RESEARCH ARTICLE

Procalcitonin: Study of its efficacy as a potent biomarker in non-sepsis bacterial infection

Asim Kumar Basak[1*], Dipankar Das[2]

ABSTRACT

Background: The calcitonin hormone plays a crucial role in regulating calcium and phosphorus homeostasis. Moreover, during systemic inflammation, particularly in bacterial infections, procalcitonin production occurs in various tissues, such as the lung, liver, kidney, and adipose tissue. This rise of procalcitonin levels becomes detectable as early as 2 to 4 hours post-stimulation, peaking within 6 to 24 hours. This is why, unlike C-reactive protein (CRP), procalcitonin is considered the earliest and most stable marker, as its concentration is unaffected by neutropenia, immunodeficiency conditions, and the use of nonsteroidal or steroid anti-inflammatory drugs. However, the reports are mainly based on sepsis-induced bacterial infection. Thereby the aim of the present study was to evaluate the procalcitonin test's ability to discriminate different bacterial (Non- sepsis) etiology in a large population of patients. *Materials and Methods:* This longitudinal observational study was conducted using clinical and routine laboratory data collected from the Clinical Microbiology Unit of the Narayana Multispeciality Hospital, Barasat, Kolkata, from 2nd August 2022 to 15th January 2023, to evaluate the significance of serum biomarker C reactive protein, total leucocyte count, and procalcitonin test in early detection of bacterial infection. *Result:* In the present study, it was observed that the prevalence of elevated procalcitonin levels is higher with the gram-negative bacterial infection, especially among *Escherichia coli* and *Klebsiella pneumoniae,* in comparison to the gram-positive bacterial infected population.

Keywords: Serum PCT in non-sepsis infection, Biomarkers in bacterial infection, Biomarkers in Gram-negative bacteria infection

Indian Journal of Physiology and Allied Sciences (2024); DOI: 10.55184/ijpas.v76i03.257 **ISSN:** 0367-8350 (Print)

INTRODUCTION

In healthy individuals, procalcitonin (PCT) is synthesized in the thyroid C cells through the CALC-1 gene located on chromosome 11. This mRNA product is termed preprocalcitonin, which undergoes further processing to yield first procalcitonin and eventually three molecules: active calcitonin, ketacalcitonin, and N-terminal procalcitonin.[1] The calcitonin hormone plays a crucial role in regulating calcium and phosphorus homeostasis.[2,3] Normally, elevated calcium levels, glucocorticoids, calcitonin gene-related peptide (CGRP), glucagon, gastrin, or β-adrenergic stimuli induce the CALC-1 gene in thyroid C cells. Consequently, all procalcitonin synthesized in these cells is converted to calcitonin, minimizing procalcitonin released into circulation and maintaining very low levels (0.05 ng/mL) in healthy individuals.[4,5] During systemic inflammation, particularly in bacterial infections, procalcitonin production occurs in various tissues such as the lung, liver, kidney, and adipose tissue primarily through two pathways. The direct pathway is triggered by lipopolysaccharides (LPS) or other toxic microbial metabolites, while the indirect pathway is initiated by inflammatory mediators such as IL-6, TNF-α, etc.[6] Though the exact mechanism of procalcitonin production during infection remains unclear, it is believed that bacterial LPS and sepsis-induced cytokines influence liver and peripheral blood mononuclear cells to produce procalcitonin.[7] Microbial infections lead to increased CALC-1 gene expression, resulting in elevated procalcitonin levels, sometimes up to 1000 times depending on disease severity and mortality.[7,8] This rise of procalcitonin levels becomes detectable as early as 2 to 4 hours post-stimulation and reaches a peak within 6 to 24 hours. This is why, unlike C-reactive protein (CRP), procalcitonin is considered the earliest and stable marker as its concentration is unaffected by neutropenia, immunodeficiency conditions, and the use of nonsteroidal or steroid anti-inflammatory drugs.[8,9] Moreover, there are numerous reports that the commonly used systemic inflammatory markers, like C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), have poor sensitivity and specificity in diagnosing bacterial infections.[10,11] Hence, a biomarker that can rapidly and accurately identify the underlying bacterial infection is warranted for use in clinical settings to prevent mortality especially due to bacterial infection-induced sepsis. Though the role of PCT as a potential biomarker for early detection of bacterial septicemia is well established, the role of PCT as a potential candidate of serum biomarker in case of non-sepsis bacterial infection is lacking. The influence of different commonly occurring bacterial infections that alter the PCT level is also not well established. The present study, therefore, aims to:

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How to cite this article: Basak AK, Das D. Procalcitonin: Study of its efficacy as a potent biomarker in non-sepsis bacterial infection. *Indian J Physiol Allied Sci* 2024;76(3):23-28.

Conflict of interest: None

Submitted: 08/06/2024 **Accepted:** 18/07/2024 **Published:** 30/09/2024

- Study the prevalence of different bacterial infections in IPD and OPD patients.
- Study of the efficacy of different biomarkers among the patients
- It is also known that different gram-positive or gram-negative bacteria stimulate different signaling pathways that may lead to the release of different proinflammatory cytokines, which eventually is responsible for PCT release.[12-15]
- This suggests that different pathogens could result in different levels of PCT production. Thereby the aim of the present study is also to evaluate PCT's ability to discriminate different bacterial (Non- sepsis) etiology in a large population of patients with documented bloodstream infection.

MATERIALS AND METHODS

Selection of Population

This longitudinal observational research was conducted using clinical and routine laboratory data collected from the Clinical Microbiology and Serology Unit of the Narayana Multispeciality Hospital, Barasat, Kolkata from 2nd August 2022 to 15th January 2023 to evaluate the significance of serum biomarker C reactive protein (CRP), total leucocyte count (TLC), and procalcitonin count (PCT) in early detection of bacterial infection. Data of a total of 485 patients (having more than 18 years) were collected who were admitted to the hospital with a primary diagnosis of infection-related issues like fever, chills, and hypothermia following the inclusion and exclusion criteria mentioned later. Among 485 patients, male patients were 272, and female patients were 213.

Inclusion criteria

- The patients with the presentation of fever, chills, and hypothermia
- Ages in between 18 to 45 years of both sex
- Diagnosed with acute bacterial infection without having septicemia
- TLC, CRP, and PCT values of more than 11000 cells/cu.mm of blood, 10 mg/l of blood, and 0.25 ng/mL, respectively.
- >80% of neutrophil count in DLC

Exclusion criteria

- Any type of chronic bacterial infected patient, including tuberculosis
- Any organic diseases like hypertension, diabetes mellitus, heart disease, kidney disease any hormonal diseases.
- Any malignant and auto-immune diseases like rheumatoid arthritis, etc

Collection of the Sample

Patients diagnosed with acute bacterial infection without having septicemia were considered for this study. Different samples like wound swabs, respiratory suction, blood, and urine were used to determine the types of bacterial infection. The isolated colonies from incubated culture media were also stained by gram staining method to determine the type of bacterial infection -gram-positive or gram-negative. In both types of bacterial infection- gram positive and gram negative the data of serum biomarkers were collected for comparative analysis of the contribution of different biomarkers in different types of bacterial infection.

Analysis of the Serum Marker

For PCT the lower limit of detection of the assay was considered as 0.25 ng/mL, whereas, in the case of CRP and TLC, the laboratory standard cut-off values for abnormal levels were considered as 10 mg/l for CRP and >11000 cells/cu.mm of blood, respectively. All parameters (PCT, CRP, and TLC) were estimated using the conventional autoanalyzer instrument method.[16,17]

Statistical Analysis

Values were expressed as counts and percentages or mean values wherever applicable. Statistical significance was assumed if a null hypothesis could be rejected at a *p-value* of <0.05 in the Student's t-test between test variables and its normal levels. To analyze whether there is any association between the change of serum markers in both GNB and GPB-infected patients data the ANOVA[18] was used.

Ethic Statement

Samples were collected as part of standard care from the patient record of the concerned department and those included in the database were deidentified before access. Neither any personal information was stored nor was any kind of intervention executed for the patients while doing this study. For these reasons, the study was exempted from the institutional ethics committee.

RESULTS

Sex-wise Prevalence of Different Gram-negative bacteria (GNB) and Gram-positive Bacteria (GPB) Infected Patients

The present study indicates that out of a total of 485 patients data 272 male and 213 female subjects were considered for this study (Figure 1A) and among them, 334 patients were found GNB infected and 151 patients were found with GPB infection as presented in Figure 1B.

In our study, *E. coli* and *Klebsiella pneumoniae* were found dominant among GNB (Figure 2A) and *Staphylococcus* sp. was found dominant among GPB infection (Figure 2B) from all the varieties of the samples. Only in urine samples besides the *Staphylococcus*, *Enterobacteria* was also predominantly found.

Prevalence of Raised Serum TLC, CRP, and PCT Among GNB Infected Population

The present study shows that the PCT is raised in a relatively small number of infected populations when the culture is grown from the swab and blood sample but when the

Total number of Patients: 485
- Male 272
- Female 213

Among the 485 bacterial infections of patient's
- Gram-negative Organism- 334
- Gram-positive Organism- 151

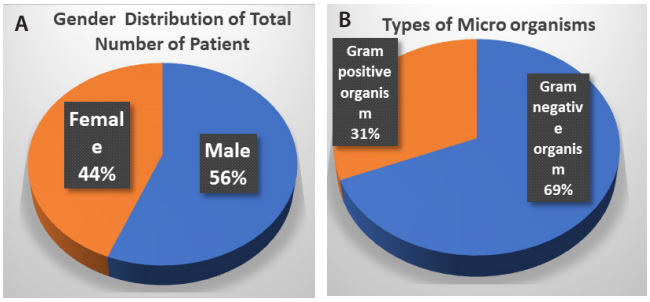
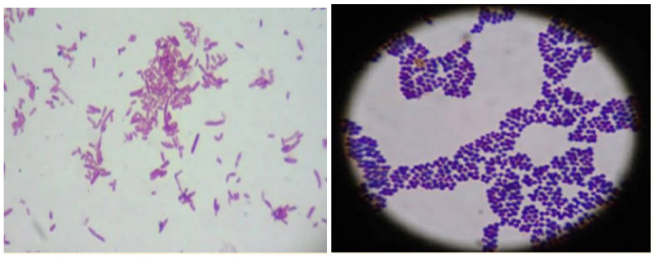


Figure 1: (A) Sex-wise prevalence of Bacterial infection (B) Prevalence of GNB & GPB infection



A B

Figure 2: Microscopic view of (A) *E. coli* and (B) *Staphylococcus* sp

culture was grown from respiratory suction or urine sample large number of population showed the raised level of serum PCT. Whereas raised CRP level and TLC levels are maximum in respiratory suction and urine samples as shown in Fig 3. This also indicates that raised PCT level was found only in 52 GNB-infected persons whereas the raised CRP was found in approx. 240 GNB-infected persons.

Prevalence of Raised Serum TLC, CRP, and PCT Among Different Samples of GPB Infection

The present study also shows that the serum PCT was found in less number of GPB-infected persons irrespective of any sample variations as shown in Figure 4.

Comparative Study of Different Serum Markers Between GNB and GPB Infected Patients

It is found in this study that the percentage rise of CRP, PCT, and TLC was significantly high in comparison to their reference normal levels in both GNB and GPB populations. The one-way ANOVA analysis shows that the percentage rise of CRP, TLC, and PCT in the entire data from all types (both GNB and GPB) bacterial infected patients is significantly different (at the level of $p < 0.05$, f-ratio value is 20.99) from their respective normal cutoff as presented under Table 1.
It was also found that there is no significant difference in

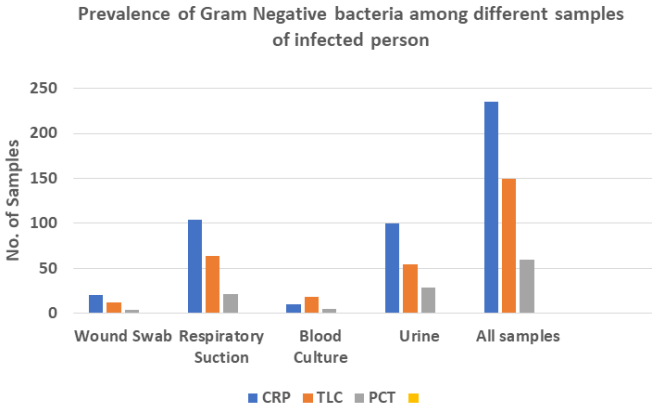


Figure 3: Histogram showing the prevalence of gram-negative bacteria among different samples

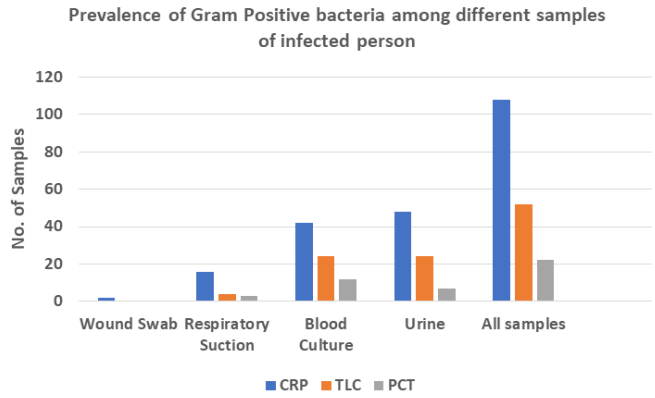


Figure 4: Histogram showing the prevalence of gram-positive bacteria among different samples

Table 1: ANOVA analysis to compare the significance level of change of different serum biomarkers in bacterial-infected patients

	CRP	*TLC*	*PCT*	*Total*
N	197	163	164	540
ΣX	34079	3135	13854.6	51068.6
Mean	351.3299	87.0833	216.4781	259.231
ΣX^2	17105111	392995	7017165.5	24515271.5
Std. Dev.	31.21	18.55	52.54	

the percentage rise of CRP among GNB, infected males and females (Figure 5). There is also no sex-wise significant difference in the percentage rise of TLC and PCT (Figure 5). A similar type of observation was found even in the case of GPB-infected patients (Figure 6) i.e., no sex-wise difference in percentage rise of CRP, TLC, and PCT was present.
Whereas when the percentage rise of CRP, TLC, and PCT among the infected population was compared with the GPB infected population it was found that there is no significant difference in the percentage rise of CRP and TLC (Figure 7) between the two variables but the percentage rise of PCT is more profound among GNB infected population (Figure 7).

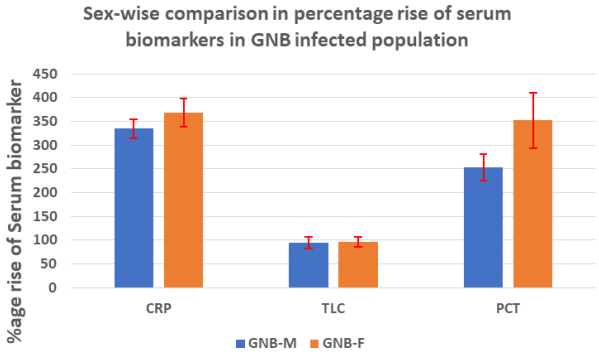


Fig 5: Histogram showing a sex-wise comparison of the percentage rise of different serum biomarkers among GNB infection

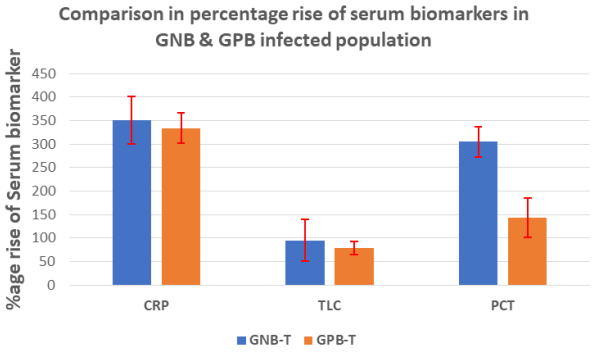


Fig 7: Histogram showing a comparison of the percentage rise of different serum biomarkers among GNB and GPB infections

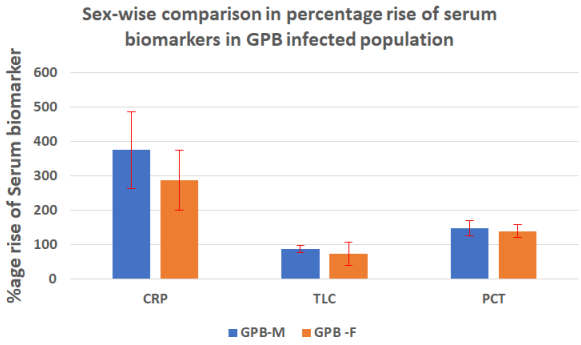


Fig 6: Histogram showing a sex-wise comparison of the percentage rise of different serum biomarkers among GPB infection

Moreover, the change in PCT level among the data of the GPB-infected population is not always in a positive direction. It was found from the data that a significant number of GPB infected population (14% %) has no change in PCT level from its cut-off level i.e. 0.25 ng/mL.

DISCUSSION

An ideal biomarker for bacterial infections should have criteria like the capacity of quick early detection, and the capacity of accurate prediction of disease's progression and outlook, and that can help to make the decisions regarding appropriate antibiotic recommendation. Various markers like leukocyte count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), soluble triggering receptor expressed on myeloid cells 1 (sTREM-1), pro-adrenomedullin (ProADM), serum procalcitonin (PCT), pro-atrial natriuretic peptide (ANP), different interleukins like IL-6, IL-8, IL-27, soluble urokinase-type plasminogen activator receptor (suPAR), etc, have been well established as potential biomarkers for appropriate diagnosis and prognostication in bacterial sepsis.[19,20] Among these, PCT has proven valuable due to its broad biological range, rapid induction (2–4 hours) following bacterial stimuli, and long half-life (22–26 hours).[21-22] This is why it is now used as a useful biomarker for identifying bacterial infections and supporting antibiotic stewardship, especially in the bacterial infection that ultimately results in septicemia.[21-23] In the present study, it is evident that there is a rise in PCT levels even in the case of non-septic bacterial infections like the rise of TLC and CRP which are also considered as gold standard of serum biomarkers for any bacterial infection. It was also found that the percentage rise of all these biomarkers among GNB & GPB-infected patients is the same irrespective of sex.

Earlier in different studies,[12,13] it was shown that serum levels of procalcitonin (PCT) are significantly raised in patients with gram-negative bacterial-induced septicemia compared to those with gram-positive. Similar findings are also observed in the present study in which it is evident that the prevalence of elevated PCT level is higher with the gram-negative bacterial infection especially among *E. coli* and *K. pneumonae* in comparison to gram-positive bacterial infected population data.[24,25] However, the exact mechanism responsible for the differing PCT levels in response to gram-negative and gram-positive bacteria remains unsettled. This may be due to the difference in their membrane composition. In the case of gram-negative bacteria, lipopolysaccharide (LPS) acts as a major membrane component that releases endotoxin in response to their infestations. On the other hand, gram-positive bacteria are characterized by the presence of peptidoglycan (PGN) in their membrane.[26-28] Both LPS and PGN can act as pathogen-associated molecular patterns (PAMPs) recognized by pattern recognition receptors (PRRs) in the innate immune system, including toll-like receptors (TLRs) and C-type lectin receptors (CLRs).[29-30] It was also reported[31] that TLRs, crucial for bacterial recognition, respond differently to these distinct membrane components. Specifically, TLR4 detects LPS, while TLR2 senses PGN. Activation of TLR4 by LPS triggers the MyD88-dependent signaling pathway, leading to the release of proinflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).[32]

Furthermore, the stimulation of TLR2 by gram-positive pathogens tends to elicit comparatively lower levels of proinflammatory cytokines (TNF-α and IL-6) due to TLR2-dependent signaling pathways.[33] So, it can be presumed that

this difference in percentage rise in PCT level between GNB and GPB-mediated infections found in this study may be due to the difference in their involvement in signaling pathways that modulate the PCT release.

CONCLUSION

From the present set of studies, it may be opined that there is a rise in PCT levels even in the case of non-septic bacterial infections like the rise of TLC and CRP which are considered as gold standard of serum biomarkers for any bacterial infection. It was also found that the percentage rise of all these biomarkers among GNB & GPB-infected patients is the same irrespective of sex. It is also observed in the present study that the prevalence of elevated PCT level is higher with the gram-negative bacterial infection especially among *E. coli* and *K. pneumonae* in comparison to gram-positive bacterial infected population.

FUNDING SOURCE

None.

ACKNOWLEDGMENT

The active cooperation of doctors and staff of Narayan Superspecialty Hospital, Barasat, Kolkata is sincerely acknowledged. The active cooperation of Mr. Sayan Das, BMLT Intern. Dept. of Allied Health Sciences, Brainware University for technical assistance and data acquisition (partial) is sincerely acknowledged.

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PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.