The Role of Procalcitonin As an Early Biomarker in Diagnosis of Sepsis

Ekram Yasein Ebid, Sarah Younes AbdelAziz, Mai Abuzied Mohamed Abuzied*

Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University

***Correspondence author:** Mai Abuzied Mohamed Abuzied, **Mobile:** (+20)01000957143,

E-mail: tarek.abuzaid24@gmail.com

ABSTRACT

Backgound: Sepsis is the systemic response to infection by microbial organisms. A differential diagnosis of infection caused by either bacteria or other microbial organisms is essential for effective treatment and prognostic assessment. Procalcitonin (PCT) is an accurate biomarker that is effective in identifying patients with sepsis and can be used in the diagnosis of bacterial infections.

Objictive: The aim of the current study is to explore the utility of PCT in early diagnosis of sepsis and determine its prognostic role in assessment of treatment response.

Patients and Methods: This study included patient group (40 patients with diagnosis of sepsis; 20 males and 20 females with ages ranged from 5-98 years) and 40 control group who matched well with patients as regard age and sex. Study groups was subjected to complete history taking, clinical examination for signs of sepsis, laboratory investigations as Complete Blood Count, C reactive protein, Blood culture, serum Procalcitonin level.

Results: The PCT is significantly higher in patients more than the control group. When the CRP values are compared with those of PCT in patients, they both show similar effects in diagnosis of sepsis. PCT indicates an early response for patients to treatment (values were less than 105, reaching the normal level in serum) while CRP values were not indicating any treatment response.

Conclusion: Procalcitonin is an important biomarker in the diagnosis of sepsis and it is a useful indicator for the effectiveness of treatment.

Keywords: Procalcitonin, biomarkers for sepsis, C-reactive protein, comparative study.

INTRODUCTION

Sepsis is a global public health problem and generates more than 3,000,000 hospitalizations per year. Despite advances in modern medicine, it remains the leading cause of death in the critically ill patient in noncoronary intensive care units, with an estimated overall mortality of about 30%, corresponding to 5.3 million deaths a year. To improve survival, early recognition of sepsis has become the primary goal of many societies, promoting research and thus promoting the development of new biomarkers [1].

An ideal biomarker should allow, with high diagnostic accuracy, an early and rapid recognition of sepsis. Procalcitonin (PCT) is a recently rediscovered biomarker that fulfills many of these requirements, especially in comparison to "older" and commonly used biomarkers, as CRP. PCT has demonstrated superior diagnostic accuracy for a variety of infections, including sepsis [2].

Procalcitonin is a protein of 116 amino acids with a molecular weight of 13 kDa, produced in the C cells of the thyroid gland, from its precursor preprocalcitonin. In response to a bacterial infection, the molecular patterns associated with pathogens stimulate the C cells, beginning their production. In sepsis the major producers of PCT are macrophages and monocytic cells of different organs, especially in the liver. In the presence of bacterial endotoxin, PCT can be identified in the plasma within 2–3 h, peaking after 12–48 h [3].

Procalcitonin can be useful for antimicrobial stewardship, which is a systematic approach to optimize the use of antibiotics in the hospitals, and its utilization may safely lead to significant reduction of unnecessary antimicrobial therapy [2].

AIM OF THE WORK

The aim of the current study is to explore the utility of PCT in early diagnosis of sepsis and determine its prognostic role.

SUBJECTS AND METHODS

I- Subjects:

This is a case-control study. It was conducted in Faculty of Medicine, Al-Azhar for Girls University and Misr University for Science and Technology hospitals during the period from Febrauary 2018 to March 2019.

Ethical approval:

Informed consent was obtained from all individual and **the study protocol was approved by Al-Azhar Medical Research Ethical Committee.**

Eighty subjects were included in this study; they were classified into two groups as follows:

Patient group: It included 40 patients with diagnosis of sepsis. They were 20 males and 20 females. Their ages ranged from 5-98 years with median age of 62.58 years.

Control group: It included 40 apparently healthy volunteers. They were 20 males and 20 females. Their ages ranged from 5-93 years with median age of 60.5

years. They matched well with patients as regard age and sex.

Study groups was subjected to the following:
A- Patient group:
1. **Complete history taking**, including: Age, sex, symptoms of anemia, fever, bleeding tendency, bone aches and history of previous treatment, together with the onset and duration of the clinical course.
2. **Clinical examination**, for signs of sepsis.
3. **Routine laboratory investgations**: Complete Blood Count (Hb, WBCs, and Platelets)
4. **Specific laboratory investigations:**
   a. C reactive protein CRP
   b. Blood culture
   c. Serum Procalcitonin level by ELISA

B- Control group:
1. **Routine laboratory investigations:** Complete Blood Count (Hb, WBCs, and Platelets).
2. **Specific laboratory investigations:**
   a. C reactive protein CRP
   b. Serum Procalcitonin level by ELISA

II-Methods:
A-Routine laboratory Investigations including:
   Complete blood count (CBC): A sample of 1 mL of fresh venous blood was collected from peripheral veins by sterile venipunctures and put in a sterile vacutainer containing K2 EDTA as anticoagulant, complete blood counts were performed electronically on automated cell counter, model XS 500i (Sysmex, Japan).
B- Specific laboratory investigations including:
1. *C reactive protein CRP :*
   Serum samples were used (1 mL blood were put in a sterile plain vacutainer and let to clot naturally then serum was separated for CRP measurement). C-reactive protein is measured in milligrams of CRP per liter of serum (mg/L).
   A reading of less than 6 mg/L indicates a normal control, while if the reading is above 10 mg/L, this may signal a need for further testing to determine the cause of such significant inflammation in patient's body.

2. *Blood Culture Collection:*
   The Bactec 9050 fluorescent instrument was used to detect positive blood cultures. This is a closed system that uses a chemical sensor to detect increases in carbon dioxide production produced by the growth of microorganisms. The sensor is automatically monitored every 10 minutes for increased fluorescence, which is proportional to the amount of carbon dioxide produced by viable microorganisms in the vials that enables the instrument to determine positive vials (positive blood culture).
   A volume of 20 ml/set for adults and 1-20 ml/set for children, depending on weight, of whole blood was added per blood culture bottle using the

adult and pediatric sample sized blood culture bottles. Subcultures of the positive Bactec samples on blood agar, chocolate agar, and MacConkey agar media were done, and identification of isolated organisms was done by colony morphology, microscopic examination and conventional biochemical reactions.

3. *Serum Procalcitonin level by ELISA:*
   - **Human PCT (Procalcitonin) ELISA Kit**
   - **Size:** 48T/96T
   - **Reactivity:** Human
   - **Range:** 31.2-2000pg/ml
   - **Sensitivity:** < 10pg/ml
   - **Application:** For quantitative detection of PCT in serum, plasma, tissue homogenates and other biological fluids.
   - **Storage:** 4°C for 12 months

Principle of the Assay
   This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti- PCTantibody was pre-coated onto 96-well plates. And the biotin conjugated anti- PCT antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the PCT amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of PCT can be calculated.

Sample Collection and Storage
   The serum samples were separated soon after collection. Then these samples were store at -20°C taking into consideration avoiding any multiple freeze-thaw cycles. These samples are then analyzed.

Assay Procedure
   Before adding to wells, the SABC working solution was equilibrated and TMB substrate for at least 30 min at room temperature (37 °C). When diluting samples and reagents, they were mixed completely and evenly.
1. Standard, test sample and control (zero) wells were setted on the pre-coated plate respectively, and then, their positions were recorded. Each standard and sample were measured in duplicate. The plate was washed 2 times before adding standard, sample and control (zero) wells.
2. 0.1ml of 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, standard solutions were aliquoted into the standard wells.

3. 0.1 ml of Sample / Standard dilution buffer was added into the control (zero) well.
4. 0.1 ml of properly diluted sample (Human serum, plasma, tissue homogenates and other biological fluids.) was added into test sample wells.
5. The plate was sealed with a cover and incubated at 37 °C for 90 min.
6. The cover was removed and the plate content was discarded. the plate was clapped on the absorbent filter papers. The wells were set on a completely wet status all the time and the plate was not subjected to any wash.
7. 0.1 ml of Biotin- detection antibody working solution was added into the above wells (standard, test sample & zero wells). The solution was added at the bottom of each well without touching the side wall.
8. The plate was sealed with a cover and incubated at 37°C for 60 min.
9. The cover was removed, and the plate was washed 3 times with Wash buffer.
10. 0.1 ml of SABC working solution was added into each well, the plate was covered and incubated at 37°C for 30 min.
11. The cover was removed and the plate was washed 5 times with Wash buffer, and each time the wash buffer was let to stay in the wells for 1-2 min.
12. 90 μl of TMB substrate was added into each well, the plate was covered and incubated at 37°C in dark within 15-30 min. 50 μl of Stop solution was added into each well and mixed thoroughly.
13. The O.D. absorbance at 450 nm was read in a microplate reader immediately after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve was plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The PCT concentration of the samples was interpolated from the standard curve. The dilution factor was multiplied to the concentrations from interpolation to obtain the concentration before dilution.

Statistical Analysis
Data were collected and coded to facilitate data manipulation and double entered into Microsoft Access and data analysis was performed using SPSS software version 18 in windows 7.

Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement, standard deviations as measure of dispersion for quantitative parametric data, and inferential statistic test:

In-depended student t-Test used to compare measures of two independent groups of quantitative data.

Chi square test to compare two or more than two qualitative groups.

Level of significance: For all above mentioned statistical tests done, the threshold of significance is fixed at 5% level (P-value). The smaller the P-value obtained the more significant are the results.
- P value of >0.05 indicates non-significant results.
- P value of ≤0.05 indicates a significant results.
- The P-value ≤0.01 was considered highly significant.

RESULTS
The present study included two groups: control group (40 normal individuals) and patient group (40 diagnosed with sepsis patients). Patients were diagnosed at clinical pathology departments, Faculty of Medicine, Al-Azhar University. The results were statistically analyzed and came to the following:

Table (1): Demographic data among Patient and Control Groups

Variable	Control Group (No=40)		Patient Group (No=40)		P-Value	Significance
Age: (years) *Mean ± SD* *(Range)*	60.5 ± 20.34 5 – 93		62.58 ± 20.78 5 – 98		0.36	NS
	No:	**%**	**No:**	**%**		
Sex: *Male:* *Female:*	20 20	50 50	20 20	50 50	-	-

SD: standard deviation No: number of subjects

Table (1) shows the mean ± SD and range of the age and sex of the patient and control groups. The calculated P-Value for the ages shown in the above table illustrates that the age is not significant in our study.

Table (2): Types of Bacterial Infection in the Patient Group

Variable	Patient group (No=40)	
	No	%
Citrobacter	2	5%
Klebsiella	5	13%
Ecoli	3	8%
Pseudomonas	5	13%
Staph Coagulase -ve	12	30%
Staph Aureus	4	10%
MRSA	5	13%
Ecoli + Proteus	1	3%
Enterococcus	2	5%
Acinetobacter	1	3%
Total	40	100

No: number of subjects

Table (2) show the types of bacterial infection in the Patient Group. Staph Coagulase -ve was the commonest presenting existence of (30%), followed by Klebsiella, Pseudomonas, and MRSA (13%), then Staph Aureus (10%), Ecoli (8%), Citrobacter & Enterococcus (5%), Ecoli + Proteus & Acinetobacter (3%).

Table (3): Laboratory data of Patient and Control Groups

Variable	Control Group (No=40)	Patient Group (No=40)	P-Value	Significance
CRP (mg/l) *Mean ± SD*	<6	109.66 ± 7.23	-	HS
PCT (Pg/ml) *Mean ± SD*	36.96 ± 5.57	282.96 ± 46.85	5.6137E-06	HS
Hb (gm/dl) *Mean ± SD*	12.79 ± 1.42	9.35 ± 1.51	1	NS
Platelets (10^9/L) *Mean ± SD*	256.68 ± 6.81	216.5 ± 14.70	0.05838995	NS
WBCs (mm^3) *Mean ± SD*	7.21 ± 1.94	11.62 ± 1.62	0.52794639	NS

No: number of subjects

Table (3) shows the laboratory data of patient and control groups. The calculated P-Value for PCT shown in the above table illustrates that PCT has high significant effect in diagnosis of sepsis. These results are illustrated also.

The results of the CRP are compared with those of PCT results for the patients group. Figures 1 and 2 show the comparison of the results of CRP & PCT for patients during early treatment and those patients without any treatment.
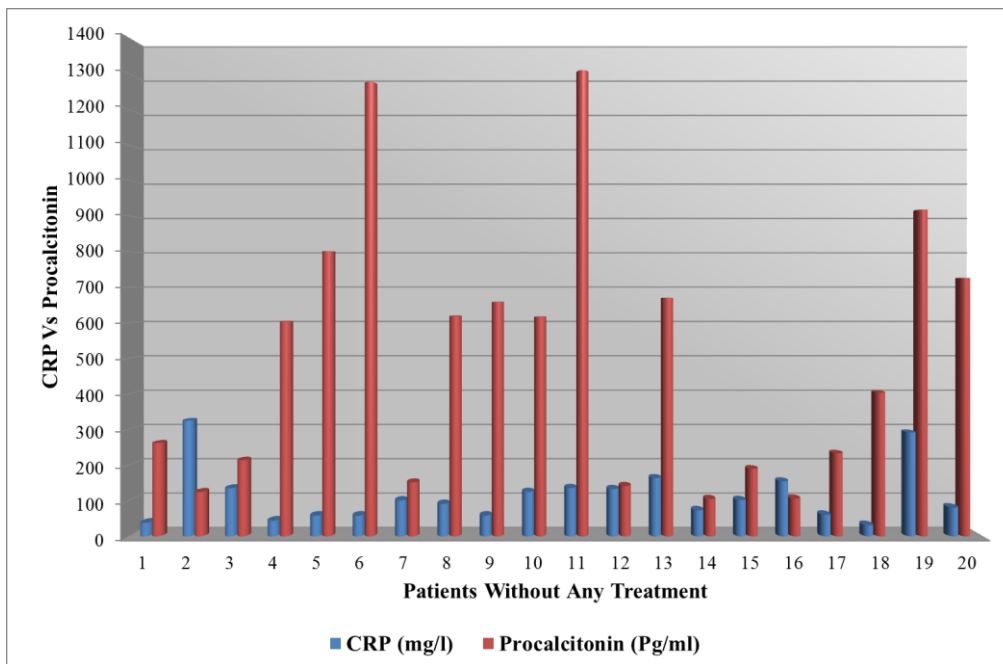
Figure (1): CRP & PCT results for patients without any treatment
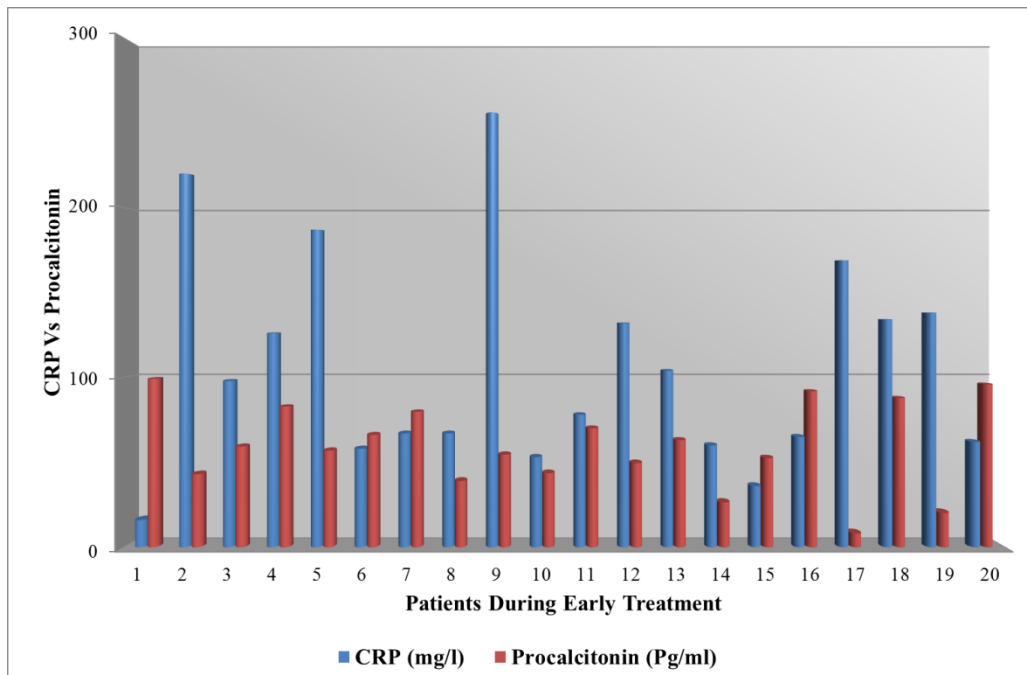

Figure (2): CRP & PCT results for patients during early treatment

Figure (1) indicates that PCT results are similar to CRP results as biomarkers for sepsis. While the CRP values are positive, the PCT values are greater than 105. Accordingly, both biomarkers are indicating the existence of sepsis.

Figure (2) indicates an early response for PCT during early treatment than the CRP values. While the CRP values are still positive, the PCT values are less than 105 (reaching the normal level in serum). Accordingly, PCT indicates an early response for patients to treatment in the same time that CRP values are still showing the bacterial infection for patients and not indicating any treatment response.

Figure (3) shows treatment response for PCT for different types of bacterial infections.
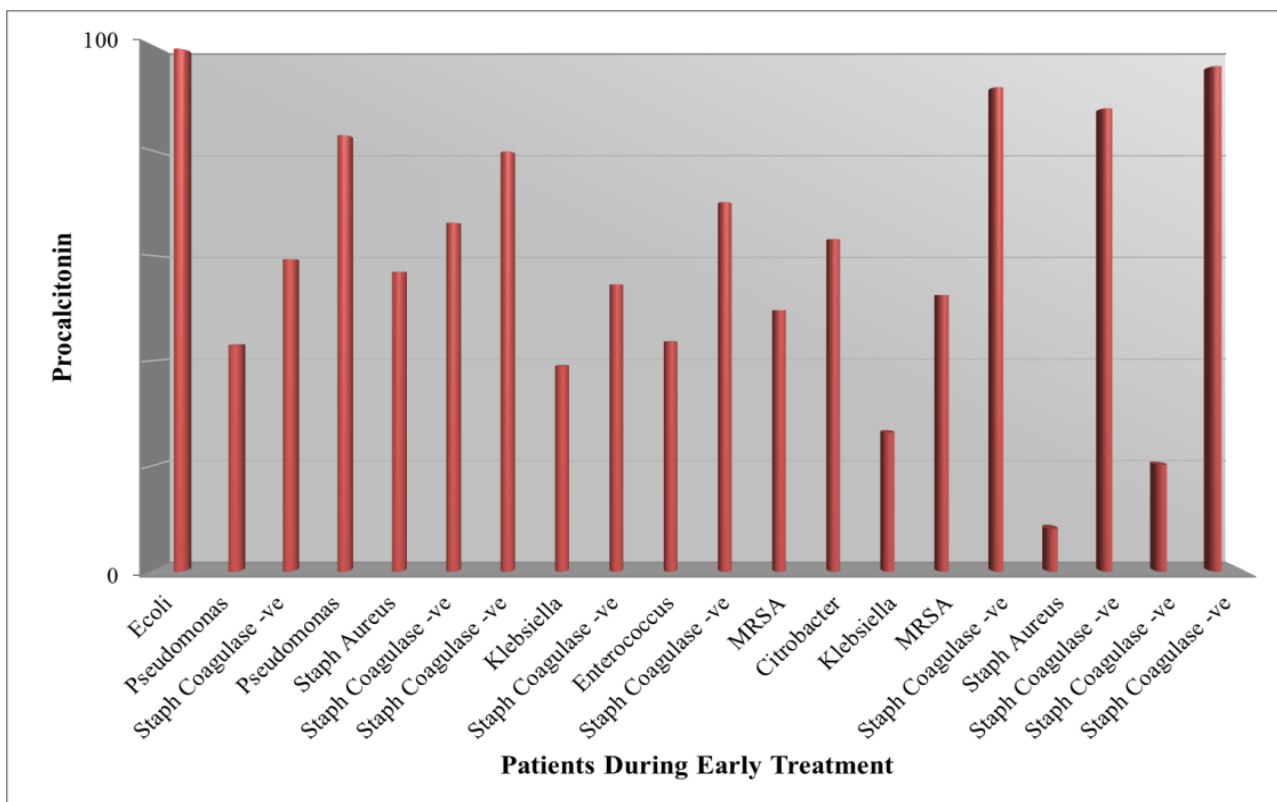
Figure (3): PCT results for patients during early treatment for different bacterial infection

Figure (3) indicates that PCT results are less than 105 for different type of bacterial infection for patients during early treatment. This means that the PCT can be taken as an early response biomarker during early treatment for different types of bacterial infection.

On the same context, the values of PCT for the patients during early treatment are compared with WBCS. The results of comparing the PCT values with WBCs illustrated that the WBCs values for some patients are not showing any response of patients to the early treatment as the PCT values as shown in figure (4).
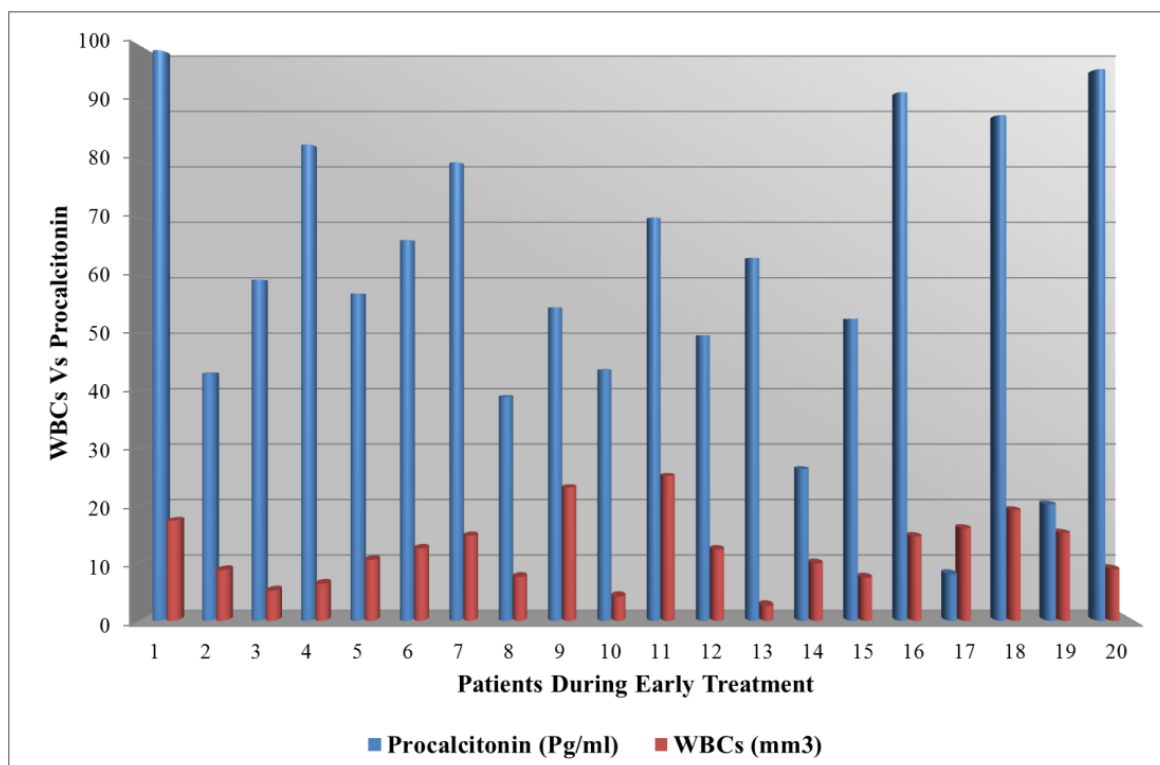

Figure (4): PCT results Vs WBCs results for patients during early treatment

Table (4): Results of patient without treatment and patient during early treatment Groups

Variable	Patient Without Treatment Group 20	Patient During Early Treatment Group 20	P-Value	Significance
CRP (mg/l) *Mean ± SD*	113.02 ± 7.881	106.3 ± 6.515	0.3496619	NS
PCT (Pg/ml) *Mean ± SD*	506.41 ± 75.738	59.5 ± 5.418	3.1136E-14	HS
Hb (gm/dl) *Mean ± SD*	9.64 ± 1.722	9.06 ± 1.233	0.99687443	NS
Platelets (10^9/L) *Mean ± SD*	234.95 ± 55.613	198.05 ± 27.609	0.15629637	NS
WBCs (mm^3) *Mean ± SD*	11.09 ± 2.155	12.15 ± 2.142	0.25750611	NS

No: number of subjects

The calculated P-Value for PCT shown in the above table illustrates that PCT has high significant effect in the early response for patients during early treatment. These results are illustrated also in figure (5).
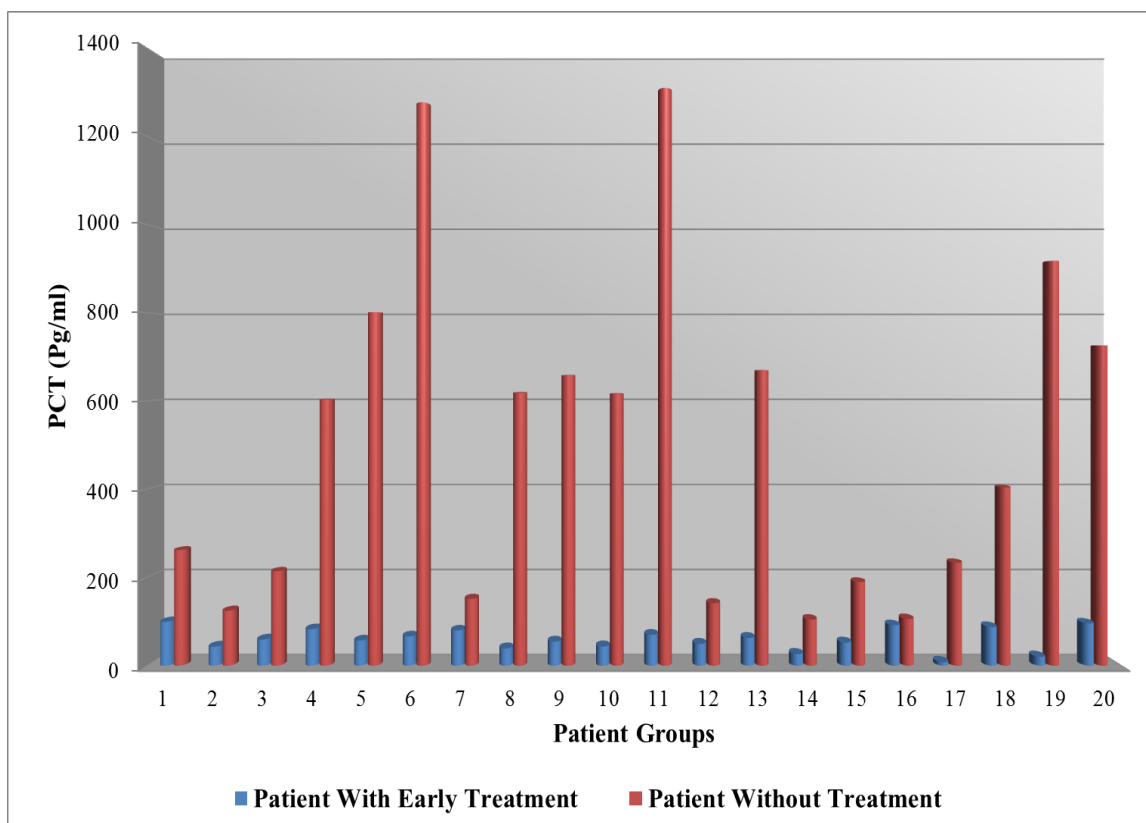

Figure (5): PCT comparison between Patients during early treatment in comparison with Patient without any treatment.

DISCUSSION

The global epidemiological burden of sepsis is difficult to ascertain but it is estimated to affect more than 30 million people worldwide every year, potentially leading to 6 million deaths. The burden of sepsis is most likely highest in low- and middle-income countries [4].

As early treatment of sepsis is associated with improved outcomes so that rapid diagnosis is important. Procalcitonin is a biomarker that allows high diagnostic accuracy, an early and rapid recognition of sepsis [1].

In our study, we aimed to explore the utility of PCT in early diagnosis of sepsis and determine its prognostic role. In our study, we found that there was no statistical significance difference between cases and controls as regards gender and age which indicated proper matching between both groups.

In the current study, we found that the hemoglobin level in the patients was lower than that of the control group but this difference was not statistically significant. Our results were in agreement with **Jansma et al.** [5] who reported that the difference in Hb between the Sepsis group and Control group was not significant. However, this result disagrees with that of **Matthew et al.** [6] who reported a significant decrease in Hb level in patients with sepsis but that was related to the volume of intravenous fluid administered by the patients.

In the present study, we found that the platelet count was lower in the patient group than the control group but that decrease was statistically insignificant. We also found that platelet count was not affected by antibiotic treatment response in the treated patients. This result was in agreement with **Kansuke et al.** [7] who reported that platelet count was maintained in the acute phase of sepsis and also in agreement with **Guclu et al.** [8] who stated that platelet count in sepsis patients was lower than control group but the difference was not significant.

However our results were in disagreement with **Venkata et al.** [9] who stated that thrombocytopenia was significantly found in patients admitted to the ICU with severe sepsis and septic shock and also disagree with **Antoine et al.** [10] reported that thrombocytopenia is a predictor of adverse outcome in sepsis, and may provide potential avenues for management and therapy.

That could be explained by the fact that thrombopoietic activity is generally normal in the acute phase of sepsis when we collected our samples. Then a decrease in absolute immature platelet counts occurs after that and is independently associated with the development of severe thrombocytopenia and mortality, suggesting the importance of suppressed thrombopoiesis in the pathophysiology of sepsis-induced thrombocytopenia [7].

Our study showed that the total white blood cell count was higher in cases than the control group but this difference was not statistically significant. Also there was no significant difference between treated and non-treated cases as regards the white blood cell count. This was in agreement with **Marik** [11] who stated that the total white blood cell count and neutrophil count are poor predictors of sepsis while an increased neutrophil to lymphocyte count ratio has been shown to be a useful marker of sepsis.

In this study, CONS was the most commonly organism detected (30%). This agrees with **Abdulhadi et al.** [12] who reported that CONS was the most common organism detected representing 44.8% of the total positive blood cultures of the study.

In the present study, both Procalcitonin and CRP levels were significantly higher in cases in comparison with the controls. These findings were remarkably similar to the results of a meta-analysis performed by **Wacker et al.** [13] who reported that procalcitonin is a helpful biomarker for early diagnosis of sepsis in critically ill patients. Also **Su et al.** [14] reported that PCT was the variable with the best diagnostic accuracy while CRP had a modest accuracy.

This study showed that PCT had decreased significantly in patients who had early antibiotic treatment while the CRP level didn't show that decrease in level in treated patients conveying the role of PCT as a predictor of cure.

This finding was in agreement with **Bartoletti et al.** [15] who stated that PCT is appropriate for determining antibiotic de-escalation and discontinuation but initiation or escalation of antibiotic therapy in specific scenarios should not be based solely on PCT serum levels. Clinical and radiological findings, evaluation of severity of illness and of patient's characteristics should be taken into proper account in order to correctly interpret PCT results. Another study by **Chanu et al.** [16] made use of the prognostic role of PCT in determining patient cure and demonstrated procalcitonin-based algorithms which can safely reduce antibiotic use in 2 clinical scenarios. First, in stable, low-risk patients with respiratory infections, procalcitonin levels of <0.25 µg/L can guide the decision to withhold antibiotics or stop therapy early. Second, in critically ill patients with suspected sepsis, clinicians should not initially withhold antibiotics, but procalcitonin levels of <0.5 µg/L or levels that decrease by ≥80% from peak can guide discontinuation once patients stabilize.

CONCLUSION

- The PCT is a unique biomarker having wide range of application in the medical field.

- It is useful in diagnosis of sepsis and in differentiating between microbial and non-microbial infection cases.
- PCT can be widely used in clinical practice and can be more useful to rule out infection, monitor the effectiveness of therapy and guide early stopping of antibiotics.
- PCT guided antibiotic stewardship could be properly designated to develop a safer and affordable strategy for diagnosis of sepsis and its prognosis.
-

RECOMMENDATIONS

Procalcitonin can be used to guide antibiotic therapy in individual patients as an effective biomarker as its level increase upon bacterial infection and decrease upon recovery.

Further studies are needed to better understand the application of PCT in the diagnosis of sepsis and determining the therapeutic approaches for sepsis.

As it is unlikely that a single biomarker serve as an effective diagnosis tool, a combination of emerging new biomarkers with PCT may be more functional in the case of clinical judgement based on which antimicrobial therapy may suggested, thus reducing the prescription and duration of antibiotic treatment.

REFERENCES

1. **Rhodes A, Evans L, Alhazzani W** *et al.* **(2017):** Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: Intensive Care Med., 43:304–77.
2. **Riedel S, Carroll KC (2013):** Laboratory detection of sepsis: biomarkers and molecular approaches. Clin Lab Med., 33(3):4134–4137.
3. **Zhang Z, Ni H (2011):** C-reactive protein as a predictor of mortality in critically ill patients: a meta-analysis and systematic review. Anaesth Intensive Care, 39:787–988.
4. **Fleischmann C, Scherag A, Adhikari NK** *et al.* **(2016):** Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. Am J Respir Crit Care Med., 193(3): 259-72.
5. **Jansma G, Buter H, Gerritsen RT** *et al.* **(2013):** Is hemoglobin concentration affected by sepsis in the acute phase.Crit Care, 17(2): 10.
6. **Matthew JM, Mark EF, Sandra P** *et al.* **(2018):** Haemoglobin concentration and volume of intravenous fluids in septic shock in the ARISE trial. Crit Care, 22: 118-22.
7. **Kansuke K, Shinshu K, Tomohiro M** *et al.* **(2018):** Time course of immature platelet count and its relation to thrombocytopenia and mortality in patients with sepsis. https://journals. plos.org/ plosone/ article? id=10.1371/ journal.pone.0192064
8. **Chanu R (2017):** Using procalcitonin to guide antibiotic therapy. Open Forum Infect Dis., 4(1): 249.
9. **Venkata C, Kashyap R, Farmer JC** *et al.* **(2013):** Thrombocytopenia in adult patients with sepsis: incidence, risk factors, and its association with clinical outcome. J Intensive Care, 1(1):9-13.
10. **Antoine G, Roussel M, Sauvadet E** *et al.* **(2012):** The sensitivity of neutrophil CD64 expression as a biomarker of bacterial infection is low in critically ill patients. Intensive Care Med., 38(3):445-52.
11. **Marik PE (2014):** Don't miss the diagnosis of sepsis!. Crit Care, 18(5): 529-32.
12. **Abdulhadi HA (2009):** Incidence and Clinical Significance of Coagulase Negative Staphylococci in Blood. Journal of Taibah University Medical Sciences, 4(2): 137-147.
13. **Wacker C, Prkno A, Brunkhorst FM** *et al.* **(2013):** Review Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis., 13(5):426-35.
14. **Su CP, Chen TH, Chen SY** *et al.* **(2011):** Predictive model for bacteremia in adult patients with blood cultures performed at the emergency department: a preliminary report. J Microbiol Immunol Infect., 44(6):449-55.
15. **Bartoletti M, Antonelli M, Bruno BFA** *et al.* **(2018):** Procalcitonin-guided antibiotic therapy: an expert consensus Clin Chem Lab Med., 56(8):1223-1229.
16. **Guclu E, Durmaz Y and Karabay O (2013):** Effect of severe sepsis on platelet count and their indices. Afr Health Sci., 13(2): 333–338.