

Original Research Article

Clinical and bacteriological relevance of procalcitonin: a single center, retrospective observational study.

Abstract

Introduction: Clinical relevance of procalcitonin levels in cases of sepsis due to different pathogens and the relationship between procalcitonin levels and patient outcome has not been widely studied. This study aims to highlight the significance of procalcitonin in sepsis and patient prognosis.

Methods: In this study 348 cases of sepsis were analysed and their procalcitonin levels were compared with the different pathogens isolated. The patient outcome as 28 day mortality was also compared with different procalcitonin levels which was divided into four groups (group1: <0.5ng/ml, group 2: 0.5 - < 2ng/ml, group 3: 2 - < 10ng/ml, group 4: >= 10ng/ml).

Results: The procalcitonin levels were significantly higher in cases of sepsis due to Gram negative bacilli (14.5ng/ml) compared to Gram positive cocci (8.59ng/ml) and yeast (2.96ng/ml). Multiple logistic regression showed significant difference between 28 day mortality and Multidrug resistant bacteria (MDR) pathogens (p=0.006) and group 4 PCT levels (p=0.033).

Conclusion: The PCT levels were higher in Gram negative bacilli compared to Gram positive cocci, Gram positive bacilli and yeast. The patient clinical outcome studied as 28 day mortality was also higher in group 4 PCT levels. Thus PCT is a reliable marker for bacteremia with Gram negative bacilli and for patient prognosis.

Key words- Procalcitonin, sepsis, pathogens, marker, 28 day mortality

Introduction

Procalcitonin is a prohormone of calcium homeostasis hormone calcitonin, it is produced in the neuroendocrine medullary C cells of thyroid gland at a very low concentration of <0.05ng/ml [1]. Bacterial infections selectively induce PCT production from multiple parenchymal tissues including liver, kidney, lung, intestine, lung and fat tissues [2]. This results in accumulation of PCT because unlike neuroendocrine cells, parenchymal cells lack the ability to cleave PCT into mature form calcitonin [1]. In case of bacterial sepsis the bacterial endotoxin and other inflammatory cytokines induces the release of PCT [1]. There is production of different proinflammatory cytokines by different Toll like receptor signalling pathway by Gram negative bacteria, Gram positive bacteria or fungi which activates PCT [3]. Procalcitonin is a helpful marker in differentiating bacterial and viral infections [1] and also cases of true bacteremia and blood samples with contamination [(4, 5]. This may be a reason for different levels of PCT in different pathogens. PCT is more specific diagnostic biomarker for bacterial infections compared to CRP [6]. There are several studies which have reported the relevance of PCT in differentiating Gram positive, Gram negative bacteremia and fungemia [7,8]. The PCT levels in different pathogens and its correlation with severity and patient outcome is still not clear. This study evaluates clinical relevance of PCT levels in relation to sepsis due to various pathogens and its importance as a prognostic marker.

Materials and methods

Study design and population

It was a retrospective study conducted in a tertiary care hospital. This study analysed data from positive blood samples showing growth of bacteria and/or fungus, during a period of one year from April 2021 to May 2022. This study was a part of a project and ethical clearance was taken from the institute ethics committee (PGI/DIR/RC/917/2021 dated 31/12/2021). Informed consent was waived off as no intervention was done and patient confidentiality was maintained.

Exclusion criteria- Among the positive blood culture samples, samples showing normal skin commensals including *coagulase-negative Staphylococcus spp.* (CONS), *Propionibacterium acnes*, *Bacillus spp.*, *Corynebacterium spp.*, *Micrococcus spp.*, or “viridans”-group, streptococci in single blood culture was considered as contaminant and not included in the study. Also paediatric samples of age <18 years and samples with incomplete lab reports or clinical data were excluded from study analysis.

Data collection

The data analysed in this study including the clinical details, laboratory tests and patient outcome were procured from the hospital information system and clinical records of the patient. For duplicate samples for each episode of bacteremia only the data for first positive sample was included in the study. A new episode of bacteremia in a same patient was defined if after 14 days of blood culture sample being negative, blood culture comes positive with the same or new organism. The data of new episode of bacteremia was included in the study. PCT levels were measured using the Roche Elecsys B.R.A.H.M.S. PCT test (Basel, Switzerland) (reference range, <0.05 ng/mL). Blood cultures were performed using the BACTEC blood culture bottles (Becton Dickinson, Sparks, MD). Blood culture bottles when flagged positive were removed for Gram stain and subcultured on blood agar and McConkey

agar. The isolated colonies were identified by automated system MALDI (matrix assisted laser desorption ionization time-of-flight [Biomerieux, USA]).

Statistical Analysis

Statistical analysis was performed using the software SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean \pm standard deviation. Kruskal–Wallis nonparametric analysis of variance was used for multi-group comparisons. Categorical variables were compared using the Chi-square test. All tests were performed as two-tailed tests. Multiple logistic regression analysis was used to determine the risk factors for mortality within 28 days. A p value of less than 0.05 was considered as statistically significant.

Results

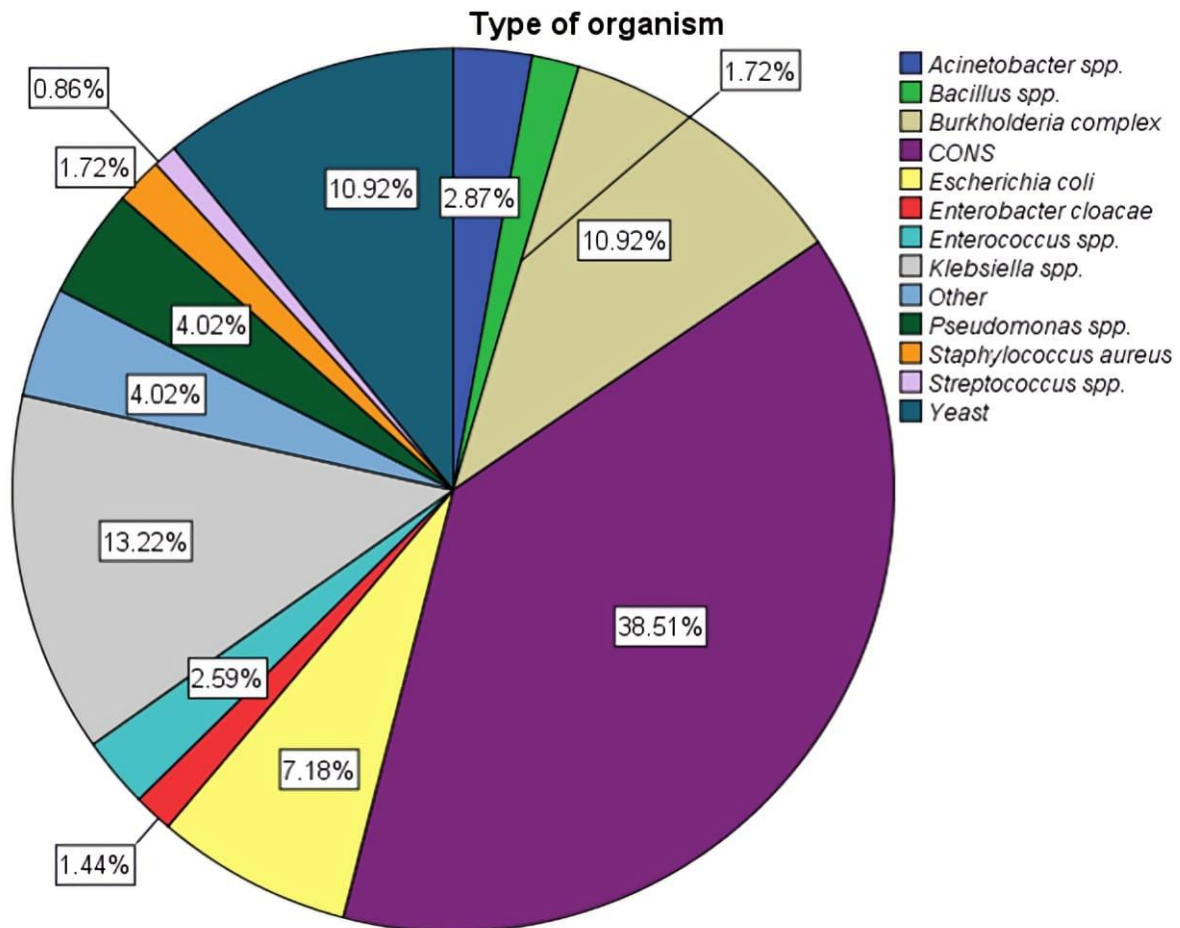
During the study period, 378 blood culture bottles were positive out of which 10 samples with contamination, 5 with polymicrobial infection and 15 with incomplete lab results or clinical information were excluded from the study. After exclusion 348 positive blood samples with mono microbial growth of bacteria or fungus were included in the study. In this study, 106 male and 242 females were included. Mean age of the patient included in the study 36.35 ± 22 years.

Pathogens isolated

Among the pathogen isolated Gram positive cocci were 151, Gram negative bacilli were 152, Gram positive bacilli were 7 and yeast isolates were 38. Most common Gram positive cocci was CONS (38.5%) and in Gram negative bacilli most common isolate was *Klebsiella species* (13.2%), some rare isolated pathogens like *Stenotrophomonas maltophilia*, *S.Typhi*,

Chryseobacterium indologenes, *Serratia marcescens* were included in a category named other .The percentage of different pathogen isolated is shown in figure 1 .

Fig .1 The percentage of different pathogen isolated



PCT levels in different pathogens- On comparison of the PCT levels among different isolates significant difference was obtained between PCT values were significantly higher for Gram negative bacilli (14.5ng/ml) than Gram positive cocci (8.59ng/ml) and yeast (2.96ng/ml) shown in figure 2. On pairwise comparison of PCT values between different organism PCT values of CONS and *Klebsiella species* and also PCT values were significantly higher for *Klebsiella species* and *Escherichia coli* than yeast. Also the PCT values were significantly higher in *Burkholderia complex* and *Acinetobacter species* than CONS (p=0.036 and p=0.012 respectively). The PCT values were significantly different for

yeast and *Acinetobacter species* ($p= 0.032$), shown in figure 3. No statistical difference was seen on intercomparison between the PCT values of other isolates included in the study.

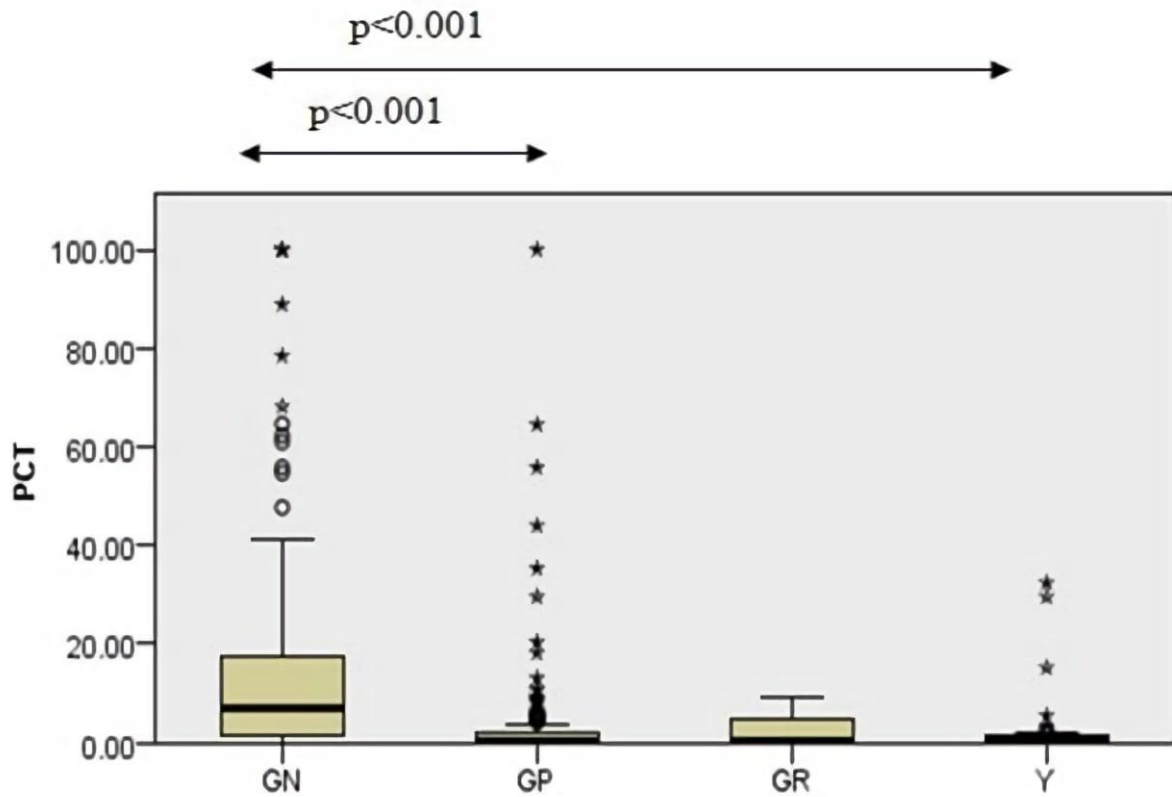


Figure 2 Distribution of PCT in GN: Gram negative bacilli, GP: Gram positive cocci, GR: Gram positive bacilli and Y: Yeast

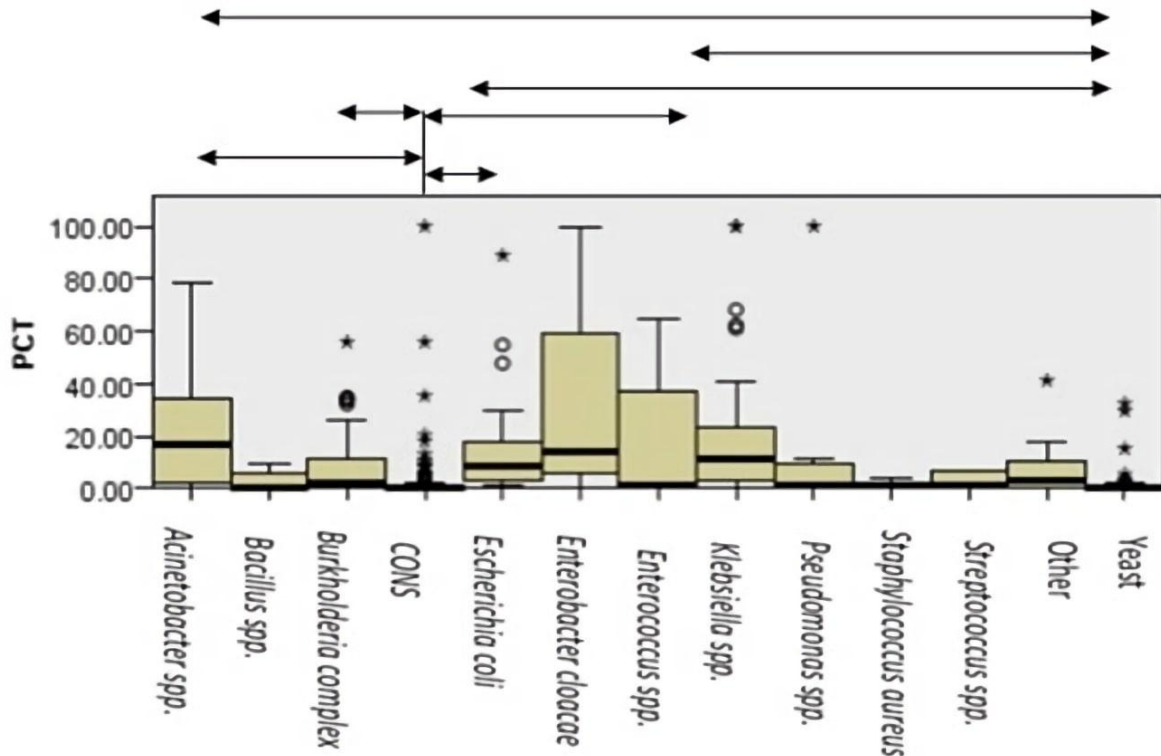


Figure 3. Distribution of PCT levels according to each pathogen. Arrow shows significant difference between PCT values of two pathogens ($p < 0.05$)

Comparison was done between patient characteristics including age, gender, comorbidities and clinical course including length of hospital stay and mortality in patients with low procalcitonin level ($< 0.5 \text{ ng/ml}$) and high procalcitonin level ($> 0.5 \text{ ng/ml}$). Statistically significant difference was found in procalcitonin levels of patient with malignancy. Patient mortality was also significantly different in low and high Procalcitonin levels shown in Table 1.

Table1. Multiple logistic regression analysis for 28-day-mortality in patients with bacteremia

Independent variable	Odds ratio	Lower limit	Upper Limit	P value
Gender	0.56	0.272	1.156	0.117
Age	1.082	0.843	1.994	0.349
Malignancy	0.292	0.134	0.635	0.002
GPC	2.202	0.956	5.069	0.064
GPR	1.006	0.166	6.092	0.995
GNR	1.386	0.588	3.264	0.455
Yeast	0	0	-	-
Multidrug resistant bacteria(MDR)	3.683	1.449	9.360	0.006
PCT(0.5- <2.0 ng/ml)	0.800	0.380	1.686	0.558
PCT(2.0 - <10 ng/ml)	0.573	0.258	1.276	0.173
PCT(>=10.0 ng/ml)	0.41	0.184	0.931	0.033

28 day Mortality

On multivariate analysis no significant difference was found between 28 day mortality and gender, age while a significant difference was seen in mortality rates ad MDR pathogen (p=0.006) and higher PCT level >=10 ng/ml (p=0.033), shown in Table 2.

Table 2. Comparison of Patient Characteristics Between Low-Procalcitonin (<0.5 µg/L, and High-Procalcitonin (≥0.5 µg/L, Group B) Patient Groups

Patient characteristics	Procalcitonin(<0.5ng/ml)n=95	Procalcitonin(>0.5ng/ml)n=253	P value
Age, Mean(S.D.)	37.6(12.4)	39.8(11.2)	0.78
Male	72(75.7)	188(74.3)	0.59
Comorbidity			
Diabetes mellitus	29(30.5)	82(32.4)	0.633
Chronic lung disease	15(15.8)	42(16.6)	0.550
Malignancy	9(9.4)	47(18.6)	0.022
Heart Failure	11(11.6)	31(12.3)	0.95
Chronic Kidney disease	9(9.4)	26(10.2)	0.64
Acute Pancreatitis	7(7.4)	19(7.5)	0.88
Length of hospital stay median in days (IQR)	7(4-10)	12(6-14)	0.61
Mortality	8(8.42)	67(26.4)	0.03

Correlation between 28 day mortality and type of pathogen

In this study, there was no statistical difference of 28 day mortality rates between different types of pathogen isolated. Highest mortality was seen in *Klebsiella* species (23.46%).

28 day mortality and PCT levels

The different PCT values in sepsis cases by different pathogens were classified into four groups i.e., group 1 (<0.5 ng/mL), group 2 (0.5 to <2.0 ng/mL), group 3 (2.0 to <10 ng/mL), and group 4 (\geq 10 ng/mL) in accordance with one the study [9]. The 28-day mortality rates in groups 1, 2, 3, and 4 were 18.2%, 24.69%, 23.46%, and 33.33%, respectively. The 28-day mortality rate was significantly higher in group 4 than those of group 3 and 2.

Discussion and Conclusions

The aim of present study was to investigate the level of PCT in cases of bacteremia and fungemia and also to find out PCT as a prognostic marker for patient outcome. The most predominant bacteria isolated in our study was CONS (38.62%) which was higher than other studies [10, 11]. In our study CONS positive cases from single blood culture positive cases was considered as contaminant and excluded from study. However complete exclusion of contaminants may not be possible, leading to higher isolation of CONS. In Gram negative bacteria sepsis with most common bacteria *Klebsiella species* (13.2%) followed by *Burkholderia complex* (10.9%), and *Escherichia coli* (7.2%) and yeast was isolated in 10.9% cases. In this study we found that the PCT values was significantly higher in cases of Gram negative bacteremia than the PCT values in Gram positive bacteremia and fungemia with yeast isolates. The PCT values in cases of *Klebsiella species* and *Escherichia coli* was found to be significantly higher than in CONS positive bacteremia, this finding is similar to other studies [12,13].

There are several studies on contributing factors to mortality in sepsis. In one of the study the contributing factors included age, causative pathogen, primary source of infection and co morbities in the patient [14]. In our study on multivariate analysis 28 day mortality was seen in high PCT levels (≥ 10 ng/mL) and also in multidrug resistance (MDR) bacteremia including ESBL in *Klebsiella* species and *Escherichia coli*. The PCT levels was significantly higher in Gram negative sepsis and a better marker of sepsis and prognosis, compared to Gram positive bacteremia and fungal sepsis which is similar to other study [15]. The different PCT levels in response to sepsis due to Gram-negative bacteria and Gram-positive bacteria is still not very clear. The most likely cause might be the difference in composition of cell membrane of Gram-negative and Gram-positive bacteria. The major difference is in the cell membrane composition is that Gram-negative bacteria have lipopolysaccharide (LPS) while it absent in Gram-positive bacteria which have a thick peptidoglycan (PGN) layer [16]. One of the study by Oberhoffer *et al.* [17] demonstrated that both LPS and sepsis-related cytokines increased PCT expression in human peripheral blood mononuclear cells (PBMCs) whereas in Gram positive bacterial sepsis due to absence of LPS there is poor production of cytokine levels (TNF- α and IL-6) leading to poor PCT expression eventually [18,19]. Among the different causative pathogens isolated, highest mortality was seen in *Klebsiella species* (23.46%). It is studied that PCT levels are increased in normal neonates, in malignancy, trauma and in cases of major surgery, severe burns [10, 20]. Thus during interpretation of the PCT results the conditions where PCT values can be increased in absence of sepsis needs to be assessed carefully. In our study we have excluded the paediatric population to avoid false positive results.

The present study reported the high PCT values correlated better with the Gram negative sepsis as compared to Gram positive bacteria sepsis and fungal sepsis. PCT is also a better prognostic marker and should be followed properly for better patient outcome. PCT values

≥ 10 ng/ml has been found to have significantly higher 28 day mortality rates. Thus antimicrobial therapy based on the hospital antibiogram should be started and follow up PCT values should be evaluated for escalation or de-escalation of antibiotics.

In this study we have not included the CONS positive cases from single blood culture positive samples, while CONS positive from two blood cultures were included as a causative pathogen. Procalcitonin is a reliable marker of sepsis with high sensitivity in Gram negative sepsis compared to Gram positive bacterial sepsis or fungal sepsis. In cases of sepsis with raised PCT levels routine monitoring of the PCT levels to go down will be helpful for better evaluation of patient outcome, as elevated or declined PCT levels will help the clinicians to escalate or de-escalate the antibiotics. This will further reduce the unnecessary antibiotic usage burden and will also helpful in emergence of MDR pathogens in hospital settings. However in high risk patient groups with suspicion of sepsis low procalcitonin levels should not be associated with delay in receipt of empirical antibiotics. Antibiotic stewardship programmes in a hospital settings must correlate with the procalcitonin values, along with the blood culture and antibiotic sensitivity reports for avoidance of inappropriate administration of antibiotics to the admitted patients.

Ethical approval

This study was a part of a project and ethical clearance was taken from the institute ethics committee (PGI/DIR/RC/917/2021 dated 31/12/2021).

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