score and the presence of septic shock were considered (p=0.477 and

Table 1. Characteristics of Gram-positive and Gram-negative bacteraemia groups

	Gram (+)	Gram (-)	
	Media (Q_1-Q_3)	Median (Q_1-Q_3)	p
Gender (M/F), n (%)	24 (39.3) /37 (60.7)	24 (38.1) / 39 (61.9)	[a]0.886
Age, mean±SD	52.29±20.91	58.84±18.57	[b]0.071
SOFA score, mean±SD	6.13±4.10	6.52±4.66	[b]0.477
Septic shock, n (%)	25 (52)	41(53.9)	[a]0.839
Leucocyte	10500 (5900-18825)	10500 (7700-13900)	[c]0.743
Lymphocytes	800 (700-1700)	1000 (400-1200)	[c]0.264
PML/lymphocyte	7.83 (5.26-17)	8.40 (6-23)	[c]0.160
PCT	0.46 (0.25-2.30)	7.31 (2.12-20.72)	[c]<0.001#
CRP	95.15 (39.30-180)	150.5 (76-212.40)	[c]0.033*
Creatinine	0.88 (0.55-2.31)	1.01 (0.47-2.81)	[c]0.552

[a]Pearson's chi-square test, [b]Student's t-test, [c]Mann–Whitney U test. *p<0.05, #p<0.001. Q_1: first quartile, 25th percentile; Q_3: third quartile, 75th percentile; SOFA: Sepsis-related Organ Failure Assessment; PML: polymorphonuclear leucocyte; PCT: procalcitonin; CRP: C-reactive protein.

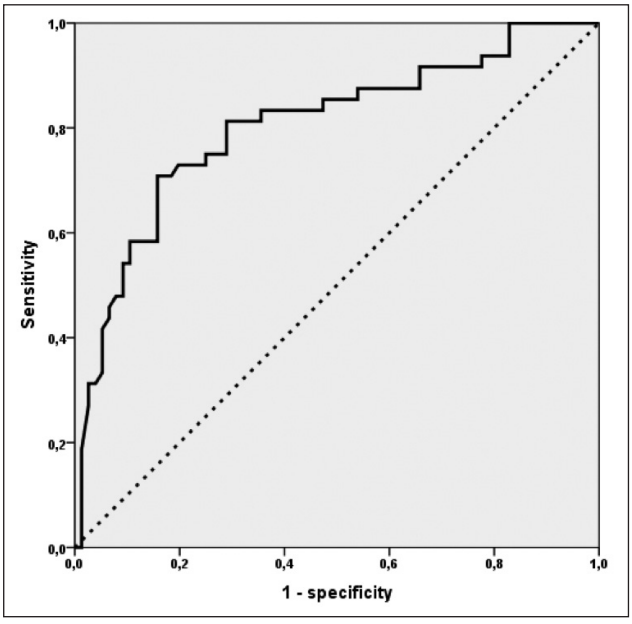

Figure 1. ROC curve for PCT values
ROC: receiver operating characteristic; PCT: procalcitonin

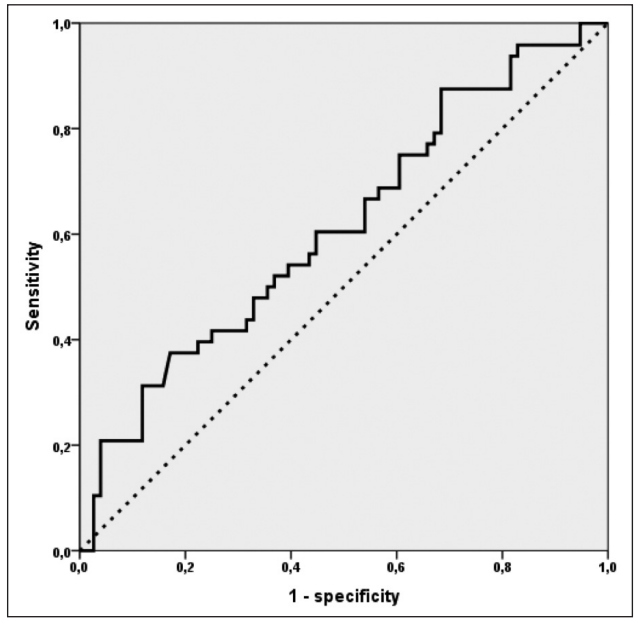

Figure 2. ROC curve for CRP values
ROC: receiver operating characteristic; PCT: procalcitonin

p=0.839, respectively). The levels of leucocytes, creatinine, lymphocytes and PML/lymphocytes ratio were similar in two groups (Table 1).

Sensitivity and specificity values were calculated at different cut-off levels for PCT to distinguish between GN and GP cases. The threshold value was determined as ≤1.3 with a sensitivity of 70.83% and a specificity of 84.21% for PCT. The area under the ROC curve for the PCT value was calculated as 0.80 (95% CI: 0.722–0.887, p<0.001) (Figure 1).

Sensitivity and specificity values for different cut-off levels for CRP were calculated to distinguish between GN and GP cases. The threshold value was determined as ≤51.8 with a sensitivity of 82.89% and a specificity of 58.1% for CRP. The area under the ROC curve for the CRP value was calculated as 0.61 (95% CI: 0.512–0.716, p=0.033) (Figure 2). The difference between the areas under the ROC curves obtained for the PCT and CRP values (mean: 0.119, 95% CI: 0.081–0.300) was found to be significant (p<0.001).

Discussion

In our study evaluating the relationship between the PCT values and the causative pathogen group in patients with sepsis with bacteraemia, the PCT levels in patients with

GN bacteria in blood culture were significantly higher than those with GP bacteria. To distinguish between GP and GN bacteraemia, the PCT threshold value was found to be 1.3 ng mL^{-1}.

The host inflammatory cascade that plays a role in the pathogenesis of sepsis is activated by the GN and GP agents. The activation of the inflammatory cascade first occurs with the recognition of the pathogen-associated molecular patterns by Toll-like receptors (TLRs). TLR2 GP bacteria recognise lipoteichoic acid, whereas TLR4 GN bacteria recognise lipopolysaccharide. Triggering of the different pathways of the inflammatory cascade with the nuclear factor kB and other mediators after TLR activation leads to the synthesis of proinflammatory cytokines and acute phase reactants (14). GN infections cause an increase in tumour necrosis factor alpha, interleukin (IL)-1, IL-6, IL-8 and IL-10 more than GP infections do (15). Cytokine increments at different plasma levels in GP and GN infections have also been demonstrated for the PCT levels (16). At the same time, elevated PCT levels correlate with the severity of inflammation (11). GN bacteraemia was shown to induce inflammatory response more than GP bacteraemia, and it was suggested that this is due to higher PCT values in GN bacteraemia (17).

In our study, the PCT median values were found as 7.31 ng mL^{-1} in patients with sepsis with GN bacteraemia and as 0.46 ng mL^{-1} in patients with sepsis with GP bacteraemia; the PCT threshold value was found to be 1.3 ng mL^{-1} to distinguish between GN and GP bacteraemia. In a study involving 166 patients with sepsis with positive blood culture in the ICU, the PCT levels were found as 8.90 ng mL^{-1} in the GN group and as 0.73 ng mL^{-1} in the GP group (11). In a study in which 292 patients diagnosed with sepsis and 31 patients with suspected sepsis were included and in which 328 bacteraemia attacks were examined, the PCT values were found to be 7.47 ng mL^{-1} and 0.48 ng mL^{-1} in GN and GP bacteraemia, respectively, and the PCT threshold value was calculated as 2.44 ng mL^{-1} for the differentiation of GN bacteraemia and GP bacteraemia (18). These values are consistent with those in our study.

In a study, 39% of the study population comprised patients in the emergency department, and it concluded that high PCT value in sepsis was a marker for Gram-negative bacteraemia; the PCT values were found as 26.7 (0.09–188.3) ng mL^{-1} in GN bacteraemia and 0.48 (0.05–18.8) ng mL^{-1} in GP bacteraemia. The PCT threshold value was calculated to be 6.47 ng mL^{-1} for the distinction between GN and GP bacteraemia. The SOFA score was calculated as 6 in the GN bacteraemia group and 4 in the GP bacteraemia group, and there was a significant difference between the two groups in terms of disease severity ($p = 0.06$) (12). While the median PCT value of the patients with GP bacteraemia is similar to that of our study, it is much higher in patients with GN bacteraemia than the value in our study. In the previous study, the method used for PCT measurement is different from that used in our study. If the PCT value is >100 in the results obtained through the method used in our study, it is expressed as >100, and the direct numerical value is not specified. The PCT value was found to range between 0.09 and 188.3 in patients with GN bacteraemia, and the values >100 were numerically included in the statistical evaluation. The PCT value was found to range between 0.05 and 18.8 in patients with GP bacteraemia, and no patient had a value >100. In addition, there is a difference between the two groups in terms of disease severity in the present study. Depending on these factors, the PCT median and threshold values are not similar to those of our study. In the study by Charles et al. (19) in which 92 intensive care patients with bacteraemia attacks were included, the median PCT values were found as 39.0 (0.41–746) ng mL^{-1} in the GN group and as 5.42 (0.07–169) ng mL^{-1} in the GP group; the PCT threshold value for the distinction between GN and GP bacteraemia was 16 ng mL^{-1}. The PCT values show similarity with our study. In the present study, the PCT level was measured with a different device, and the PCT values were given numerically >100, and there are values >100 in both GN and GP groups. Heterogeneous and different groups of patient populations, differences in the severity of the disease and different laboratory methods influence the consequences of studies. It is very important to interpret the studies by considering these characteristics.

Blood culture is negative in 30% of patients with sepsis due to reasons such as concurrent use of antibiotics and due to pathogens that multiply slowly or that are not appropriate to multiply in culture. Pathogens can be detected independently before they multiply in the blood culture using PCR-based systems that detect pathogenic DNA (5). Since these systems are very expensive, they have a limited use. In a study investigating the role of PCT values in selecting patients in whom PCR-based systems would be used, it was shown that the PCT values in patients with suspected sepsis could predict the outcomes of the PCR-based system and that the threshold value was 0.37 ng mL^{-1} for this. In the present study, the PCT values were found as 35.42±61.03 ng mL^{-1} in those whose PCR-based system result was positive, as 23.14±51.56 ng mL^{-1} in those who had positive blood culture, as 0.84±1.67 ng mL^{-1} in those whose PCR-based system result was negative and as 2.79±16.64 ng mL^{-1} in those who had negative blood culture (20). The PCT values that were detected are different from our study. Patients with sepsis with positive blood culture in the ICU were included in our study. Since the use of PCR-based systems will increase the rate of detection of causative pathogen, using different systems may impact the outcome in terms of pathogen detection.

In our study, the CRP values of those with GN-induced bacteraemia were found to be significantly higher than those with GP-induced bacteraemia. Although the CRP values have been shown to be higher in GN-induced bacteraemia in various studies (11, 18, 21), there are also studies reporting similar CRP values (12, 19). In our study, the area under the ROC curve was 0.80 for PCT and 0.61 for CRP, and the difference was determined to be significant; accordingly, our results show that the PCT values are more successful for the distinction between GN and GP bacteraemia. Similarly, in patients with suspected bloodstream infections, the area under the curve was reported as 0.75 for PCT and 0.60 for CRP for the distinction between GN and GP bacteraemia (21).

We need to consider the limitations in our study. Our results should not be generalised for all patients with sepsis because only patients with sepsis with bacteraemia have been included in the study. Blood culture was used for the diagnosis of bacteraemia. More patients could be included in the study with the recognition of more bacteraemia through PCR-based rapid molecular diagnostic tests that recognise pathogenic DNA. Another limitation is that the study was conducted retrospectively.

Conclusion

The PCT values were found higher in patients with sepsis with GN bacteraemia than those with GP bacteraemia. Our results suggest that the PCT values can be a useful tool for the distinction of GN and GP bacteraemia. However, it should not be forgotten that the PCT values should be used as an assistive tool for predictive purposes in microbiological diagnostic tests. Owing to the possibility of different outcomes in different patient populations, there is a need for studies in which the efficacy of PCT is separately assessed in various patient groups for the purpose of pathogen distinction.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Marmara University School of Medicine (09.2016.0243/70737436-050.06.04).

Informed Consent: Written informed consent was obtained from the parents of the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – B.B.; Design – B.B., M.H.; Supervision – I.C., B.B.; Data Collection and/or Processing – İ.S., M.S.A., U.S.K.; Analysis and/or Interpretation – B.B., M.H.; Literature Search – İ.S., M.S.A., U.S.K.; Writing Manuscript – I.C., B.B., M.H.; Critical Review – I.C.

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