Procalcitonin (ProCT): A Reliable Marker of Sepsis. A Single Centre Experience

Shabnam Dildar, Jawad Hassan*, Tahir Sultan Shamsi

Department of Pathology, National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan.

Abstract: Objective: Sepsis occurs in hematological disorders due to neutropenia, clinical signs and symptoms are not specific for its diagnosis. Early biomarkers are potentially helpful for the diagnosis in these patients. This study was done to assess the utility of Procalcitonin (ProCT) as a marker of sepsis in patients with hematological disorders.

Methods: A cross sectional study was conducted in the section of clinical chemistry, department of laboratory, National institute of Blood disease and Bone marrow transplantation (NIBD) Hospital in Karachi Pakistan. Total 366 patients with age groups of 15 to 60 years with suspected sepsis undergone ProCT test were enrolled in the study, their blood cultures results and biomarkers including; White blood cell (WBC) count, C-reactive protein (CRP) and Absolute Neutrophil count (ANC) were also recorded. Sepsis was labeled as per surviving sepsis campaign (SSG) international guidelines for management of severe sepsis and Septic Shock 2012 criteria.

Results: Biomarkers of culture positive patients were compared with culture negative patients. The statistically significant difference was noted in ProCT (p value < 0.001) and CRP (p value < 0.003). However, no significant differences were noted in WBC count (p value > 0.05) and ANC count (p value > 0.05). ProCT had outstanding diagnostic accuracy with area under receiver operator characteristic curve (AUC) was 0.95 ; (95%CI0.89 - 1.00), that for CRP was modest (AUC 0.74; 95%CI 0.61-0.87), while that for WBC and ANC were poor with AUC 0.43; (95%CI0.31-0.58), AUC 0.44(95%CI 0.28-0.54) respectively.

Conclusion: ProCT alone showed the excellent diagnostic power on ROC analysis which proves it as a stronger diagnostic biomarker for sepsis in hematological disorders.

Keywords: Procalcitonin (ProCT), Suspected sepsis, Hematological disorders, White blood cell (WBC), C-reactive protein (CRP), Absolute neutrophil count (ANC), Neutropenia.

INTRODUCTION

Sepsis is a massive immune response to bacterial infection, which affects many organs and without prompt management can lead to shock and multisystem organ failure [1]. It is the second most common cause of death after myocardial infarction in patients admitted to intensive care units [2]. Clinical, laboratory and microbiologic strategies are used for its diagnosis. Laboratory tests ranging from white blood cell count (WBC), neutrophil count and erythrocyte sedimentation rate (ESR), to more complex biomarkers such as blood cultures, Procalcitonin (ProCT), cytokine levels and circulating cell-free DNA(cfDNA) are used. The common approach to detect sepsis is by blood cultures and they are considered as gold standard, but it has certain limitations as they are positive in up to 30% of patients and takes 2 to 3 days for the results to be available [3, 4]. Several studies have reported negative culture results even in severe sepsis, resulting in difficulty in diagnosing such cases.

The diagnosis of infection is complex and sometimes impossible because of limited sign and symptoms in patients receiving intensive chemotherapy for hematopoietic stem cell transplantation (HSCT) which leads to long-term granulocytopenia. Fever is the only sign of infection in these patients [5]. Identifying the origin of a fever is very essential to start appropriate therapy. The working group of the German Society of Hematology and Oncology suggest that in neutropenic patients, the white blood cell count should not be used in criterion to define sepsis. The Third International Consensus Definitions for Sepsis and Septic Shock stated that sepsis is a clinical diagnosis with no gold-standard diagnostic test [6].

For the above mentioned reasons, a reliable biomarker is needed to identify sepsis, that must be sensitive, specific, predict early diagnosis and easy to measure and useful in neutropenic patients as well. Since recent decades ProCT has emerged as a highly sensitive biomarker for the diagnosis of sepsis. Normal serum ProCT levels in the blood are very low. However its concentration instantaneously elevates in 6 to12 hours of infection and after treatment, its levels declines and with undetectable levels in patients with complete recovery [7, 8]. Its levels are proportional to the severity of infection, higher levels associated with more severe disease and vice versa. In 2001 International Sepsis Definitions Conference added ProCT and C-reactive protein to the new diagnostic criteria of sepsis [9]. Centers for Medicare & Medicaid Services (CMS) regulations have stated that ProCT is a...
The diagnosis of infection is complex and sometimes impossible in patients. Positive blood cultures take 2 to 3 days to detect sepsis, and they are considered the criterion to define sepsis. The Third International Consensus Conference of the American College of Chest Physicians/Society of Critical Care Medicine. Procalcitonin (ProCT) was 0.40 ng/ml (IQR: 0.121-1.2), median WBC was 3.1 ng/ml (IQR: 0.94-20) was significantly higher in blood cultures positive patients in comparison to blood culture negative patients by using Mann-Whitney U test. ProCT was 0.93 (95% CI 0.87-0.99), CRP was 0.71 (95% CI 0.59-0.84). ProCT <0.5 ng/ml was labeled as infection. WBC and Neutrophil count was analyzed by flow cytometry technique. The cut off used for ANC was <2x10⁹/L considered as neutropenia, and >7x10⁹/L considered as neutrophilia. For Internal quality control; two levels of manufacturer provided controls (low and high) were run of ProCT, CRP, WBC, and Neutrophil count (ANC) laboratory also participated in RIQAS (Randox International quality assurance scheme) as External quality assurance program for proficiency testing.

**RESULTS**

Statistical software SPSS version 19 was used for analysis of data. Frequency and percentages were calculated for gender, while mean and standard deviation were calculated for age. Median and interquartile (IQR) were calculated for ProCT, CRP, WBC, and ANC count. The ProCT, CRP, WBC and ANC levels were compared between culture positive and cultures negative patients by using Mann-Whitney U test. Kappa statistic was used to find the agreement between blood culture and Procalcitonin. Diagnostic accuracy of ProCT, CRP, WBC and ANC to predict sepsis was calculated against gold standard as blood culture. Receiver operator curve and area under the curve was derived. A p-value of <0.05 was considered assignificant.

**EXCLUSION CRITERION**

Pregnant females, patients with sepsis and already started on antibiotic treatment, the death of patients due to sepsis and unavailability of blood culture reports were excluded.

**Biomarkers Analysis**

Three to five milliliter of blood was collected in yellow top (gel tube) and ProCT analyzed by electrochemiluminescence technique on Roche Cobas e-411 analyzer, cut off used as per Consensus conference of the American College of Chest Physicians/Society of Critical Care Medicine. ProCT levels <0.5 ng/ml considered as low risk for sepsis, ProCT levels 0.5ng/ml but <2ng/ml considered as moderate risk for sepsis, ProCT levels ≥2 and <10ng/ml considered as high risk for sepsis, ProCT levels ≥10ng/ml consider as severe sepsis or septic shock [14-16]. CRP was analyze by Particle enhanced immunoturbidimetric assay on Roche Cobas c-111 analyzer, cut off used CRP<10mg/L labeled as no infection while CRP>10mg/L labeled as infection. WBC and Neutrophil count was analyzed by flow cytometry technique.

| Table 1. | ProCT | CRP | WBC | ANC |
|----------|-------|-----|-----|-----|
| n=24     | 0.30  | 12  | 5.0 | 7.0 |
| n=46     | 0.60  | 16  | 6.0 | 8.0 |

There were (n=24) 34.2% culture positive, while (n=46) 65.7% were cultures negative patients. Out of 24 cultures positive patients, ProCT correctly identified 22 (91.6%) patients. Out of 46 cultures negative patients ProCT correctly identified 37 (80.4%) that had ProCT levels less than 0.5 ng/ml as shown in Table 1. ProCT, CRP, WBC and ANC were compared between positive and negative blood culture groups, a significant difference was seen with ProCT (p-value <0.001), and CRP (p-value 0.003), whereas no significant differences were seen in WBC count (p-value 0.16) and ANC (p-value 0.23) as shown in Table 2. On ROC analysis, the AUC of serum
ProCT was 0.93 (95% CI 0.87-0.99), CRP was 0.71 (95% CI 0.58-0.85), WBC0.39 (95% CI 0.25-0.54) and for ANC was 0.41 (95% CI 0.26-0.56) as shown in Fig. (1). At a cut off of 0.5 ng/ml ProCT had sensitivity of 95.5% specificity of 78.2%, PPV of 69.7% and NPV of 97.3%, the positive likelihood ratio for Procalcitonin 4.5 (95% CI 2.58-7.85) while the negative likelihood ratio 0.06 (95% CI 0.01-0.80) with accuracy of 84.29 (95% CI 73.62-91.89). There were positive agreement between blood cultures and Procalcitonin with kappa value of 0.676 with p-value of <0.001.

Table 1. Accuracy of Procalcitonin (ProCT) at Cut off 0.5ng/ml.

| Test                | Blood culture positive (n=24) | Blood culture negative (n=46) | Total (n=70) |
|---------------------|-------------------------------|-------------------------------|--------------|
| Procalcitonin Positive | 22                            | 10                            | 33           |
| Procalcitonin Negative | 01                            | 37                            | 37           |

Table 2. Sepsis Markers of Patients with Positive and Negative Blood Cultures.

| Sepsis Markers | Blood culture positive Median (IQR) | Blood culture negative Median (IQR) | P-value |
|----------------|------------------------------------|-------------------------------------|---------|
| ProCT (ng/ml) | 3.1 [0.94-20]                      | 0.2 [0.09-0.44]                    | <0.001  |
| CRP (mg/L)    | 79.9 [15-171]                      | 7.2 [3.0-41.6]                     | 0.002   |
| WBC x10^9/L   | 1.34 [0.38-5.0]                    | 2.4 [1.1-7.0]                      | 0.16    |
| ANC x10^7/L   | 0.34 [0.0-3.8]                     | 1.7 [0.24-4.2]                     | 0.46    |

DISCUSSION

ProCT is 116-amino acid polypeptide prohormone of calcitonin synthesized by the C-cells of the thyroid gland, and in small amount by neuroendocrine tissue, lungs and intestines. Normally ProCT levels in the blood are very low; its production is stimulated by inflammatory cytokines and bacterial endotoxins causing increased levels in the blood. It has superior biokinetics, characterized by increased levels earlier in bacterial infection or sepsis and decrease faster when infection is controlled.

In this study, the diagnostic utility of serum ProCT for the diagnosis of sepsis in hematological disorders was evaluated. All subjects with suspected sepsis with hematological disorders undergoing serum ProCT test were included in the study. It was observed that median serum ProCT level, 3.1 ng/ml [IQR 0.94-20] was significantly higher in blood culture positive patients in comparison to blood culture negative patients, 0.2 ng/ml [IQR 0.09-0.44] (p-value <0.001). A study performed by M. Assicotand his colleagues in 1993 found very high concentrations of serum ProCT at diagnosis in severe bacterial infections patients (range 6-53 ng/mL) [17].

We used a cut off of serum ProCT 0.5 ng/mL for sepsis diagnosis. Similar Cut-offs used by Vincenzi I. and his colleagues in 2016 and Romano his colleagues in 2019 [18, 19]. We reported a sensitivity of 96% and specificity of 22% at a cutoff point of 0.5 ng/mL sepsis diagnoses. However, by lowering the cutoff point to 0.1 ng/mL led to increased sensitivity and specificity, 100% and 70% respectively. Romano and his colleagues in 2018 reported similar sensitivity and specificity of 100 and 22% respectively at a similar cut-off of 0.5 ng/mL [19].

We found that ProCT performed better than CRP, WBC and ANC for the diagnosis of sepsis. A systematic review of randomized controlled trials done by Schuetz P and his colleagues in 2011 stated that for optimal performance, serum ProCT cut-offs must be used according to patient risk level and clinical setting. Low-risk patients, typically those with respiratory tract infections presenting to an emergency department (ED), a serum ProCT cut-off of ≤ 0.25 ng/mL or 0.1 ng/mL should be used. While in high-risk patients, typically those admitted to the intensive care unit (ICU) serum ProCT level of 0.5 ng/mL or ≥ 0.26 ng/mL should be used [6].

Normally, serum ProCT levels are very low <0.01 ng/mL. In sepsis its levels increases significantly, sometimes to more than several hundred nanograms per milliliter. Paul E. Marik in 2014 stated that ProCT level >0.5 ng/mL is highly suggestive of a bacterial infection whereas a level <0.1 ng/mL less likely to be bacterial infection [4].

A multicenter randomized trial done by De Jong (2016) and his colleagues on Stop antibiotics on procalcitonin guidance Study (SAPS), in patients with suspected infection or proven infection admitted to the ICU. They recommended that...
Procalcitonin (ProCT): A Reliable Marker of Sepsis:... National Journal of Health Sciences, 2019, Vol. 4, No. 4 151

clinicians should stop antibiotics if ProCT is ≤0.5 μg/L or if it decreased by ≥80% of the peak value [7]. Riedel in 2011 stated that ProCT is extremely sensitive biomarker for sepsis diagnosis or to rule out the bacterial sepsis, with a high negative predictive value of more than 95% [20].

The study has many limitations as 1) it was conducted at single institute with a small sample size 2) Serum ProCT mislabeled 14.2 % (n=10) out of 70 patients as septic and their levels were between 0.5 and 2 ng/ml. This could probably be due to low levels of bacteremia and bacteria failed to grow in culture, anaerobic bacterial infection or patients had some other cause of infection. One patient was missed by serum ProCT; it may be due to skin flora contaminant or maybe empirically antibiotic was started prior to blood culture, which resulted in a reduction of the serum ProCT values. 3) Most of the patients were post chemotherapy and post Bone Marrow Transplant (BMT) patients so their ANC and WBC did not rise significantly.

The strength of the study was a strict inclusion criterion of patients with suspected sepsis and one type of population included in study.

CONCLUSION

In conclusion, ProCT is a reliable and specific sepsis biomarker. It can be used as a single reliable test and it has about the same accuracy as blood cultures and more accurate than CRP, ANC, and WBC for sepsis diagnosis in patients with hematological disorders.

STUDY APPROVAL

Study commenced after the approval of Institutional Review Board (IRB)/Ethical Committee of NIBD Hospital with protocol no. NIBD/RD-181/2018.

DATA AVAILABILITY

Data is confidential.

FUNDING SOURCE

No.

AUTHORS’ CONTRIBUTIONS

1. Shabnam Dildar (SD) planned the study, collected and analyzed the data, and wrote the manuscript
2. Jawad Hassan (JH) critically reviewed the manuscript for intellectual content.
3. Tahir Sultan Shamsi (TS) critically reviewed the manuscript for intellectual content.

ABBREVIATIONS

AUC: Area under Curve.
cfDNA: Circulating Cell-Free DNA.
CI: Confidence Interval.
CRP: C-Reactive Protein.

ProCT: Procalcitonin.
SIRS: Systemic Inflammatory Response Syndrome.
BMT: Bone Marrow Transplant.
ANC: Absolute Neutrophil Count.

CONFLICT OF INTEREST

Declared none.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Angus DC. The lingering consequences of sepsis: A hidden public health disaster. JAMA 2010; 304(16): 1833-4. DOI: 10.1001/jama.2010.1546
[2] Sunilkumar SN, Dinakaran A, Hyabat K, et al. Diagnostic and prognostic role of Procalcitonin in sepsis in a tertiary care hospital. Biomed Res 2016; 27 (1): 79-83.
[3] Mancini, Nicasio, Roberto, et al. Microbiological Diagnosis of sepsis: The confounding effects of a "gold standard". Methods Mol Biol 2015; (1237): 1-4. DOI: 10.1007/978-1-4939-1776-1_1
[4] Marik PE. Don't miss the diagnosis of sepsis. Crit Care 2014; 18(5): 529. DOI: 10.1186/s13054-014-0529-6
[5] Wacker C, Prkno A, Brunkhorst FM, et al. Procalcitonin as a diagnostic marker for sepsis: A systematic review and meta-analysis. Lancet Infect Dis 2013; 13(5): 426. DOI: 10.1016/S1473-3099(12)70323-7
[6] Schuetz P. Procalcitonin algorithms for antibiotic therapy decisions: A systematic review of randomized controlled trials and recommendations for clinical algorithms. Arch Intern Med 2011; 171(15): 1322-31. DOI: 10.1001/archinternmed.2011.318
[7] De Jong E. Efficacy and safety of procalcitonin guidance in reducing the duration of antibiotic treatment in critically ill patients: A randomised, controlled, open-label trial. Lancet Infect Dis 2016; 16(7): 819-27. DOI: 10.1016/S1473-3099(16)00053-0
[8] Becker KL, Nylen ES, White JC, et al. Clinical review 167: Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: A journey from calcitonin back to its precursors. J Clin Endocrinol Metab 2004; 89(4): 1512-25. DOI: 10.1210/jc.2002-021444
[9] Levy MM, Fink MP, Marshall JC, et al. SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Intensive Care Med 2003; 29(4): 530-38. DOI: 10.1007/s00134-003-1662-x
[10] Singer M, Deutschman CS, Seymour CW, et al. The third...
The diagnosis of infection is complex and sometimes impossi-
difficulty in diagnosing such cases.

Positive in up to 30% of patients and takes 2 to 3 days for the
gold standard, but they are

Sepsis is a massive immune response to bacterial infection,

INTRODUCTION

Services (CMS) regulations have stated that ProCT is a
criteria of sepsis [9]. Centers for Medicare & Medicaid
versa. In 2001 International Sepsis Definitions Conference
[7, 8]. Its levels are proportional to the severity of infection,
Identifying the origin of a fever is very essential to start appro-
cut–off of ≤ 0.25 ng/mL or 0.1 ng/mL should be used. While
difference was seen with ProCT (p-value <0.001), and CRP

Romano and his
cutoff point to 0.1ng/mL led to increased sensitivity and
diagnostic marker for sepsis: A systematic review and
The strength of the study was a strict inclusion criterion of

ProCT; it may be due to skin flora contaminant or maybe

American college of chest physicians/society of critical care

[1] Levy MM, Fink MP, Marshall JC, et al. Review Article: Clinical applications of procalcitonin in pediatrics: An advanced biomarker for inflammation and infection—can it also be used in trauma? Int Scholar Res Notices 2014; (10): 1155. DOI: 10.1155/2014/286493

[2] Plevin R, Callcut R. Update in sepsis guidelines: What is really new? Trauma Surg Acute Care Open 2017; 0: 1-6. DOI: 10.1136/tsaco-2017-000088

[3] Andriolo BN, Andriolo RB, Salomão R, et al. Effectiveness and safety of Procalcitonin evaluation for reducing mortality in adults with sepsis, severe sepsis or septic shock. Cochrane Database Syst Rev 2017; 1: CD010959. DOI: 10.1002/14651858.CD010959.pub2

[4] Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock. Crit Care Med 2013; 41(2): 580-637. DOI: 10.1097/CCM.0b013e31827e83af

[5] Bone RC, Balk A, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM consensus conference committee.

[10] Koutroulis I, Loscalzo SM, Kratimenos P, et al. Review Article: Clinical applications of procalcitonin in pediatrics: An advanced biomarker for inflammation and infection—can it also be used in trauma? Int Scholar Res Notices 2014; (10): 1155. DOI: 10.1155/2014/286493

[11] Plevin R, Callcut R. Update in sepsis guidelines: What is really new? Trauma Surg Acute Care Open 2017; 0: 1-6. DOI: 10.1136/tsaco-2017-000088

[12] Andriolo BN, Andriolo RB, Salomão R, et al. Effectiveness and safety of Procalcitonin evaluation for reducing mortality in adults with sepsis, severe sepsis or septic shock. Cochrane Database Syst Rev 2017; 1: CD010959. DOI: 10.1002/14651858.CD010959.pub2

[13] Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock. Crit Care Med 2013; 41(2): 580-637. DOI: 10.1097/CCM.0b013e31827e83af

[14] Bone RC, Balk A, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM consensus conference committee.