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Comparison of Serum Procalcitonin Levels in Patients with Gram-Negative and Gram-Positive Sepsis

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ABSTRACT

Objective: To compare rise of serum Procalcitonin levels in patients with culture-proven Gram-negative and Gram-positive sepsis.

Study Design: Cross-sectional study

Place and Duration of Study: Department of Chemical Pathology in collaboration with the Department of Microbiology, Army Medical College at Pak Emirates Military Hospital, Rawalpindi, Pakistan, from Nov 2022 to Nov 2023.

Methodology: Five hundred and twenty-four blood samples were analyzed. Blood culture samples were analyzed on the fully automated Biomerieux Bact /Alert 3D system. Bacterial growth was confirmed after incubation at 35°C±2°C ambient air as per Clinical Laboratory Standards Institute guidelines. All samples which showed no growth were discarded after five days.

For Procalcitonin analysis, 3ml of peripheral venous blood was collected in a serum plain tube. The serum was separated within 1 hour after collection by centrifugation at 4000rpm. Procalcitonin levels were then measured by Electro-Chemiluminescence immunoassay on the Roche COBAS 6000 chemistry analyzer as per reagent kit literature.

Three hundred and seventy-two samples showed growth on blood culture and were included.

Results: Out of 372 blood cultures, 195(52.4%) were positive for Gram-negative and 177(47.6%) for Gram-positive isolates. The median serum Procalcitonin levels in Gram-negative group were significantly higher than Gram-positive group (*p*-value<0.001). ROC curve analysis revealed PCT has a sensitivity of 65.6% and specificity of 78% at a cut-off of 3ng/mL for distinguishing Gram-negative from Gram-positive sepsis.

Conclusion: Serum PCT levels were significantly elevated in Gram-negative compared to Gram-positive septicemia.

Keywords: Antimicrobial Resistance, Blood Culture, Procalcitonin, Sepsis

How to Cite This Article: *Bukhari SB, Naeem U, Bibi A, Usman J, Qurat ul ain Mustafa QUA, Bashir S. Comparison of Serum Procalcitonin Levels in Patients with Gram-Negative and Gram-Positive Sepsis. Pak Armed Forces Med J 2024; 74(SUPPL-2): S289-S294. DOI: https://doi.org/10.51253/pafmj.v74iSUPPL-2.12190*

INTRODUCTION

Sepsis is a life-threatening condition which is becoming increasingly prevalent and continues to challenge healthcare providers in our community despite the availability of critical care in many hospital setups all across the country.[1] Identifying patients requiring immediate diagnostic workup and selecting suitable antimicrobial therapy according to the relevant pathogen can drive substantial improvements in the prognosis of patients with sepsis.[2]

Studies have revealed that sepsis may lead to severe organ failure and prove more lethal for the patient, particularly when Gram-negative bacteria are implicated.[3] The host inflammatory cascade is triggered when bacteria enter the bloodstream and cause the synthesis of proinflammatory cytokines and acute phase reactants.[4] Gram-negative bacteria cause a more pronounced increase in inflammatory mediators such as TNFα, Interleukin-1, Interleukin-6, Interleukin-8, and Interleukin-10 as compared to Gram-positive bacteria.[5] Researchers report that the same increase has also been demonstrated for serum Procalcitonin (PCT) levels.[6]

A growing body of data suggests that Procalcitonin is a promising diagnostic tool that can complement clinical signs and lab parameters for bacterial infection.[7] There is also a substantial amount of literature supporting the use of PCT for rational prescription of antibiotics.[8] Results of various studies have indicated variation in peak levels of Procalcitonin in Gram-negative and Gram-positive infections.[9]

The current study aims to evaluate the ability of PCT to discriminate between septicemia based on a rise in its serum level in two groups of Gram-negative and Gram-positive isolates based on culture results. The potential of this study is rapid detection of the presence or absence of bacterial sepsis and broadly identifying the bacterial pathogen group responsible

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Received: 07 May 2024; revision received: 05 Jul 2024; accepted: 08 Jul 2024

for sepsis. This is so that accurate, well-timed, and justified prescriptions of antibiotics can be initiated on an empirical basis.

METHODOLOGY

The study was conducted at the Department of Chemical Pathology in collaboration with the Department of Microbiology, Army Medical College at Pak Emirates Military Hospital (PEMH), Rawalpindi. The study was completed from November 2022 to November 2023 after ethical review board (ERC) approval (ERC/ID/235).

A minimal sample size of 312 was calculated using the WHO (World Health Organization) calculator, keeping a 5% margin of error, 95% confidence interval, and prevalence of sepsis in critically ill patients requiring Intensive care unit (ICU) admission in Pakistan as 28.3%.10 Sampling was done using the nonprobability convenience sampling technique.

Inclusion Criteria: Indoor medical patients of both genders with clinical suspicion of sepsis above 12 and up to 80 years of age having positive blood culture results were included in the study.

Exclusion Criteria: Patients <12 years of age or those already on antibiotic therapy were excluded. Moreover, immunosuppressed patients or those with a history of recent surgery/trauma, active fungal infection, severe heart, liver, or renal insufficiency, malignancy, or organ transplantation were excluded from the study.

A total of 524 clinical samples were collected. Out of these, 372 samples having positive blood culture results were included in the study analysis. The enrolled patients were divided into two groups based on blood culture results and Gram stain: Gram-negative group and Gram-positive group. Informed consent was obtained from all participants as part of ethical practice. All samples were collected under aseptic measures and duly labelled with the patient's name and medical record number. For blood culture, paired samples were collected each having 10ml of peripheral venous blood in Bact/Alert culture media bottles from two separate sites and the bottles were then incubated in an automated BacT/ALERT BD Biomerieux microbial identification system (USA). The prototype BacT/Alert system is based on the principle of colorimetry and functions as an independent incubator (temperature remains between 35-37°C ± 0.5°C), shaker, and detector. Specimens from signal-positive blood culture bottles were sub-cultured on appropriate culture plates (blood agar and MacConkey's agar). The culture plates were then incubated overnight at ±35 °C in ambient atmospheric conditions and later examined for bacterial growth. Bacterial isolates were initially identified by colony morphology, Gram stain, catalase, oxidase, and other biochemical tests using the Analytical Profile Index (API-20 E) appropriate for that isolate. Identical isolates distinguished from both sets of samples were defined as causal organisms. To reduce the possibility of contamination as a confounding factor, Coagulase-negative staphylococci (CoNS) and other skin commensals were not considered as etiology of infection when isolated from one blood culture set alone, and in absence of clinical data supporting a pathogenic role. ATCC controls, E-coli 25922, and Staphylococcus aureus 25923 were used for validating the results.

For every blood culture sample included in the study, PCT, TLC, and C-reactive protein (CRP) values were also recorded simultaneously from the same patient before initiation of antibiotic therapy and sent to the concerned lab for analysis. Three ml of peripheral venous blood was collected from an antecubital vein in a serum red-top plain tube for PCT and CRP and 3ml in an EDTA vacutainer for TLC. Serum was separated within 1 hour after blood sample collection by centrifugation at 4000rpm.[11] Roche Elecsys BRAHMS kit for PCT was used. Serum PCT was measured via an electrochemiluminescent immunoassay on the Roche COBAS 6000 chemistry analyzer in accordance with the manufacturer's recommendations.[12] Serum C-Reactive Protein (CRP) was measured on the principle of immuno-turbidimetry. For Total Leucocyte Count (TLC) measurement, whole blood was analyzed on the Sysmex XN-3000 seven-part analyzer based on the principle of fluorescence flow cytometry.

Data analysis was done by using SPSS (Statistical Package for Social Sciences) for Windows version 25. Test of normality (Shapiro Wilk test) was applied to assess the distribution of data. Median and IQR were calculated for quantitative variables. Mann Whitney U test was used to compare median PCT levels between the Gram-negative and Gram-positive group. ROC curve analysis was done to determine the cutoff value for PCT for gram negative septicemia. The threshold for significance was set at $p< 0.05$.

RESULTS

A total of 524 samples were collected. From these, 372(71%) clinical samples with bacterial growth were included in the final study analysis. The number of samples which showed negative growth on blood culture after five days was 120(22.90%). Another 32(6.10%) patients who did not fulfil the inclusion criteria were excluded from the study.

The median age of the study population was 51(IQR: 32) years. Gram-negative blood culture isolates were found to be more in number 195(52.4%) than Gram-positive blood culture isolates 177(47.6%). Escherichia coli and Klebsiella pneumoniae were the two most common species identified on blood culture results accounting for 42(11.3%) and 41(11%) respectively. Among Gram-positive species, Staphylococcus aureus was the most common species identified accounting for 67(18%) followed by Coagulase-negative Staphylococci which accounted for (16.1%) of the total samples.

PCT levels were raised in 276(74.20%) samples. The remaining 96(25.80%) had PCT levels below the recommended cut-off of 0.15ng/mL. Nonparametric comparisons were done with the Mann-Whitney U test which showed a significant difference in median PCT, CRP, and TLC levels between the Gram-negative and Gram-positive groups. The p-value for PCT was highly significant as compared to TLC and CRP which implies that serum PCT levels can better discern Gram-negative from Gram-positive sepsis (Table-1). Gram-negative isolates showed significantly higher median values of PCT (10.3ng/mL) compared to Gram-positive isolates (0.96ng/mL).

Table-I: Comparison of Median (Iqr) Of Biomarkers of Sepsis In Patients with Gram-Negative and Gram-Positive Septicemia (n=372)

	Gram Negative Median (IQR) n = 195	Gram Positive Median (IQR) n = 177	*p*-value
PCT (ng/mL)	10.30 (22)	0.96 (2.40)	< 0.001
CRP (mg/dL)	98.80 (142)	90.00 (143)	0.03
TLC (x 10*9/L)	11.60 (98)	13.30 (8.70)	0.01

[*IQR = Interquartile range, PCT = Procalcitonin. CRP = C-Reactive Protein, TLC = Total Leucocyte Count*]

The area under the curve (AUC) of a receiver operating characteristic curve (ROC) was calculated. PCT had the highest AUC of 0.73 (95% CI, 0.674-0.779, *p*=<0.001) which makes it a better marker in discerning Gram-negative from Gram-positive bacteremia as compared to CRP and TLC (AUC; 0.56 and 0.42, *p*< 0.001) respectively (Table II).

The sensitivity and specificity of PCT at different cut-off levels were calculated to distinguish between Gram-negative and Gram-positive cases. The optimal cut-off point according to our study results was found to be 3ng/mL with a sensitivity of 65.6% and specificity of 78% (TableIII).

Table-II: Comparison of area under curve (AUC) and p-value for PCT, CRP and TLC (n=372)

Parameter	AUC	*p*-value
PCT (ng/mL)	0.73	<0.001
CRP (mg/dL)	0.56	0.03
TLC (x 10*9/L)	0.42	0.01

[*AUC = Area under curve, PCT = Procalcitonin. CRP = C-Reactive Protein, TLC = Total Leucocyte Count*]

Table-III: Performance of Procalcitonin (ng/mL) for diagnosing septicemia at various cut-offs (n=372)

Test Result Variable(s)	Positive If Greater Than or Equal To	Sensitivity	1 – Specificity
	2.92000	.656	232
	2.95000	.656	226
	3.05000	656	220
	3.13000	656	209
	3.205000	656	203
	3.30000	651	203
	3.33500	651	198
	3.35500	646	198
	3.41000	646	192
	3.45500	641	192
	3.46500	636	192
	3.47500	636	186
	3.52000	631	186
	3.59000	631	181
	3.63500	631	175
	3.72000	626	175

DISCUSSION

The chief focus of our study was the clinical question of whether PCT is beneficial in identifying bacteremia caused by Gram-positive versus Gram-negative bacteria in a cohort of patients with positive blood cultures. We also conducted a basic evaluation of the diagnostic abilities of PCT, TLC, and CRP.

Analysis revealed that bloodstream infections due to Gram-negative pathogens were dominant in our cohort (GN in 52.4 %, GP in 47.6 % cases). Similar distribution has been noted in studies done previously.[13] Furthermore, a male predominance was noted (Males = 64%, Females = 36%). This finding is also correlated with many studies done in the past on male gender predominance in sepsis. In 2018, Campanelli *et al*., analyzed 34 large multicentre randomized control trials and reported that out of

498,146 septic adult patients admitted to ICU, the majority were males.[14]

Studies conducted over the past two decades have confirmed a rise in Gram-positive bacteremia as compared to Gram-negative bacteremia.[15] The most common pathogen isolated from the bacterial cultures in our study was also Gram-positive Staphylococcus aureus (18%) followed by Coagulase-negative staphylococci (16.1%). We also recorded 11.3 % cultures positive for E. coli and 11% cultures positive for K. pneumoniae. Numerous studies conducted nationally as well as internationally have reported Escherichia coli and Klebsiella pneumoniae as the most common cause of Gram-negative bacteremia.[16] Our study concluded the same. According to the SENTRY Antimicrobial Surveillance Program (1997-2016), out of 264,901 bloodstream infection isolates gathered, S. aureus and Escherichia coli were the most prevalent pathogens overall, followed by Klebsiella pneumoniae, P. aeruginosa, and Enterococcus faecalis.[17]

In our study, we established the diagnostic value of PCT for distinguishing Gram-negative sepsis from Gram-positive sepsis by using ROC analysis. ROCs were plotted and AUCs were calculated for PCT, CRP, and TLC in Gram-negative and Gram-positive bacteremia. The utility of PCT for diagnosing and differentiating the type of sepsis was also reflected by a significant *p*-value (<0.001). According to our analysis, PCT has sensitivity of 65.6% and specificity of 78% at a cutoff of 3 ng/mL for identifying Gram negative from Gram positive sepsis. Furthermore, our study reported median serum PCT levels of 10.3 and 0.96 for Gram-negative and Gram-positive bacteremia respectively. Receiver operating characteristic curves were used to further investigate the diagnostic efficacy of PCT in contrast to CRP and TLC. The area under the curve for differentiating Gram-negative from Gram-positive sepsis was maximal for PCT (AUC 0.727). This is in concordance with a study done by Guo, S.Y *et al*., who recorded data from 122 diagnostic episodes from patients with positive blood culture results and analyzed an area under the receiver operating characteristic curve (AUROC) of 0.73 (95% confidence interval [CI], 0.66–0.81,*p*< 0.001) for PCT to detect Gram negative bacteremia. They established that a cutoff value of 3.39 ng/mL for PCT had a diagnostic accuracy of 0.60, a sensitivity of 80%, a specificity of 71%, and median serum PCT values of 26.7 and 0.84 for Gram-negative and Gram-positive bacteria respectively.[18] As in our study, AUROC of CRP to detect Gram-negative bacteremia was also analyzed for the sake of comparison with PCT and was found to be 0.46 (95% CI, 0.46–0.67,*p*= 0.03) which is less than that for PCT.

Similar conclusions were reported in a fairly recent study conducted in Wuhan, China by Li *et al*., who established an optimal cut-off value of 3.11 ng/mL for PCT for distinguishing Gram-negative sepsis from Gram-positive sepsis (sensitivity = 63.9% and specificity of= 93.3%) and reported median serum PCT levels of 7.47 for Gram-negative and 0.48 for Gram-positive bacteremia.

In our study, although the CRP values of those with Gram-negative induced bacteremia were found to be raised than those with Gram-positive induced bacteremia, the area under the ROC curve was 0.73 for PCT and 0.56 for CRP. Therefore, it can be said that the diagnostic ability of PCT is greater than that of CRP.[19] Many studies have reported similar CRP values in both Gram negative as well as Gram positive bacterial infections such as those conducted by Guo *et al*., and Charles *et al*. In our study, however, the ROC curve analysis has elaborated that PCT was superior to CRP in predicting Gram-negative bacteremia. Same was the case with TLC.

In light of recent data and findings from our study analysis, serum PCT levels rise significantly in Gram-negative bacteremia and can therefore aid in diagnosis and management of patients with sepsis.

Per our knowledge, this is the first study reconnoitering PCT's ability to distinguish between Gram-negative and Gram-positive bacterial sepsis in moderately to severely ill adult patients in the ICUs and medical wards from Pakistan. The majority of studies done previously on adults as well as on neonates have aimed to determine the diagnostic accuracy of serum PCT for determining sepsis as a whole.

ACKNOWLEDGMENT

To all those who participated directly or indirectly in the study.

LIMITATION OF STUDY

The patients included in this study were mainly critically ill medical and surgical patients. The results might not be suitable to apply to other populations, such as trauma patients or those who are not critically ill. Also, the association between serum PCT measurement at initial ICU admission and sepsis was considered. The serial changes of serum PCT levels after management were not taken into consideration.

RECOMMENDATIONS

PCT is an effective complementary tool that, when combined with good clinical practice and other diagnostic parameters, can serve as a promising approach to tackling diagnostic uncertainties. The results from our study emphasize that elevated serum PCT levels correlate well with Gram-negative isolates on blood culture so they can be used to predict blood culture results; further confirming their diagnostic value. Keeping in view these observations, it can be stated that if bacterial cultures are negative and a definite source of infection cannot be identified, repeat low serum PCT levels could be a compelling argument for discontinuing antimicrobial therapy and exploring a substitute diagnosis. By skipping numerous days of broad-spectrum antibiotic prescriptions per patient, such a strategy is likely to reduce costs and enhance the therapeutic approach ove.

CONCLUSION

Patients with culture-positive Gram-negative bacteremia had higher PCT concentrations than those with Gram-positive bacteremia. This makes PCT a useful tool for distinguishing the type of causative pathogen in patients with septicemia and therefore may help to facilitate the selection of an appropriate empiric antibiotic regimen.

Conflict of interest: None.

Discolure: The author discloses that this abstract has not been presented or published at a conference, or published in an abstract book or any other publication.

Funding Source: None

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SBB & UN: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

AB: & JU: Data acquisition, data analysis, approval of the final version to be published.

QUAM: & SB: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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