Comparison of Procalcitonin and C-Reactive Protein Values in *Brucella Spp* and Other Gram-Negative Bacteremias

Muhammet Güzel Kurtoğlu, Meral Kaya, Ayşegül Opus, Şerife Yüksekay, Asuman Güzelant

*Konya Research and Education Hospital, Department of Microbiology and Clinical Microbiology, Konya, Turkey*

**Özet**  
Bu çalışmada *Brucella spp.*, Escherichia coli, Klebsiella spp. ve Acinetobacter spp. gibi Gram negatif bakteriyemisi olan hastalarda prokalsitonin (PCT) ve C-reaktif protein (CRP) düzeylerinin karşılaştırılması amaçlanmıştır. Hastalardan alınan kan örneklerinde PCT, CRP ve kültür eş zamanlı çalışılmıştır. Bu örnekler Konya Eğitim ve Araştırma Hastanesinde çalışılmıştır. PCT ve CRP değerlerinin >5 mg/L idi. Acinetobacter spp. simdi 24 hastada PCT değerlerinin Grup 2 (0.5-2.0 ng/ml)'de iken 40 hastanın tümünde CRP değerleri >5 mg/L idi. Klebsiella spp. saptanan hastaların 34'ünde PCT değerleri Grup 3 (>2 ng/ml)'de, 46'sında CRP değerleri >5 mg/L idi. E. coli saptanan hastalara PCT değerleri Grup 1 (<0.5 ng/ml)'de, 92 hastada CRP değerleri ise >5 mg/L idi. E. coli saptanan hastaların 34'ünde PCT değerleri Grup 3 (>2 ng/ml)'de, 46'sında CRP değerleri >5 mg/L idi. Klebsiella spp. saptanan hastaların 47’sinde PCT değerleri Grup 3 (>2 ng/ml)'de, 39’unda CRP değerleri >5 mg/L idi. Acinetobacter spp. saptanan 4 hastada PCT değerleri Grup 2 (0.5-2.0 ng/ml)'de iken 24 hastanın tümünde CRP değerleri >5 mg/L idi. Başı Gram negatif bakteri infeksiyonlarında PCT ve CRP değerleri artırmakta iken, kan kültüründe Brucella spp. üreyan hastalarda ise CRP değerlerinin artışı ancak PCT değerlerinin ise artmadığı saptanmıştır.

**Anahtar kelimeler:** Prokalsitonin, C-reaktif protein, *Brucella spp.*, Gram-negatif bakteriler

**Abstract**  
In this study, we aimed to compare procalcitonin (PCT) and C-reactive protein (CRP) levels in patients with gram-negative bacteremia, as *Brucella spp.*, Escherichia coli, Klebsiella spp., and Acinetobacter spp. Simultaneous studies on culture, PCT and CRP were done on the blood samples obtained from the patients. Samples were investigated in Konya Research and Education Hospital, Konya, Turkey. Samples were collected and incubated in BACTEC 9120 blood culture system. Phoenix-100 automated identification panels were used. For *Brucella spp.*, identification, Brucella polyvalent serum was used in addition to conventional methods. CRP values >5 mg/L and PCT values >0.5 ng/ml were considered pathological. (Cut of value of PCT is 0.02-50 ng/ml.) PCT values were evaluated in three groups as: Group 1 (<0.5 ng/ml), Group 2 (0.5-2.0 ng/ml) and Group 3 (>2.0 ng/ml). *Brucella spp.* were detected in 100 patients, while E. coli in 50 patients, Klebsiella spp. in 50 patients, and Acinetobacter spp. in 40 patients, and these 240 patients were included in the study. For *Brucella spp.* PCT was in Group 1 (<0.5 ng/ml) in all 100 patients, and CRP was >5 mg/L in 92. For E. coli, PCT was in Group 3 (>2 ng/ml) in 34 patients and CRP was >5 mg/L in 46. For Klebsiella spp., PCT was in Group 3 (>2 ng/ml) in 47 patients and CRP was >5 mg/L in 39. For Acinetobacter spp., PCT was in Group 2 (0.5-2.0 ng/ml) in 24 patients and CRP was >5 mg/L in all 40. In some of the gram-negative bacterial infections, PCT and CRP levels were increased, but in patients in whom *Brucella spp.* were grown in blood culture, CRP level increased while PCT level did not.

**Key words:** Procalcitonin, C-reactive protein, *Brucella spp.*, Gram-negative bacteria.
bacterial endotoxin. Secreted PCT concentration remains high for 24-48 hours, and drops to base level after two days (2,4). The identification of PCT is simplified by its stability at room temperature, its durability in response to heat, freezing and melting, and the availability of simple laboratory techniques for its determination (3,11). Plasma concentrations of PCT in healthy individuals are at low levels (as picogram), and these are below the levels that can be determined by the current PCT measuring methods (<0.1 ng/ml). PCT values >0.5 ng/ml are considered pathological. Control of infection with antibiotic treatment produces a reduction in PCT levels (4,6,12). PCT plasma values for illnesses that are not bacterial or parasitic are generally <2 ng/ml, and in serious bacterial infections and sepsis, values range between 1 ng/ml and 1000 ng/ml (4,13-16).

In the studies carried out, an increase in PCT values in acute bacterial infections or in the 4-6 hours after endotoxin injection was observed, while no increase in C-reactive protein (CRP) values are found. However, it was reported that at the end of inflammation, PCT values reduced immediately, while the drop in CRP values was late (4,8). CRP, which has the structure of globulin, is the most frequently used acute phase reactant in practice and is synthesized in the liver. While CRP is a very sensitive parameter of inflammation, it can also be induced by stimulants that are not specific. CRP can increase 1000-fold in 24-48 hours, and it increases at a slower rate than PCT, which is determined at higher levels for longer periods, and is inadequate to distinguish bacterial inflammation from others (4,17-19). Brucella bacteria are a pathogen for humans and animals, and the preferred reproductive environment is the host’s intracellular. In contrast with other pathogen bacteria, Brucella do not have the classic virulence factors such as exotoxin, cytolysin, capsule, fimbriae, plasmid, and endotoxic lipopolysaccharide. Instead, molecular determinants play the role of virulence factors that enable the Brucella bacteria to invade the host cell, remain vital and multiply in the intracellular (20). In this study, we aimed to compare the PCT and CRP levels in patients in whom gram-negative and Brucella bacteremia was determined on blood cultures.

MATERIALS AND METHODS

Many of data miners think that association rule (AR) is an unsuThe study is a prospective one. A total of 240 patients with axillary fever ≥37 °C in various clinics and who were determined to have Brucella spp. (100), Escherichia coli (50), Klebsiella spp. (50), and Acinetobacter spp. (40) on blood sample cultures were included in the study. Only one strain was evaluated in each patient’s sample. Simultaneous studies on culture, PCT and CRP were done on the blood samples obtained from the patients. In some patients with C-cell carcinoma of the thyroid, serum PCT levels was checked, and no malignancy was determined. The blood taken from patients with fever was inoculated in BACTEC Plus Aerobic/F blood culture vials (Becton Dickinson, USA) and incubated at 37 °C for at most 10 days in BACTEC 9120 (Becton Dickinson, USA) blood culture system. When blood cultures became positive, the broth was Gram-stained and subcultured onto Columbia agar with 5% defibrinated sheep blood agar (Difco, USA) Chocolate agar, Mac Conkey agar, Eosin Methylene Blue agar (Difco, USA) media. When growth was detected, the bacteria were identified by using conventional methods and Phoenix 100 (Becton Dickinson, USA) identification panels. In Brucella identification, Gram-negative coccobacilli were observed in the gram stain of the smooth, small, round and daw-drop-like colonies grown in blood agar and chocolate agar. Catalase and oxidase activities were positive. Pure colonies taken from these colonies that were thought to be Brucella spp. were verified with Brucella polyvalent serum (Refik Saydam National Public Health Agency, Ankara). COBAS E411 system (ROCHE, Japan) and BRAHMS PCT kits (ROCHE, Germany) were used for PCT measurement. Normal PCT value <0.5 ng/ml and all values >0.5 ng/ml were considered pathologic. Plasma concentration of PCT is proportional to inflammatory reaction. Values in the range 0.5-2.0 ng/ml are considered to be slightly elevated, and those >2.0 ng/ml are considered high. PCT values were evaluated in three groups as: Group 1 (<0.5 ng/ml), Group 2 (0.5-2.0 ng/ml) and Group 3 (>2.0 ng/ml) (21).

Dade Behring/Siemens BN II analyzer (Germany) and Dade Behring/Siemens kits (Germany) were used for CRP measurement. CRP levels >5 mg/L were considered pathologic (21). The SPSS 15.0 package program was used for the statistical evaluations. For the analysis of data, Chi-square (χ²) and McNemar tests and hypothesis were used.

RESULTS

A total of 240 patients in whom Brucella spp. (100), E. coli (50), Klebsiella spp. (50), and Acinetobacter spp. (40) were detected on blood cultures were included in the study. The age range of the patients with Brucella spp. was 12-86 years (mean: 54.44 ± 1.70), and 52 (52%) were males and 48 (48%) were females. The age range of patients with gram-negative bacteria was 6-82 years (mean: 53.96 ± 4.84), and 71 (51%) were males and 69 (49%) were females. In all 100 (100%) patients in whom Brucella spp. were detected, PCT level was in Group 1, and CRP was >5 mg/L in 92 patients (92 %). For the patients in whom E. coli was detected (n: 50), PCT levels were in Group 3 in 34 patients (68 %), and CRP was >5 mg/L in 46 patients (92 %). Among the patients in whom Klebsiella spp. were identified (n: 50), PCT values were in Group 3 in 47 patients (94 %), and CRP was >5 mg/L in 39 patients (78 %). Finally, 24 (60 %) of 40 patients with Acinetobacter spp. had PCT values in Group 2 and all 40 (100 %) had CRP >5 mg/L (Table I). Distributions of PCT groups by age and sex are shown in the figure I and II.

Table 1. The distribution of PCT and CRP values according to the bacteria determined (n, %).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>PCT Group 1</th>
<th>PCT Group 2</th>
<th>PCT Group 3</th>
<th>CRP &lt;5 mg/L</th>
<th>CRP &gt;5 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucella spp. (100)</td>
<td>100 (100 %)</td>
<td></td>
<td></td>
<td>8 (8 %)</td>
<td>92 (92 %)</td>
</tr>
<tr>
<td>E. coli (50)</td>
<td>0</td>
<td>16 (32 %)</td>
<td>34 (68 %)</td>
<td>4 (8 %)</td>
<td>46 (92 %)</td>
</tr>
<tr>
<td>Klebsiella spp. (50)</td>
<td>0</td>
<td>3 (6 %)</td>
<td>47 (94 %)</td>
<td>11 (22 %)</td>
<td>39 (78 %)</td>
</tr>
<tr>
<td>Acinetobacter spp. (40)</td>
<td>0</td>
<td>24 (60 %)</td>
<td>16 (40 %)</td>
<td>0</td>
<td>40 (100 %)</td>
</tr>
</tbody>
</table>

*a: The total number of patients taken into the study in the group*
Brucella spp. and procalcitonin

The distribution of PCT and age groups (n)

Among the 100 patients with Brucella spp. and 140 patients who had been treated with Gram-negative bacteria, 54.44 ± 1.70 and 53.96 ± 4.84, respectively. When PCT and CRP values were compared, no significant correlation was seen (0.95 significance level and 2 degrees of freedom (df), X² table: 5.9 and calculated X²: 3.7, p>0.05). There was a significant correlation between PCT and the bacteria grown (0.95 significance level and 6 df, X² table: 16.812 and calculated X²: 282.83; p<0.01). A comparison between CRP and the bacteria grown demonstrated a significant correlation (0.99 significance level and 3 df, X² table: 11.34 and calculated X²: 15.06; p<0.01). A comparison between PCT and age groups demonstrated a significant correlation (0.95 significance level and 6 df, X² table: 1.635 and calculated X²: 9.208; p>0.05). A comparison between PCT and gender demonstrated a significant correlation (0.95 significance level and 2 df, X² table: 0.352 and calculated X²: 217.043; p>0.05).

Statistical Findings

The mean ages of the 100 patients with Brucella spp. and 140 patients with Gram-negative bacteria were 54.44 ± 1.70 and 53.96 ± 4.84, respectively. When PCT and CRP values were compared, no significant correlation was seen (0.95 significance level and 2 degrees of freedom (df), X² table: 5.9 and calculated X²: 3.7, p>0.05). There was a significant correlation between PCT and the bacteria grown (0.95 significance level and 6 df, X² table: 16.812 and calculated X²: 282.83; p<0.01). A comparison between CRP and the bacteria grown demonstrated a significant correlation (0.99 significance level and 3 df, X² table: 11.34 and calculated X²: 15.06; p<0.01). A comparison between PCT and age groups demonstrated a significant correlation (0.95 significance level and 6 df, X² table: 1.635 and calculated X²: 9.208; p>0.05). A comparison between PCT and gender demonstrated a significant correlation (0.95 significance level and 2 df, X² table: 0.352 and calculated X²: 217.043; p>0.05).

DISCUSSION

It has been stated that since CRP alone is inadequate for the diagnosis of sepsis, other markers must be evaluated together with this parameter (22.23). In many studies, it has been stated that PCT, which is a new marker, increases markedly in conditions like severe sepsis and septic shock. However, in non-bacterial systemic inflammations such as viral infections, allergic reactions, autoimmune illnesses, neoplastic illnesses, minor surgical procedures, and local bacterial infections, it was found that the increase in PCT is not significant [4]. Simon et al. (24), based on the results of a meta-analysis, concluded that the measurement of PCT is more successful than of CRP for the diagnosis of bacterial infections of patients admitted to the hospital. It has been reported that the sensitivity and specificity of PCT in bacterial infections are 92.6% and 97.5%, respectively (25,26). Moreover, it was reported that the sensitivity and specificity values reach 100% in delayed bacterial infections (3-30 days) (2). In the study done by Gendrel et al. (3) with burned patients, it was observed that PCT secretion is moderate, but reaches very high levels in patients with septic complications. It was reported that PCT serum concentration can range from 20 ng/ml to 200 ng/ml in severe systemic infections of bacterial origin, and the increase in serum levels is compatible with the severity of illness (27,28). In a study done by Clech et al. (29), PCT level was found high in patients with septic shock as compared to those without septic shock, but a difference in PCT ratios was not seen in gram-negative and gram-positive bacterial infections. It was emphasized in their work that PCT can be used as both a diagnostic and prognostic factor in patients with septic shock of bacterial origin.

In his study, Ghorbani (30) demonstrated a relationship between high PCT and positive blood culture. Different researchers reported that PCT is high in cases in which growth has been seen in blood culture (31,32). Lee et al. (33) reported that PCT level can also be used to predict mortality due to sepsis. A study done by Niederman (34) on nosocomial pneumonia recommended not starting antibiotics in patients lacking clinical symptoms of severe illness and with low PCT levels (<0.25 µg/L). He stated that a series of PCT measurements is important for the follow-up of the response to treatment and that the treatment should be discontinued after a short period. Seow et al. (9) found a PCT level of 0.5 ng/ml in a brucellosis patient with 38 °C fever and Delevaux et al. (35) reported PCT as 0.1 ng/ml. The findings of our study was found to be consistent with the results determined by these researchers.

While it is known that PCT is a good criterion for diagnosis of other bacterial infections, there are only a few publications on the significance of PCT in infections due to Brucella, which is a gram-negative bacterium. In this study, it was found that PCT and CRP levels generally increased in gram-negative bacterial infections, in agreement with the data of other researchers, and that PCT level did not increase in patients in whom Brucella spp. were grown, but CRP level increased 92 %. Brucella is an intracellular bacteria and continues its presence by settling in both a diagnostic and prognostic factor in patients with septic shock of bacterial origin. The lack of increase in PCT level. Since it is not fully known why PCT levels are low in Brucella infections, our work is important because it is the first study in this field and will thus contribute valuable data to the new studies on this issue.

Acknowledgment

For his contributions, we thank Nurettin Kaya for statistical analysis.

REFERENCES

5. Muller B, White JC, Nylen ES, et al. Ubiquitous expression of the calcitonin-I gene in multitissue in response to sepsis. L Clin Endocrinol Metab...