Araştırma Yazısı
Selçuk Tıp Derg 2014;30(3): 104-107

SELÇUK TIP DERGİSİ

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

Brucella spp ve Diğer Gram Negatif Bakterilerde C-Reaktif Protein ve Prokalsitonin Değerlerinin Karşılaştırılması

Muhammet Güzel Kurtoğlu, Meral Kaya, Ayşegül Opus, Şerife Yüksekkaya, 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 çalışıldı. Örnekler BACTEC 9120 kan kültür sisteminde inkübe edildi. Phoenix-100 otomatik identifikasyon panelleri kullanıldı. Brucella spp.'in tanımlanmasında konvansiyonel metodlara ilaveten Brucelle polivalan serum kullanıldı. CRP değerlerinin >5 mg/L ve PCT değerlerinin ise >0.5 ng/ml olması patolojik olarak kabul edildi (PCT'in Cut of değeri: 0.02-50 ng/ml). PCT değerleri üç grupta değerlendirildi. Grup 1 (<0.5 ng/ml), Grup 2 (0.5-2.0 ng/ml) ve Grup 3 (>2.0 ng/ml). Brucella spp. saptanan 100, E. coli saptanan 50, Klebsiella spp. saptanan 50, ve Acinetobacter spp. saptanan 40 hasta olmak üzere 240 hasta çalışmaya alındı. Brucella spp. saptanan 100 hastada da PTC 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 (>2.0 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 24 hastada PCT değerleri Grup 2 (0.5-2.0 ng/ml)'de iken 40 hastanın tümünde CRP değerleri >5 mg/L idi. Bazı Gram negatif bakteri infeksiyonlarında PTC ve CRP değerleri artmakta iken, kan kültüründe Brucella spp. üreyen hastalarda ise CRP değerlerinin arttığı 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 (>2.0 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.

INTRODUCTION

A quick distinction between systemic inflammations due to infection or otherwise and initiation of proper treatment will help to reduce morbidity, mortality and the cost of care of patients. Procalcitonin (PCT), which has recently come to be used for this purpose, is a new parameter added to the infection markers (1). PCT has the structure of glycoprotein and is the precursor of calsitonin. It becomes active in normal metabolic conditions and is released by C cells of the thyroid gland (2-5). Recent studies have shown that blood levels of PCT are increased in infection, stemming from extrathyroidal origin (4). The high PCT levels observed in patients, who have undergone thyroidectomy, support this theory (3,4). Nowadays, there are increasing numbers of studies aimed to show that PCT can be used as an early indicator of infection (6-8). It differentiates bacterial infections from other causes of fever or sepsis (9). PCT is not only a better indicator for the rapid diagnosis of serious infections like sepsis, it is also used for prognosis and during the follow-up of response to treatment (1,8,10). Induction of PCT was realized in experiments with healthy volunteers with the injection of a small amount of intravenous

Yazışma Adresi: Muhammet Güzel Kurtoğlu, MD, Konya Research and Education Hospital, Department of Microbiology and Clinical Microbiology, Konya, Türkiye
e posta: kurtoglumg@hotmail.com
Geliş Tarihi: 11.03.2014 Yayına Kabul Tarihi: 19.03.2014

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 can be high in the absence of infection [9]. Thus, thyroid malignancy was queried and investigated in all patients in whom PCT level 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 Colombia 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 dew-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 is <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 (X^2) 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, %).

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

n: The total number of patients taken into the study in the group

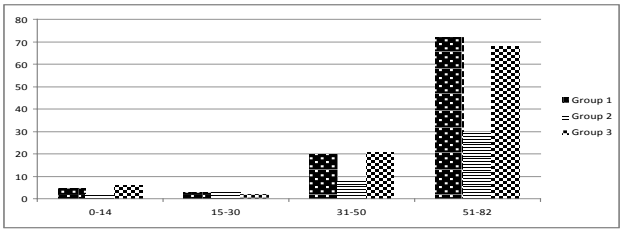

Figure 1. The distribution of PCT and age groups (n)

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), X^2 table: 5.9 and calculated X^2: 3.7, $p>0.05$). There was a significant correlation between PCT and the bacteria grown (0.95 significance level and 6 df, X^2 table: 16.812 and calculated X^2: 282.83; $p<0.01$). A comparison between CRP and the bacteria grown demonstrated a significant correlation (0.99 significance level and 3 df, X^2 table: 11.34 and calculated X^2: 15.06; $p<0.01$). A comparison between PCT and age groups demonstrated a significant correlation (0.95 significance level and 6 df, X^2 table: 1.635 and calculated X^2: 9.208; $p<0.05$). A comparison between PCT and gender demonstrated a significant correlation (0.95 significance level and 2 df, X^2 table: 0.352 and calculated X^2: 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 Clec'h 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 macrophages (20). It is not exactly known yet if this has any effect on 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.

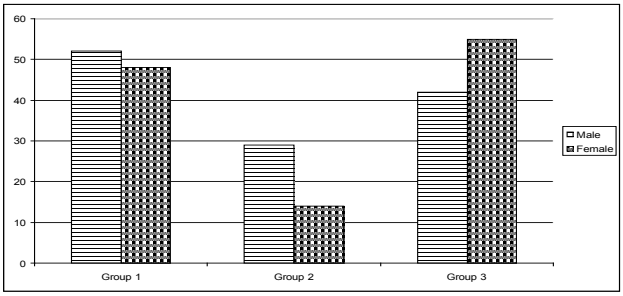

Figure 2. The distribution of PCT and gender (n)

Acknowledgment

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

REFERENCES

1. Gunal o, Barut HS. Sepsis and procalcitonin. Cumhuriyet Med J 2009; 31: 502-12.
2. Carrol ED, Thomson APJ, Hart CA. Procalcitonin as a marker of sepsis. Int J Antimicrob Agents 2002; 20: 1-9.
3. Gendrel D, Bohuon C. Procalcitonin as a marker of bacterial infection. Pediatr Infect Dic J 2000; 19: 679-88.
4. Maisner M. Procalcitonin-a new, innovative infection parameter biochemical and clinical aspects. 3. revised and expanded edition. Stuttgart, New York: Georg Thieme Verlag, 2000.
5. Muller B, White JC, Nylen ES, et al. Ubiguitous expression of the calcitonin-I gene in multible tissues in response to sepsis. L Clin Endocrinol Metab

2001; 86: 396-404.
6. Franzin L, Cabodi D. Legionella pneumonia and serum procalcitonin. Curr Microbiol 2005; 50(1): 43-6.
7. Schneider HG, Lam QT. Procalcitonin for the clinical laboratory: a review. Pathology 2007; 39(4): 383-90.
8. Van Rossum AM, Wulkan RW, Oudesluys-Murphy AM. Procalcitonin as an early marker of infection in neonates and children. Lancet Infect Dis 2004; 4 (10): 620-30.
9. Seow CJ, Barkham T, Wong PM. Brucellosis in a Singaporean with prolonged fever. Singapore Med J 2009; 50(9): 312-4.
10. Suzanne B, Cohen R, Nicholas P. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med 2006; 34(7): 1996-2003.
11. Meisner M, Tschaikowsky K, Schnabel S, et al. Procalcitonin-influence of temperature, storage, anticoagulation and arterial or venous asservation of blood samples on procalcitonin concentrations. Eur J Clin Chem Clin Biochem 1997; 35: 597-601.
12. Jones AE, Fiechtl JF, Brown MD, et al. Procalcitonin test in the diagnosis of bacteremia: a meta-analysis. Ann Emerg Med 2007; 50(1): 34-41.
13. Boussekey N, Leroy O, Alfandari S, et al. Procalcitonin kinetics in the prognosis of severe community-acquired pneumonia. Intensive Care Med 2006; 32: 469-72.
14. Heper Y, Akalın EH, Mıstık R, et al. Evaluation of serum C-reactive protein, procalcitonin, tumor necrosis factor alpha, and interleukin-10 levels as diagnostic and prognostic parameters in patients with community-acquired sepsis, severe sepsis, and septic shock. Eur J Clin Microbiol Infect Dis 2006; 25(8): 481-91.
15. Mimoz O, Benoist JF, Edouard AR. Procalcitonin and C-reactive protein during the early posttraumatic systemic inflamatory response syndrome. Intensıve Care Med 1998; 24: 185-8.
16. Korppi M, Remes S, Heiskanen-Kosma T. Serum procalcitonin concentrations in bacterial pneumonia in children: a negative result in primary healthcare settings. Peditr Pulmonol 2003; 35(1): 56-61.
17. Fernandez LA, Luaces CC, Garcia JJ, et al. Procalcitonin in pediatric emergency departments for the early diagnosis of invasive bacterial infections in febrile infants: results of a multicenter study and utility of a rapid qualitative test for this marker. Pediatr Infect Dis J 2003; 22: 895-903.
18. Meisner M. Pathobiochemistry and clinical use of procalcitonin. Clinica Chimica Acta 2002; 323:17-29.
19. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003; 107: 363–9.
20. Winn JW, Allen S, Janda WM, et al. Brucella species. In Winn JW, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Woods G, ed. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th edition. Philadelphia: Lippincott Williams & Wilkins, 2006: 482-91.
21. Güneş Başol, Zuhal Parıldar, Barutcuoglu B, et al. A new inflamatory marker procalcitonin and its relationship with renal dysfunction. Journal of Turkish Clinical Biochemistry 2008; 6 (1): 7-16.
22. Krediet T, Gerard L, Fleer A. The predictive value of CRP and I/T-ratio in neonatal infection. J Perinat Med 1992; 20: 479-85.
23. Nga PC, Cheng SH, Chui KM. Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecule, and C-reactive protein in preterm very low-birth weight infants. Arch Dis Child 1997; 77: 221-7.
24. Simon L, Gauvin F, Amre DK. Serum procalcitonin and C-rective protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis 2004; 39: 206-17.
25. Gendrel D, Raymond J, Assicot M, et al. Measurement of procalcitonin levels in children with bacterial or viral meningitis. Clin Infect Dis 1997; 24: 1240-2.
26. Rey C, Los Arcos M, Concha A. Procalcitonin and CRP as markers of systemic inflammatory response syndrome severity in critical ill children. Intensive Care Med 2007; 33: 477-84.
27. Assicot M, Gendrel D, Carsin H, et al. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993; 341: 515-8.
28. Gendrel D, Raymond J, Coste J, et al. Comparison of procalcitonin with C-reactive protein, interleukin 6 and interferon-alpha for differentitation of bacterial vs. viral infections. Pediatr Infect Dis J 1999; 18: 875-81.
29. Clec'h C, Ferriere F, Karoubi P, et al. Diagnostic and prognostic value of procalcitonin in patients with septic shock. Crit Care Med 2004; 32: 1166-9.
30. Ghorbani G. Procalcitonin role in differential diagnosis of infection stages and non infection inflammation. Pak J Biol Sci 2009; 12: 393-6.
31. Gendrel D, Assicot M, Raymond J. Procalcitonin as a marker for the early diagnosis of neonatal infection. J Pediatr 1996; 128: 570-3.
32. Mannoet G, Labaune JM, Isaac C. Procalcitonin and Creaktive protein levels in neonatal infections. Acta Pediatr 1997; 86: 209-12.
33. Lee CC, Chen SY, Tsai CL, et al. Procnostic value of mortality in emergency department sepsis score, procalcitonin and C-reactif protein in patients with sepsis at the emergency department. Shock 2008; 29: 322-7.
34. Niederman MS. Biological markers to determine eligibility in trials for community-acquired pneumonia: a focus on procalcitonin. Clin Infect Dis 2008; 47: 127-32.
35. Delevaux I, Andre M, Colombier M, et al. Can procalcitonin measurement help in differentiating between bacterial infection and other kinds of inflammatory processes? Ann Rheum Dis 2003; 62: 337-40.