



Roles of C-Reactive Protein and Procalcitonin in Empirical Treatment Approach to the Bacterial Sepsis Agent

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Objective: The primary aim of our study was to investigate the usefulness of serum C-reactive protein (CRP) and procalcitonin (PCT) levels in the differential diagnosis of causative gram-positive (Gram+) or gram-negative (Gram-) bacteria in patients with sepsis to facilitate decisions concerning the initial choice of an empiric antibiotic regimen.

Methods: Between February 2014 and February 2016, 47 patients who had sepsis diagnosed on the basis of positive blood cultures and clinical examination results were retrospectively enrolled. Serum CRP and PCT levels of 15 gram+ and 32 gram- bacterial sepsis groups were compared using the Mann-Whitney U test. The correlation between CRP and PCT levels was calculated using the Spearman's test.

Results: Among patients with bacterial sepsis, the median CRP level was 91.42 mg/L and the median PCT level was 0.46 ng/mL. There were no significant difference in CRP and PCT levels between the gram+ and gram- sepsis groups ($p=0.98$ and $p=0.21$, respectively). There was good correlation between the CRP and PCT levels ($r=0.64$, $p<0.001$).

Conclusion: Considering the changes and the status of proinflammatory/anti-inflammatory responses in the pathogenesis of sepsis, we believe that CRP and PCT levels alone are insufficient for predicting the type of causative bacteria in sepsis.

Keywords: Procalcitonin, C-reactive protein, sepsis, gram-positive bacteria, gram-negative bacteria

Introduction

Although the incidence of blood circulation infections such as bacteremia and sepsis can be reduced with appropriate antibiotic treatment when detected at an early stage (1), such infections are one of the most important causes of morbidity and mortality in hospitalized patients (2, 3). Rapid diagnosis of bloodstream infections is often difficult because traditional blood culture procedures are slow and time consuming, and the isolation of the pathogen through the antibiogram takes at least 48 hours after blood culture (4). Alternative laboratory tests such as erythrocyte sedimentation rate, serum C-reactive protein (CRP) levels, white blood cell count or percentage of neutrophils, and polymerase chain reaction (PCR) are used to detect bacteremia but are slow and tedious and lack sensitivity and specificity (1). Therefore, it is difficult to distinguish bloodstream infections from other diseases with these tests (5, 6). In fact, except for PCR, none of the other tests can on their own confirm the diagnosis of bacteremia (7, 8).

Interleukins, proatrial natriuretic peptide, copeptin, interferon- γ , resistin, and procalcitonin (PCT) have been investigated as potential sepsis biomarkers (7-14), and the most studied among these are PCT levels. Most of these studies have shown that serum PCT levels are low in healthy individuals and elevated in patients with bloodstream infection (11, 13-15). Other investigators have found inconsistent and variable findings when they conducted examinations by comparing the diagnostic and prognostic values of PCT levels with alternative parameters in the case of bacteremia (16, 17). Therefore, there is a need for more studies in order for PCT to become a diagnostic or predictive parameter that is routinely recommended.

Early identification and recognition of the first minor symptoms of infection at the onset of bloodstream infections can help determine whether patients are infected by gram-positive (gram+) or gram-negative (gram-) pathogens (4). Because serum PCT levels are influenced by lipopolysaccharides and sepsis-related cytokines (18, 19), it is expected that serum PCT levels of blood infections caused by gram- pathogens will be higher than blood infections caused by gram+ pathogens.

The aim of our study was to determine, in the differential diagnosis, if the predictive value of serum CRP and PCT levels of culture-positive sepsis patients could effectively separate gram+ from gram- bacterial infections at an earlier time point than the standard blood culture results. This would allow us to determine whether or not the correct treatment could be started by facilitat-

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ing the selection of an empirical antibiotic regimen based on the serum CRP and PCT levels at the early stage of the infection.

Methods

Cases

Patients over 18 years of age who were hospitalized in İstanbul Okmeydanı Training and Research Hospital between February 2014 and February 2016 were retrospectively scanned in the software system of our hospital. Reproduction in blood culture was detected, and the results of CRP and PCT, which were taken and studied simultaneously with blood culture, were selected in the laboratory software system. Blood cultures whose reproductions were microbiologically evaluated as contamination or those that were incompatible with the patient's clinical evaluation were excluded. The clinical records of the remaining cases with positive blood cultures were obtained through the hospital software system. Forty-seven cases that were clinically diagnosed with sepsis and in whom antibiotic therapy was started or whose antibiotic regimen was changed according to the isolated bacteria were included in the study. Gram+ bacteria were detected in 15 of these cases, and gram- bacteria were detected in 32 cases.

The study was conducted entirely in accordance with the Helsinki Declaration using the data obtained through the laboratory software system.

Blood culture

Aerobic and anaerobic blood culture bottles taken during the patients' febrile periods were loaded into a BACTEC FX blood culture device (BD Diagnostics, New Jersey, USA). MacConkey agar, sheep blood agar, and chocolate agar were inoculated, and the plaques from blood culture flasks giving positive reproductive signal were evaluated after incubation for 24 hours at 35°C-37°C. The Phoenix (BD Diagnostics, New Jersey, USA) automated microbiology system was used to identify isolated bacteria and antibiotic susceptibilities.

CRP and PCT measurements

The serum PCT levels were measured in a Cobas e411 (Roche Diagnostics, Mannheim, Germany) analyzer with the electrochemoluminescence immunoassay method using the Elecsys BRAHMS PCT kit (Roche Diagnostics, Mannheim, Germany), and the serum CRP levels were immunoturbidimetrically measured in the Cobas c501 (Roche Diagnostics, Mannheim, Germany) analyzer on the basis of latex agglutination using the CRPLX kit (Roche Diagnostics, Mannheim, Germany). All results were obtained from the laboratory software system and recorded.

The intra-day and inter-day coefficients of variation (CV) were respectively 1.0% and 1.3% on average for the CRP kit that was used, and the intra-day and inter-day CVs for the PCT kit were respectively 3.0% and 6.6% on average.

Statistical analysis

The serum CRP and PCT levels of the two groups whose sepsis factor was gram+ and gram- bacteria were described as the median (25th percentile–75th percentile) because neither of the two variables showed a normal distribution. The non-parametric Mann–Whitney U-test was performed with SPSS 17.0 (SPSS Inc.; Chicago, USA) to determine whether there was a significant difference be-

tween the two groups. The correlation between CRP and PCT levels was assessed by Spearman's test. For all tests, $p < 0.05$ was considered statistically significant.

Results

The types and numbers of the bacteria isolated from blood cultures are given in Table 1. While *Klebsiella pneumoniae* was the most common factor in blood cultures of 47 cases who were diagnosed clinically as bacterial sepsis with blood culture positivity, coagulase-negative staphylococci were the most common agent among gram+ bacteria.

There was no significant difference between serum CRP and PCT levels of sepsis patients with gram+ bacteria compared to those with gram- bacteria ($p=0.98$ and $p=0.21$, respectively) (Table 2).

Significant positive correlations were found between CRP and PCT levels in all sepsis cases ($r = 0.640$, $p < 0.001$).

Discussion

Early initiation of the appropriate antibiotic regimen is critical for good outcomes in the treatment of severe infections (20-24). Because of this, early and accurate diagnosis of bloodstream infections is very important, and the initiation of appropriate antibiotics should not be delayed until the isolation of the causative microorganism from the blood culture (25).

Table 1. Bacteria isolated from blood cultures

Bacteria	Number (%)
Gram- bacteria	32 (68.0)
<i>Klebsiella pneumoniae</i>	16 (34.0)
<i>Acinetobacter baumannii</i>	5 (10.6)
<i>Escherichia coli</i>	4 (8.5)
<i>Stenotrophomonas maltophilia</i>	3 (6.4)
<i>Pseudomonas aureginosa</i>	2 (4.3)
<i>Enterobacter spp.</i>	1 (2.1)
<i>Klebsiella pneumoniae</i> + <i>enterobacter spp.</i>	1 (2.1)
Gram+ bacteria	15 (32.0)
<i>Staphylococcus aureus</i>	6 (12.8)
<i>Staphylococcus spp.</i>	4 (8.5)
<i>Staphylococcus epidermidis</i>	2 (4.3)
<i>Staphylococcus hemolyticus</i>	2 (4.3)
<i>Enterococcus spp.</i>	1 (2.1)

Table 2. The CRP and PCT levels of the cases caused by gram+ and gram- bacteria

	Gram+ (n=15)	Gram- (n=32)	p
CRP (mg/L)	76.67 (48.71-171.0)	94.08 (22.73-215.05)	0.98
PCT (ng/mL)	0.26 (0.15-7.05)	0.85 (0.29-8.74)	0.21

CRP: C-reactive protein; PCT: procalcitonin

Mortality rates of up to 45% have been reported in patients with nosocomial bacteremia as a result of improper selection of the empiric antibiotic regimen (26). Because microbiological results can only be obtained 24–48 hours after the culture is taken, the initiation of an inappropriate antibiotic regimen during this period adversely affects the prognosis (20, 26). This suggests that there is a need for clinical and/or biochemical parameters that might signal to the clinician in the early stages of the disease that the empirical antibiotic regimen might be inappropriate (20, 26).

In our study, we could not find a significant difference between serum CRP and PCT levels in the patients with sepsis caused by gram+ and gram- bacteria. In addition, although we found a positive correlation between CRP and PCT levels, the fact that both parameters did not differ between the sepsis caused by gram+ and gram- bacteria also supports our conclusion. Nakajima et al. (27) demonstrated in their study that PCT levels were higher in sepsis patients with gram-factor than the group with gram+ factor. However, because they stated as a limitation of their work that they made this comparison with only 7 gram+ and 6 gram- cases, their results must be interpreted with caution. In contrast, and similar to our findings, Anand et al. (28) in a prospective observational study found no significant difference among culture-positive sepsis patients with gram+ and gram- factors in terms of PCT and IL-6 levels. In a meta-analysis of the available data, it was noted that PCT was not a reliable marker to distinguish bacterial sepsis in adult ICU patients from systemic inflammatory response syndrome (SIRS) occurring due to the other noninfectious causes because the PCT level has low sensitivity and specificity (29). As a result of this, they argued that this result did not support the widespread use of procalcitonin in intensive care settings (29).

It should not be forgotten that PCT levels might also be affected in the event that the patients have other underlying and additional inflammatory diseases besides bloodstream infections. In addition, high levels of PCT in culture-negative patients might be encountered depending on previous stroke, burns, trauma, liver cancer, or cardiac surgery (30-32). Because the number of cases we could use to compare PCT levels according to the infection factor by separating them into subtypes according to underlying diseases was small, prospective multi-centered studies in this respect will enable more accurate predictive values for PCT.

Another important point is that the half-life of PCT is 24 hours (33) and that PCT levels have been shown to decrease in some patients who previously received antibiotic therapy (34). For this reason, the fact that information on whether or not the patients received antibiotic treatment before their blood was taken could not be obtained from the software system is a limitation of our study. However, because the blood cultures were routinely taken during febrile periods and because only the patients in whose cultures reproduction occurred were included in the study (culture-negative sepsis cases were taken as an exclusion criterion), even if the patients were receiving antibiotic treatment it can be assumed that response to the treatment might not have been recorded. In addition, because the start of a new antibiotic regimen due to infectious diseases was among the inclusion criteria, this suggests that the previously used antibiotic regimen was not effective.

Sepsis initiates a complex immunological response that changes over time (35, 36). Although some studies show that inflammatory and anti-inflammatory responses start simultaneously, the most

common understanding is that the hyperinflammatory response occurs in the early period and that immunosuppression occurs in the later period (37). The magnitude of these responses varies depending on a large number of variables, including the number and virulence of pathogens and other diseases affecting the patient (35). This situation requires that the sepsis diagnosis not be delayed and that it be made in time in order for the PCT measurement to be used correctly and effectively for the purpose of anticipating the factor in advance and suggesting the appropriate treatment and follow-up.

When the results of our study are evaluated, it should be taken into consideration that the above-mentioned limitations might also have been effective because of the fact that there was no significant difference between serum CRP and PCT levels of sepsis patients with gram+ and gram- bacteria. However, it is also evident that the limitations mentioned above cannot be considered independently of the situations encountered in routine practice because the underlying diseases of each sepsis patient will naturally differ from each other in clinical practice. It is also highly probable that many adult sepsis patients are receiving antibiotherapy prior to the development of sepsis in an internal medicine or intensive care unit. Finally, it should not be forgotten that the time needed to take blood cultures and establish the diagnosis of sepsis might also affect the CRP and PCT levels. Therefore, despite the limitations caused by the fact that our study was retrospective, we think that it reflects the use of CRP and PCT tests in sepsis evaluation in clinical practice. We have concluded that gram+ or gram- cannot be confirmed to be the factor only by examining the CRP and PCT levels alone, and the culture results of the patients with culture-positive bacterial sepsis still need to be examined.

Because our study was conducted only on culture-positive bacterial sepsis, our results suggest that CRP and PCT alone cannot distinguish the culture-positive bacterial sepsis factor as gram+ or gram-. These results do not include bacteremia, SIRS, or culture-negative sepsis. It should be kept in mind that the immunological responses, and therefore the results, in these cases might be different, and the results of the studies carried out with regards to these issues should be evaluated.

Conclusion

Considering the underlying differences in the pathogenesis of sepsis and the difficulties in the early diagnosis of sepsis in clinical practice, we think that the CRP and PCT levels alone are not enough to predict the type of bacterial factor as gram+ or gram- in patients with culture-positive bacterial sepsis.

Ethics Committee Approval: Ethics committee approval was not received because the study was retrospectively made through the laboratory information system.

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