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.The aim of this study is tohighlight the clinical relevance of procalcitonin in sepsis due to various pathogens and in patient prognosis.

Methods: In this retrospective observational 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 ± 2.8) compared to Gram positive cocci (8.59ng/ml ± 1.5) and yeast (2.96ng/ml ± 0.56). Multiple logistic regression showed significant difference between 28-day mortality and Multidrug resistant bacteria (MDR) pathogens (p=0.006) and group 4 procalcitonin (PCT) levels (p=0.033).

Conclusion: The procalcitonin levels were significantly higher in sepsis due to Gram negative bacilli compared to Gram positive cocci, Gram positive bacilli and yeast. The patient clinical outcome observed as 28-day mortality was also higher in group 4 PCT levels (>= 10ng/ml). Thus, we found PCT is a reliable marker for sepsis 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 avery low concentration of <0.05ng/ml [1]. Bacterial infections selectively induce PCT production from multiple parenchymal tissues including liver,kidney,lung,intestineand 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]. ThePCT 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 commensalsincluding *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 bottleswhen flagged positive were removed for Gram stainand subcultured on blood agar and McConkey agar.The isolated

colonies were identified by automated system MALDI (matrix assistedlaser 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 ± 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

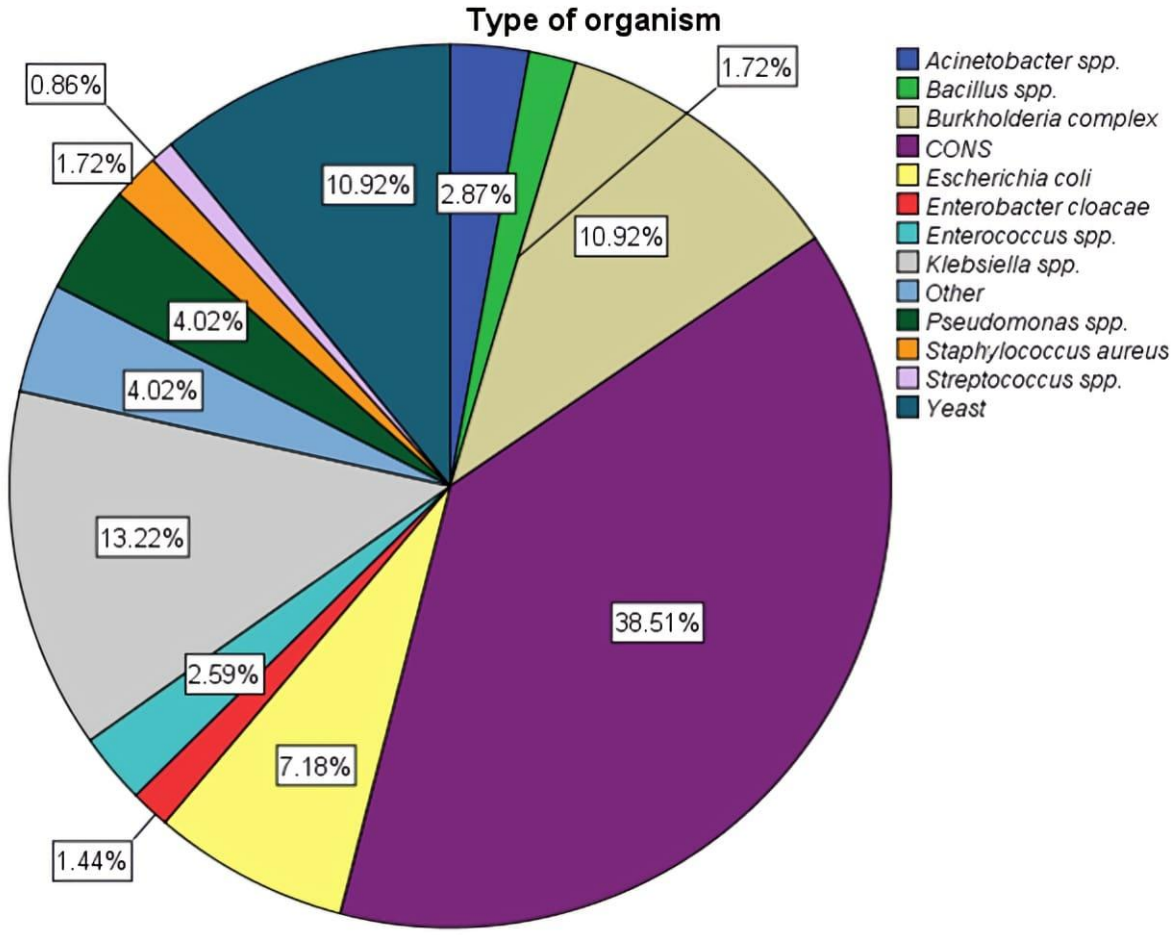
During the study period, 378 blood culture bottles were positive out of which 10 samples with contamination, 5 with polymicrobial infection and 15 with incomplete lab results or clinical information were excluded from the study. After exclusion 348 positive blood samples with monomicrobial growth of bacteria or fungus were included in the study. In this study, 106male 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 specie*s (13.2%), some rare isolated pathogens like *Stenotrophomonasmaltophila*, S.Typhi,

Chryseobacteriumindologenes,*Serratiamarcescens* were included in a category named other.The percentage of different pathogen isolated is shown in figure1 .

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 ± 2.8)) than Gram positive cocci(8.59ng/ml ± 1.5)and yeast(2.96ng/ml ± 0.56) 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.

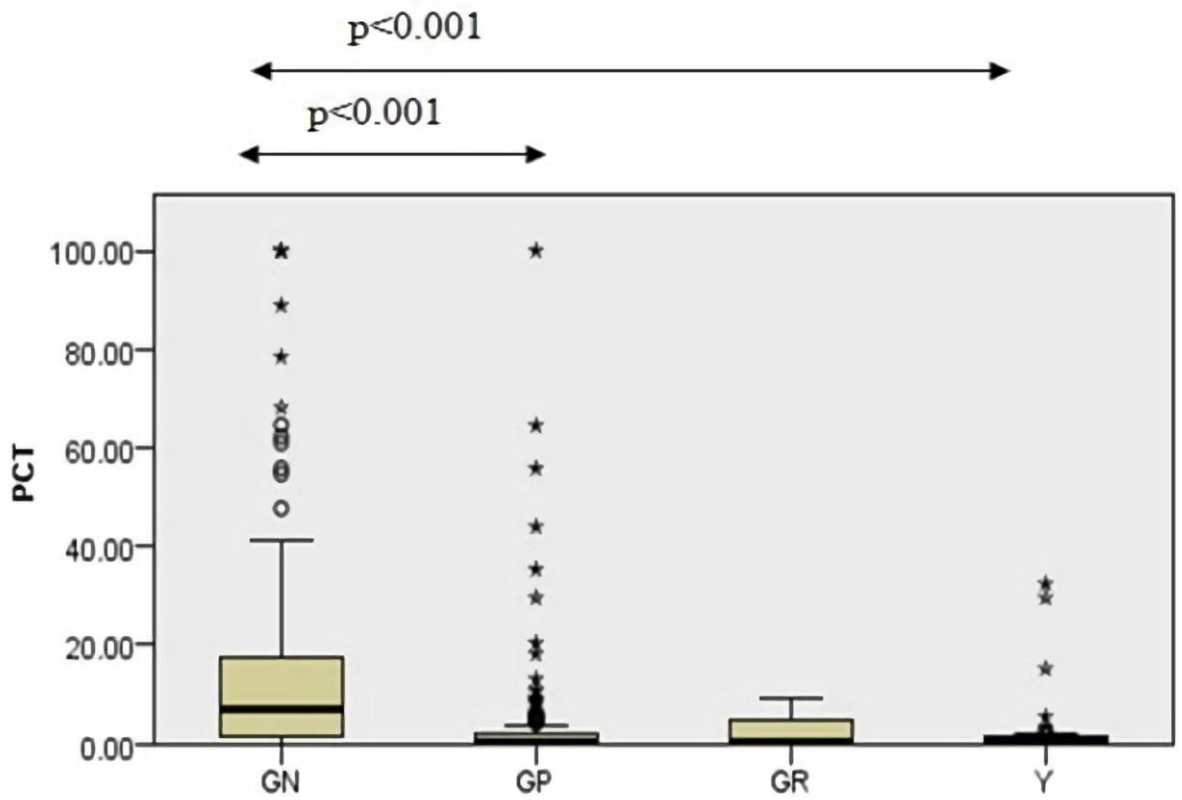


Figure 2 Distribution of PCT in GN: Gram negative bacilli, GP: Gram positive cocci, GR: Gram positive bacilli and Y: Yeast

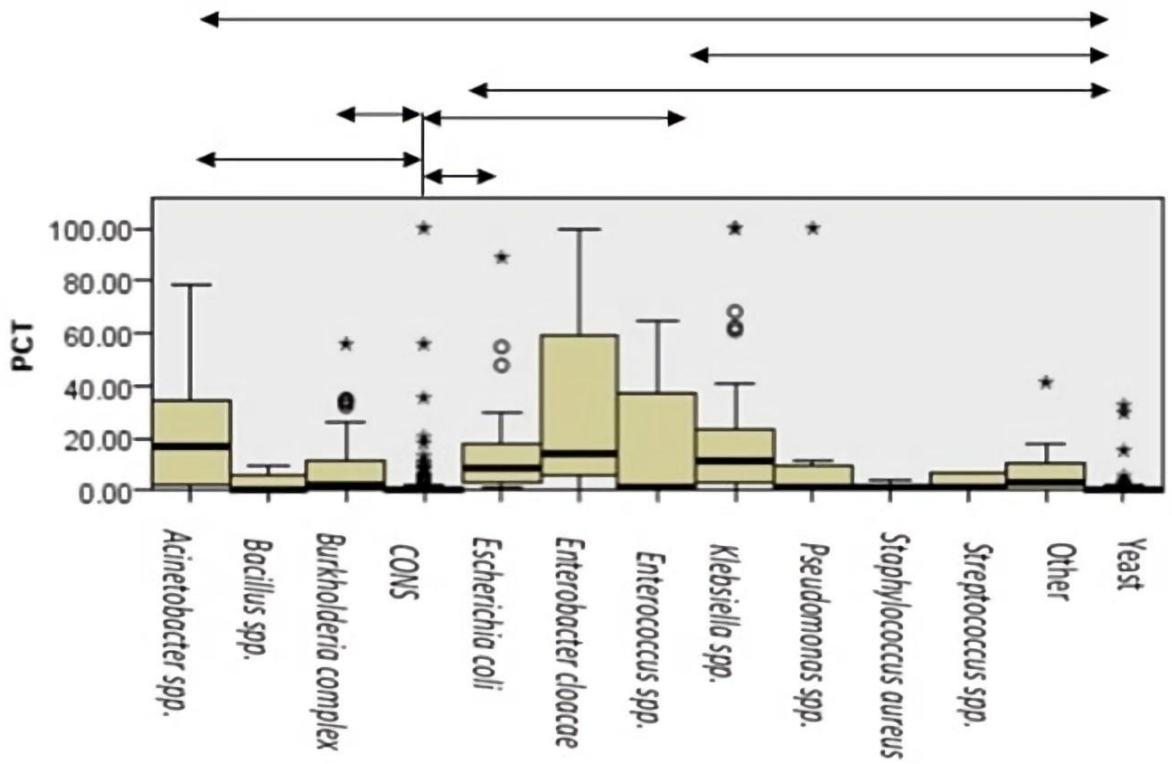


Figure 3. Distribution of PCT levels according to each pathogen. Arrow shows significant difference between PCT values of two pathogens (p<0.05)

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 (<0.5ng/ml) and high procalcitonin level (>0.5ng/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**
Gram positive cocci (GPC)	**2.202**	**0.956**	**5.069**	**0.064**
Gram positive bacilli (GPB)	**1.006**	**0.166**	**6.092**	**0.995**
Gram negative bacilli (GNB)	**1.386**	**0.588**	**3.264**	**0.455**
Yeast	**0**	**0**	**0**	**0.999**
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, 28-day mortality was found not to be associated with age and gender while it was significantly associated with multidrug resistant (MDR) pathogen

(p=0.006) and higher PCT level >=10 ng/ml (p=0.033), shown in Table 1.

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 (>= 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 infectionand comorbitiesin 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)bacteremiaincluding ESBL in *Klebsiella* speciesand *Escherichia coli*.ThePCT levels was significantly higher in Gram negative sepsis and a better marker of sepsis and prognosis, compared to Gram positive bacterial 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 is absent in Gram-positive bacteria which have a thick peptidoglycan (PGN) layer [16].One of thestudy 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.

Conclusion

The present study reported that the procalcitonin values were significantly higher in sepsis due to Gram negative bacteria as compared to Gram positive bacterial and fungal sepsis. In this study, we found procalcitoninto be a better prognostic marker, as 28-day mortality rates were significantly higher in patients with PCT levels >= 10 ng/ml. Thus, a vigilant monitoring of procalcitonin levels should be followed properly for better patient outcome. The antimicrobial therapy should be promptly started based on the hospital antibiogram and antibiotic susceptibility results.Procalcitonin values should be evaluated at regular intervals for escalation or de-escalation of antibiotics.This will further reduce the unnecessary antibiotic usage burden and will also helpful in control of emergence of multidrug resistant pathogens in hospital settings. However, inhigh-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 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).

References

[1]. L. Simon, F. Gauvin,D. K. Amre, P. Saint-Louis, and J. Lacroix,"Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis,"*Clinical Infectious Diseases*, vol. 39, no. 2, pp. 206–217, 2004.

[2]. Jin M, Khan AI. Procalcitonin: Uses in the clinical laboratory for the diagnosis of sepsis. Lab Med 2010;41:173e7.

[3].S. Kumar, H. Ingle, D. V. R. Prasad, andH. Kumar, "Recognition of bacterial infection by innate immune sensors," *CriticalReviews in Microbiology*, vol. 39, no. 3, pp. 229–246, 2013

[4].P. Schuetz, B. Mueller, and A. Trampuz, "Serum Procalcitonin for discrimination of blood contamination from bloodstream infection due to coagulase-negative staphylococci,"*Infection*, vol. 35, no. 5, pp. 352–355, 2007.

[5].W. Shomali, R. Hachem, A.-M. Chaftari et al., "Can Procalcitonin differentiate*Staphylococcus aureus*from coagulasenegative staphylococci in clustered gram-positive bacteremia?"*Diagnostic Microbiology & Infectious Disease*, vol. 76, no. 2, pp. 158–161, 2013.

[6].Enguix-Armada A, Escobar-Conesa R, García-De La Torre A, De La Torre-Prados MV. Usefulness of several biomarkers in the management of septicpatients: C-reactive protein, 13rocalcitonin, presepsin and mid-regional proadrenomedullin. Clin Chem Lab Med 2016;54:163e8

[7]. P. E. Charles, S. Ladoire, S. Aho et al., "Serum Procalcitonin elevation in critically ill patients at the onset of bacteremia caused by either gramnegative or grampositive bacteria," *BMCInfectious Diseases*, vol. 8, article 38, 2008.

[8]. Koivula, S.H¨am¨al¨ainen, E. Jantunen et al., "Elevated Procalcitonin predicts Gram-negative sepsis in haematological patients with febrile neutropenia," *Scandinavian Journal of InfectiousDiseases*, vol. 43, no. 6-7, pp. 471–478, 2011.

[9].Dipalo M, Guido L, Micca G, Pittalis S, Locatelli M, Motta A, et al. Multicentercomparison of automated 13rocalcitonin immunoassays. Pract Lab Med 2015;2:22e8.

[10].Vincent JL, Sakr Y, Charles L, Sprung CL, Ranieri VM, Reinhart K, et al. Sepsis inEuropean intensive care units: results of the SOAP study. Crit Care Med2006;34:344e53

[11].Hall KK, Lyman JA. Updated review of blood culture contamination. ClinMicrobiol Rev 2006;19:788e802.

[12].Nakajima A, Yazawa J, Sugiki D, Mizuguchi M, Sagara H, Fujisiro M, et al.Clinical utility of 14rocalcitonin as a marker of sepsis: a potential predictor of causative pathogens. Intern Med 2014;53:1497e503.

[13]. Thomas-Rüddel DO, Poidinger B, Kott M, Weiss M, Reinhart K, Bloos F. Influence of pathogen and focus of infection on 14rocalcitonin values in sepsispatients with bacteremia or candidemia. Crit Care 2018;22:128

[14].Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, ParmigianiG,et al. The clinical significance of positive blood cultures in the 1990s: a prospectivecomprehensive evaluation of the microbiology, epidemiology, andoutcome of bacteremia and fungemia in adults. Clin Infect Dis 1997;24:584e602.

[15]. Li S, Rong H, Guo Q, Chen Y, Zhang G, Yang J. Serum 14rocalcitonin levels distinguish Gram-negative bacterial sepsis from Gram-positive bacterial and fungal sepsis. *J Res Med Sci*. 2016;21:39

[16]. Kumar S, Ingle H, Prasad DV, Kumar H. Recognition of bacterial infection by innate immune sensors. Crit Rev Microbiol 2013;39:229-46.

[17].Oberhoffer M, Stonans I, Russwurm S, Stonane E, Vogelsang H, Junker U, *et al.* Procalcitonin expression in human peripheral blood mononuclear cells and its modulation by lipopolysaccharides and sepsis-related cytokines *in vitro*. J Lab Clin Med 1999;134:49-55

[18]. Beran O, Potmešil R, Holub M. Differences in Toll-like receptor expression and cytokine production after stimulation with heat-killed gram-positive and gram-negative bacteria. Folia Microbiol (Praha) 2011;56:138-42

[19].Tavares E, Maldonado R, Ojeda ML, Miñano FJ. Circulating inflammatory mediators during start of fever in differential diagnosis of gram-negative and gram-positive infections in leukopenic rats. ClinDiagn Lab Immunol 2005;12:1085-93

[20].Samsudin I, Vasikaran SD. Clinical utility and measurement of procalcitonin. ClinBiochem Rev 2017;38:59e68.