J Pediatr Res
DOI: 10.4274/jpr.47560

Original Article

Can Serial Measurement Enhance the Diagnostic Value of Procalcitonin as a Marker of Gram-Negative Bacteremia in Children with Acute Leukemia?

Zümrüt Şahbudak Bal[1], Gulinaz Ercan[2], Deniz Yilmaz Karapinar[3]

[1]Ege University Faculty of Medicine, Department of Pediatrics, Division of Infectious Diseases, İzmir, Turkey
[2]Ege University Faculty of Medicine, Department of Medical Biochemistry, İzmir, Turkey
[3]Ege University Faculty of Medicine, Department of Pediatrics, Division of Hematology, İzmir, Turkey

ABSTRACT

Aim: Despite improvements in diagnosis and treatment, infections are still major cause of morbidity and mortality in children with febrile neutropenia (FN). In these patients, due to inadequate inflammatory response and subtle clinical symptoms, to determine the source of infection can be challenging.Therefore, it is important to distinguish infections from other non-infectious causes, for both to choose appropriate antibiotic and to reduce the redundant antibiotic use.

Materials and Methods: In this retrospective study, we aim to evaluate serial procalcitonin (PCT) levels for predicting bacteremia particularly caused by Gram-negative microorganism.

Results: Among FN episodes caused by Gram-negative microorganism, the median level of second PCT sample obtained between 48 and 72 hours (PCT 2) was found to be significantly higher when compared to infections caused by Coagulase-negative Staphylococcus (CoNS) or culture-negative confirmed infections, P value was 0.003; however, fever onset PCT 1 and C-reactive protein (CRP) 1 values showed no significant difference (P>0.05). The area under curve (AUC) values demonstrated by ROC analysis for CRP 1, CRP 2, PCT 1, PCT 2 were 0.664, 0.748, 0.504 and 0.842, respectively.

Conclusion: This study showed that initial PCT levels were not significantly correlate with culture-confirmed bacterial infection. Therefore, initial PCT values do not help the clinicians in terms of administering or postponing empirical antibiotics at the time of fever onset. However, third day PCT levels present as a good diagnostic marker due to a higher sensitivity and specificity when comparing them to the initial values. Determination of serial PCT may enhance the diagnostic value of PCT diagnostic marker in FN episodes caused by Gram-negative bacteria with a high sensitivity (87.5%). This study also demonstrated that PCT could be used to rule out bacterial infections particularly caused by Gram-negative bacteria.

Keywords: Procalcitonin, febrile neutropenia, child, gram-negative bacteremia

Introduction

Infections are the most prominent causes of morbidity and mortality in children with febrile neutropenia (FN) (1,2). In these patients, due to inadequate inflammatory response and subtle clinical symptoms, to determine the source of infection can be challenging. Fever, sometimes presents as the primary and sole manifestation of infection adding to confusion. A positive microbiological culture is only found in 7–31 % of febrile episodes (3). Therefore, it is important to distinguish infections from other non-infectious causes, for both to choose appropriate antibiotic and to reduce the redundant antibiotic use. A focus of recent researches has been a search for predictors of severe infection and bacteremia. Procalcitonin (PCT) is a precursor of calcitonin hormone and is released in excessive amounts during infections (4,5). There are several studies suggesting PCT to be as a better and earlier marker than C-reactive protein(CRP) in children with infections (6-9). A recent meta-analysis evaluating 10 pediatric studies and has suggested that PCT is

Address for Correspondence
Zümrüt Şahbudak Bal MD, Ege University Faculty of Medicine, Department of Pediatrics, Division of Infectious Diseases, İzmir, Turkey
Phone: +90 505 442 31 92 E-mail: z.sahbudak@gmail.com
Received: *27.06.2018* **Accepted:** *23.09.2018*

a useful bioamarker in detecting microbiologically/clinically confirmed infections with a sensitivity of 68% and specificity of 82% (6). Another pediatric study has also showed that PCT>2 ng/dL strongly associated with increased risk of severe infection (likelihood ratio [LHR] of 26 [95% confidence interval [CI] 3.5, 190]) (9).

This study was conducted to investigate the possible use of PCT levels can be used for predicting bacteremia particularly those caused by Gram-negative microorganisms; which are respectively more frequent in our center. The second aim of this study was to determine whether serial measurements of PCT could improve the diagnostic value.

Materials and Methods

This retrospective study included 33 patients hospitalized in Hematology Subdivision, Department of Pediatrics, Faculty of Medicine, Ege University between June 2014 and February 2014. The patients aged between 1month-18-year old were receiving chemotherapy regimen for Acute lymphoblastic leukemia (ALL) [n:27] or acute myeloblastic leukemia (AML) [n:6]. During this period, 51 FN episodes were observed. The measurements of CRP 1 and PCT 1 were performed at onset of fever (0-24 hour) and re-measurement was performed within 48-72 hour (CRP 2, PCT 2). Blood cultures were taken at the onset of fever before the initiation of antibiotics. All patients had already receiving antibiotic therapy, were excluded. Neutropenia was defined as an absolute neutrophil counts [ANC] <500 cells/L or an ANC that was expected to decrease to <500 cells/microL during the next 48 hours. Fever was defined as a single axillary temperature ≥38.3^{0}C or a temperature ≥38^{0}C for longer than one hour or two elevations >38^{0}C during a 12-hour period (10). Bacteremia was defined as positive blood culture for bacteria [peripheral blood or central venous indwelling catheter], with or without septic shock. Standard practice in our center includes a through daily examination of all patients for clinical signs and sources of infections, as well as monitoring for sepsis and septic shock. Initial blood samples are obtained on the first day and the second drawn being performed on the third day at the time the reassessment of antibiotic therapy is suggested. Broad spectrum antibiotics [piperacillin-tazobactam +amikacin or meropenem +amikacin] are initiated after obtaining blood cultures. Additionally, when infection is suspected, urine culture and cerebrospinal fluid culture are obtained. In the patients with hemodynamic instability, skin and soft tissue infections, or a sign of catheter-related infection, vancomycin is initiated empirically.

We reviewed the medical records of 33 patients whose initial PCT and CRP levels were available. CRP and PCT levels of the patients were also checked and recorded if they were drawn on the third day of admission. Demographic characteristics, medical history, maximum degrees of fever on the day of presentation, fever duration, physical examination findings were recorded. Laboratory findings, including complete blood count, CRP, bacterial and fungal cultures were also recorded.

Microbiological testing

Presumptive identification of microorganism was made using VITEK MS (bioMérieux, France). This cutting edge technology uses Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) technology which is a new technology for species identification based on the protein composition of microbial cells.

Statistical Analysis

Statistical analyses were performed using MedCalc for Windows [version 15.2 Med Calc Software, Belgium] and SPSS for Windows [version 22.0 SPSS Inc., Chicago, IL, USA]. Numerical datas were expressed as median [25P-75P]. Mann-Whitney and Wilcoxon tests were used for inter-variable analysis. Comparisons were referred to as statistically significant if the p-values were <0.05. A receiver operating characteristics [ROC] curve was used to determine a cut-off level for the marker; sensitivity and specificity were assessed as equally significant. Associations between categorical variables were evaluated using Chi-square and Fisher's exact tests.

Results

This retrospective study consisted of 51 febrile episodes from 33 patients (ALL [n:27] and AML [n:6] with a median age of 92.1 months ranging between 13-216 months. Initial PCT levels of 51 episodes, and third day PCT levels of 41 episodes were available. The mean durations of fever and neutropenia were 3.82 (1-19) days and 9.33 (0-56) days, respectively. Five (9.8%) patients developed septic shock with 4 (7.8%) of them being admitted to the intensive care unit (ICU). The majority of FN episodes [13 (25.4%)] were associated with catheter related bloodstream infection (BSI). This was followed by pneumonia 3 (5.8%), catheter exit site infection 1 (1.9%) and zona zoster infection 1 (1.9%). From 13 (25.4%) catheter-related BSI episodes, blood culture revealed Gram-positive microorganism in 5 episodes and Gram-negative microorganism in 8 episodes. In all culture-confirmed episodes, the median levels of PCT and CRP on day 1, and day 3 were not found to be statistically different with the P levels of 0.494, 0.755, 0.326, 0.592, respectively. However, in the FN episodes caused by Gram-negative microorganism, the median levels of PCT 2 and CRP 2 were found to be significantly higher when compared to infections caused by CoNS or culture-negative confirmed infections, P values were 0.029 and 0.003, respectively. PCT 1, CRP 1 did not show significant difference (P>0.05) (Table 2).

In Gram-negative bacteria infected group and non-infected, the values of sensitivity, specificity, positive LHR, negative LHR for CRP 1, CRP 2, PCT 1, PCT 2 demonstrated using receiver operating characteristics [ROC] curves, are given in Table 3. The area under curve [AUC] showed a statistical significance for CRP 2 and PCT 2 levels (P=0.0015 and P<0.0001, respectively). However, CRP 1 and PCT 1 levels showed no significance (P>0.05). The AUC values for CRP 1, CRP 2, PCT 1, and PCT 2 were 0.664, 0.748, 0.504 and 0.842, respectively. This is summarized in Table 3. The optimal cut off values, calculated using ROC curve analysis, were found to be 4 mg/dL for CRP1, 1.3 mg/dL for CRP 2, 0.29 µg/L for PCT 1 and 0.17 µ/L for PCT 2.

Table I. Demographic and clinical characteristics of febrile neutropenia episodes

Characteristics of episodes	
Total number of episodes, n (%)	51 (100)
Duration of fever, median (minimum-maximum)	3.82 (1-19)
Duration of neutropenia , median (minimum-maximum)	9.33 (0-56)
Intensive care unit admission, n (%)	4 (7.8)
Septic shock, n (%)	5 (9.8)
Culture-confirmed infections, n (%)	13 (25.4)
Coagulase-negative Staphylococcus, n (%)	5 (9.8)
Klebsiella pneumoniae, n (%)	3 (5.8)
Escherischia coli, n (%)	3 (5.8)
Pseudomonas aeruginosa, n (%)	2 (3.9)

Table II. Comparison of CRP 1, CRP 2, PCT 1, PCT 2 values in febrile neutropenic patients infected with Gram-negative bacteria and non-infected

	Gram-negative bacteria infected (n:8[15.7%])	Non Gram-negative bacteria infected (n:43[84.3%])	P value
CRP 1, median (IQR)	2.80 (3.5)	4.40 (5.43)	0.152
CRP 2, median (IQR)	2.60 (1.1)	0.795 (2.53)	0.029
PCT 1, median (IQR)	0.31 (0.41)	0.295 (0.297)	1.000
PCT 2, median (IQR)	0.40 (4.64)	0.155 (0.1)	0.003

CRP 1(Fever onset): C-reactive protein; PCT 1 (Fever onset): Procalcitonin; CRP 2 (48-72 hour), PCT 2 (48-72 hour) IQR: İnterquartile range

Discussion

Children with chemotherapy induced neutropenia may develop infections rapidly progressing to sepsis. Fever can be an initial and sole manifestation of infections. Therefore, when presenting with fever, require an initiation of broad-spectrum antibiotics immediately. Using reliable bacterial infection markers can allow to make an accurate clinical decision regarding antibiotic use can be made with redundant antibiotic use avoided. PCT has been shown as a reliable marker for distinguishing bacterial/fungal infections from other non-infectious causes in patients with FN (7).

CRP and PCT are the biomarkers most commonly used in clinical practice for predicting bacteremia. In this retrospective study, PCT levels in the second drawn on the third day were found to have better diagnostic accuracy than initial values; particularly in detecting FN episodes caused by Gram-negative microorganisms. Gram-negative bacterial infections were significantly associated with higher levels of PCT and CRP on the third day of FN. Both CRP and PCT levels were found to be valuable markers due to their high NPVs. However, the PPVs were found to be low. In a recent study comparing PCT levels in pediatric patients with FN with healthy controls, PCT levels of patients with FN were showed to be significantly higher

Table III. Diagnostic accuracy of PCT and CRP for predicting Gram-negative bacteremia, results from Receiver operating curve analysis

	CRP 1 (FO)	CRP 2 (48-72 h)	PCT 1(FO)	PCT 2 (48-72 h)
Cut-off value	4 mg/dL	1.3 mg/dL	0.50 µ/dL	0.17 µg/dL
Sensitivity (%)	87.5	87.5	37.5	85.7
Specificity (%)	47.6	66.7	79.0	69.7
PPV (%)	22.6	38.9	25	37.5
NPV (%)	95.2	95.7	87.2	95.8
Positive LHR (95% CI)	1.67 (1.1-2.5)	2.62 (1.5—4.5)	1.79 (0.6-5.2)	2.83 (1.6-5.2)
Negative LHR (95% CI)	0.26 (0.04-1.7)	0.19 (0.03-1.2)	0.79 (0.5-1.4)	0.20 (0.03-1.3)
AUC (95% CI)	0.664 (0.516-0.791)	0.748 (0.588-0.870)	0.504 (0.361-0.647)	0.842 (0.692--.938)
SE	0.122	0.0763	0.119	0.0705
P value	0.1793	0.0011	0.9736	<0.0001

AUC: Area under curve; CI: Confidence interval; CRP: C-reactive protein; FO: Fever onset; h: Hour; LHR: Likelihood ratio; NPV: Negative predictive value; PCT: Procalcitonin; PPV: Positive predictive value; SE: Standard error

than controls' (P=0.001). However, the values of patients with culture-confirmed infections and fever of unknown origin were not compared (11). Malign process itself and treatment complications such as mucositis and graft versus host disease have been shown to stimulate chemokines and associated with increased inflammatory markers including CRP, ESR, and leukocyte count. Therefore, investigations focused on PCT as a promising diagnostic marker. However, there is no single proven clinical or laboratory prognostic biomarker for infection-related mortality. A recent meta-analysis evaluated 3420 FN episodes and the lowest area under curve was found in immunocompromised patients when compared with non-immunocompromised patients and ICU patients (4). Demirkaya et al (12) evaluated 50 FN episodes of 37 cancer patients and found that PCT levels were significantly higher in patients who developed sepsis when compared with clinically and microbiologically documented infections at admission and on day 3and 7. In our study, PCT did not show significant difference at day of fever onset while diagnostic value improved on day 3.

A prospective cohort study by Hemming et al. (9), which included 27 patients with 48 FN episodes, demonstrated that PCT>2g/dL was strongly associated with an increased risk of severe infection (LHR of 26 [95%CI 3.5, 190]. Several previous reports investigating the diagnostic value of PCT in children with FN, have reported sensitivity and specificity ranged between 93%-96.5% and 70.6%-97%, respectively (13-15). In contrast, another large prospective cohort evaluated 194 consecutive FN episodes in adult patients, and found that fever onset median PCT levels did not show significant difference between clinically or microbiologically documented infections and fever of unexplained origin. However, the diagnostic value did increase on the second day of fever (56% sensitivity, 90% specificity) which is similar to our findings (16). Stoma et al. (17) suggested that PCT is a good diagnostic marker with a 62% sensitivity and an 88% specificity in adult patients with Gram-negative BSI following hematopoietic stem cell transplant (HSCT). In this study, at fever onset, PCT levels showed a lower specificity (51.1%) in predicting BSI caused by Gram-negative microorganisms. The lower specificity could be attributed to the low ratio of documented infections. On the other hand, higher PCT levels were significantly associated with Gram-negative bacteremia (P= 0.018) on the third day. Fleischhack et al. (18) found that the PCT levels were significantly higher in febrile neutropenic children infected with Gram-negative bacteria. Similarly, Reitman et al. (19) reported higher PCT levels febrile neutropenic children infected with Gram-negative bacteria when compared to those infected with Gram-positive bacteria. In their study, PCT levels on admission showed a sensitivity of 50% and a specificity of 79%. However, a serial analysis of PCT showed a sensitivity of 78% and a specificity of 76% with a NPV of 96%. They also suggested PCT is a good biomarker for ruling out bacteremia due to high NPV. In this study, we also found third day PCT levels showed a NPV of 95.8%, similar to previous reports.

The limitations of this study included relatively low number of FN episodes and the retrospective design.

Conclusions

This study showed that initial PCT levels were not significantly corelate with culture-confirmed bacterial infection. Therefore, initial PCT values do not help the clinicians in terms of administering or postponing empirical antibiotics at the time of fever onset. However, third day PCT levels present as a good diagnostic marker due to a higher sensitivity and specificity when comparing them to the initial values. This study also demonstrated that PCT could be used to rule out bacterial infections particularly caused by Gram-negative bacteria. Due to low rate of culture-confirmed infections in children with chemotherapy-induced neutropenia, monitoring PCT may provide a platform which antibiotic therapy more accurately managed.

References

1. Barton CD, Waugh LK, Nielsen MJ, Paulus S. Febrile neutropenia in children treated for malignancy. J Infect. 2015;71:27-35.
2. Haeusler GM, Sung L, Ammann RA, Phillips B. Management of fever and neutropenia in paediatric cancer patients: room for improvement? Curr Opin Infect Dis. 2015;28(6):532-8.
3. de Naurois J, Novitzky-Basso I, Gill MJ, Marti FM, Cullen MH, Roila F; ESMO Guidelines Working Group. Management of febrile neutropenia: ESMO Clinical Practice Guidelines. Ann Oncol. 2010;2:252-6.
4. Hoeboer SH, van der Geest PJ, Nieboer D, Groeneveld AB. The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis. Clin Microbiol Infect. 2015;21:474-81.
5. Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis. 2013;13:426-35.
6. Wu CW, Wu JY, Chen CK, Huang SL, Hsu SC, Lee MT, Chang SS, Lee CC. Does procalcitonin, C-reactive protein, or interleukin-6 test have a role in the diagnosis of severe infection in patients with febrile neutropenia? A systematic review and meta-analysis. Support Care Cancer. 2015;23:2863-72.
7. Bruno B, Busca A, Vallero S, Raviolo S, Mordini N, Nassi L, Cignetti A, Audisio E, Festuccia M, Corsetti A, Depaoli L, Faraci M, Micalizzi C, Corcione S, Berger M, Saglio F, Caropreso P, Mengozzi G, Squadrone V, De Rosa FG, Giaccone L. Current use and potential role of procalcitonin in the diagnostic work up and follow up of febrile neutropenia in hematological patients. Expert Rev Hematol.2017;10:543-50.
8. Sbrana A, Torchio M, Comolli G, Antonuzzo A, Danova M; Italian Network for Supportive Care in Oncology (NICSO). Use of procalcitonin in clinical oncology: a literature review. New Microbiol. 2016;39:174-180.
9. Hemming V, Jakes AD, Shenton G, Phillips B. Prospective cohort study of procalcitonin levels in children with cancer presenting with febrile neutropenia. BMC Pediatr. 2017;17:2.

10. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, Raad II, Rolston KV, Young JA, Wingard JR; Infectious Diseases Society of America. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the infectious diseases society of america. Clin Infect Dis. 2011;52:56-93.
11. Sirinoglu M, Soysal A, Karaaslan A, Kepenekli Kadayifci E, Cinel I, Koç A,Tokuç G, Yaman A, Haklar G, Şirikçi Ö, Turan S, Altınkanat Gelmez G, Söyletir G, Bakır M. The diagnostic value of soluble urokinase plasminogen activator receptor compared with C-reactive protein and procalcitonin in children with febrile neutropenia. Pediatr Hematol Oncol. 2016 ;33:200-8.
12. Demirkaya M, Tugcu D, Akcay A, Aydogan G, Akıcı F, Salcioglu Z, Ekmekci H, Sevinir B, Balci Ekmekci O. Adrenomedullin--A New Marker in Febrile Neutropenia: Comparison With CRP and Procalcitonin. Pediatr Hematol Oncol. 2015;32:482-9.
13. Hitoglou-Hatzi S, Hatzistilianou M, Gougoustamou D, et al. Serum adenosine deaminase and procalcitonin concentrations in neutropenic febrile children with acute lymphoblastic leukaemia. Clin Exp Med. 2005;5:60–5.
14. Kitanovski L, Jazbec J, Hojker S, Gubina M, Derganc M. Diagnostic accuracy of procalcitonin and interleukin-6 values for predicting bacteremia and clinical sepsis in febrile neutropenix children with cancer. Eur J Clin Microbiology Infect Dis. 2006;25:413–5.
15. Hatzistilianou M, Rekleity A, Athanassiou K, DeLutiis MA, Conti P, Catriu D. Serial procalcitonin responses in infection of children with secondary immunodeficiency. Clin Invest Med. 2007;30:75–85.
16. Robinson JO, Lamoth F, Bally F, Knaup M, Calandra T, Marchetti O. Monitoring procalcitonin in febrile neutropenia: what is its utility for initial diagnosis of infection and reassessment in persistent fever? PLoS One. 2011;6:e18886.
17. Stoma I, Karpov I, Uss A, Rummo O, Milanovich N, Iskrov I. Diagnostic value of sepsis biomarkers in hematopoietic stem cell transplant recipients in a condition of high prevalence of gram-negative pathogens. Hematol Oncol Stem Cell Ther. 2017;10:15-21.
18. Fleischhack G, Kambeck I, Cipic D, Hasan C, Bode U. Procalcitonin in paediatric cancer patients: its diagnostic relevance is superior to that of C-reactive protein, interleukin 6, interleukin 8, soluble interleukin 2 receptor and soluble tumour necrosis factor receptor II. Br J Haematol. 2000;111:1093-102.
19. Reitman AJ, Pisk RM, Gates JV 3rd, Ozeran JD. Serial procalcitonin levels to detect bacteremia in febrile neutropenia. Clin Pediatr (Phila). 2012;51:1175-83.