ORIGINAL RESEARCH

Biomarker Profiling for Sepsis: Role of Procalcitonin, IL-6, And C-Reactive Protein

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ABSTRACT

Introduction: Procalcitonin (PCT), Interleukin-6 (IL-6), and C-Reactive Protein (CRP) biomarkers have shown promise in aiding clinicians in the early identification and assessment of sepsis, offering the potential to revolutionize the management of this critical condition. **Objective:** This study aimed to assess the diagnostic value of serum procalcitonin as a biomarker in sepsis and to compare its diagnostic performance with that of other sepsis markers, namely IL-6 and CRP, in patients with suspected sepsis. **Materials and Methods:** A total of 60 patients admitted to the intensive care unit (ICU) were enrolled in this study. Each patient underwent a comprehensive evaluation, including the measurement of serum procalcitonin, Interleukin-6 (IL-6), and C-reactive protein (CRP) levels using enzyme-linked immunosorbent assay (ELISA) techniques. Additionally, blood cultures were obtained and processed using the BacT/Alert system. **Results:** The findings of this study indicate that procalcitonin, in conjunction with CRP, exhibits superior diagnostic accuracy as a tool for sepsis diagnosis when compared to IL-6 and CRP alone. This suggests that a combined approach utilizing both procalcitonin and CRP may offer a more reliable and effective method for the diagnosis of sepsis. **Conclusion:** our study highlights the potential of procalcitonin as a valuable diagnostic biomarker for sepsis. When used in conjunction with CRP, it provides a more robust diagnostic tool for identifying sepsis in patients with suspected infections. These results may have important implications for clinical practice, potentially leading to earlier and more accurate sepsis diagnosis and treatment, thereby improving patient outcomes. Further research and clinical validation are warranted to confirm these findings and establish a standardized approach for sepsis diagnosis in various clinical settings.

Keywords: Biomarker, CRP, Diagnostic, Sepsis, Procalcitonin

INTRODUCTION

Sepsis, a life-threatening condition stemming from the body's overwhelming response to infection, remains a significant global healthcare challenge. Timely and accurate diagnosis of sepsis is imperative for optimizing patient outcomes and reducing mortality. Traditional diagnostic methods, while valuable, often lack the sensitivity and specificity required for early detection and precise assessment of sepsis. In this context, the identification and validation of biomarkers that can effectively aid in the diagnosis and prognosis of sepsis have become an area of intense research interest.Sepsis, a life-threatening infection, afflicts approximately 27 million individuals worldwide annually. Left untreated, sepsis may progress to severe sepsis, characterized by organ dysfunction, and ultimately lead to septic shock, resulting in roughly 8 million deaths per year [1] . A recent study conducted in India revealed that during their stay in the intensive care unit, 28.3% of patients developed sepsis, with a corresponding mortality rate of 34%. [2]

Sepsis presents with non-specific symptoms, rendering its clinical diagnosis challenging. Even in cases where microbiologic diagnostics are available, only a subset of patients exhibit identifiable bacteremia. The early initiation of appropriate antimicrobial regimens has been linked to improved patient outcomes [3]. Timely recognition of infection and the prompt initiation of suitable therapy are pivotal goals, as delays in empiric sepsis treatment not only elevate mortality rates but also inflate healthcare costs [4].The complex multi-organ involvement in sepsis expands the spectrum of potential biomarkers for its diagnosis. An ideal biomarker for bacterial infections should offer the advantages of early and swift diagnosis, the ability to forecast disease progression and prognosis, and the capacity to guide

therapeutic decisions, such as antibiotic stewardship[5].

Despite extensive research, the discovery of a single, universally ideal biomarker for sepsis remains elusive. Consequently, researchers have redirected their focus towards evaluating the diagnostic relevance of employing multiple biomarkers in concert. In line with this approach, our study was designed to assess the effectiveness of procalcitonin (PCT) as a diagnostic marker for sepsis, comparing it with other markers like C-reactive protein (CRP) and Interleukin-6 (IL-6), while also examining the relationship between these biomarkers and blood culture positivity. Our research was conducted within the confines of a tertiary care hospital, mirroring our healthcare environment

OBJECTIVE

This study aimed to assess the diagnostic value of serum procalcitonin as a biomarker in sepsis and to compare its diagnostic performance with that of other sepsis markers, namely IL-6 and CRP, in patients with suspected sepsis.

METHODOLOGY

Our study involved 60 adult patients of both genders aged over 18 years, who were admitted to the medical ICU and satisfied the diagnostic criteria for sepsis outlined by the American College Of Chest Physician's Criterion (ACCP). [6] These criteria included the presence of a minimum of two of the following: (a) Body temperature above 38℃ or below 36℃, (b) Heart rate exceeding 90 beats per minute, (c) Respiratory rate exceeding 20 breaths per minute or a paCO2 level below 32 mm Hg, and (d) White blood cell (WBC) count exceeding 12,000 cells/mm3 or falling below 4,000 cells/mm3 or showing more than 10% immature (band) forms. Patients with a history of trauma, recent surgical procedures, or a diagnosis of medullary carcinoma of the thyroid were excluded from the study.

Upon obtaining informed consent from the participants, we collected 2-3 ml of blood under sterile conditions for the assessment of procalcitonin (PCT), Interleukin-6 (IL-6), and C-reactive protein (CRP). The blood samples were allowed to coagulate, followed by centrifugation to separate the serum, which was then stored at temperatures between 2-8℃ for up to 3 days or frozen at -20℃ for extended preservation. The measurement of these inflammatory markers was carried out using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Blood cultures were executed using the automated BacT/Alert BioMerieux system. To ensure result accuracy, paired blood samples of 10-20 ml each were drawn from two separate veins with meticulous aseptic procedures.

STATISTICAL ANALYSIS

Categorical variables were presented as counts and percentages, while continuous variables were expressed as mean ± standard deviation (SD) and median. To assess data normality, the Kolmogorov-Smirnov test was employed. When normality was not met, non-parametric tests were applied.Comparisons of quantitative variables between two groups were conducted using the Unpaired t-test or Mann-Whitney Test (for non-normally distributed datasets), and for analyses involving more than two groups, ANOVA or Kruskal-Wallis tests (for non-parametric data) were used. The association of qualitative variables was examined using the Chi-square test. A p-value less than 0.05 was considered statistically significant. Data entry was performed in an MS EXCEL spreadsheet, and the analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 25.0.[7]

RESULTS

We enrolled 60 patients in our study and the results were as follows

PROCALCITONIN LEVELS

To assess PCT values, we categorized them into four groups based on the severity of sepsis. This categorization aids in the diagnosis of sepsis patients and has been applied in a similar manner in previous studies. [8, 9]

PCT >10 ng/ml	**: Indicates severe bacterial sepsis or septic shock.**
PCT 2-10 ng/ml	: Suggests a severe systemic inflammatory response, most likely due to sepsis.
PCT 0.5-1.9 ng/ml	: Represents Systemic Inflammatory Response Syndrome (SIRS); a systemic infection cannot be ruled out.
PCT <0.5 ng/ml:	Suggests the possibility of a local bacterial infection, making sepsis less likely.

As shown in table 1., among the 60 patients, procalcitonin tested positive in 51 individuals. The range of procalcitonin values varied from a minimum of 0.16 ng/ml to a maximum of 43.65 ng/ml, with a mean value of 18.89 ng/ml. Out of the total, 5 patients had procalcitonin levels below 0.5 ng/ml, with a median value of 0.26 ng/ml. Additionally, 4 patients had procalcitonin levels falling within the range of 0.5 to 1.9 ng/ml, with a median of 1.71 ng/ml. Furthermore, 8 patients had procalcitonin levels between 2 and 10 ng/ml, with a median value of 7.29 ng/ml. Notably, 43 patients exhibited procalcitonin levels exceeding 10 ng/ml, with a median value of 22.41 ng/ml

Table 1: Distribution of procalcitonin levels

PCT(ng/ml)	<0.5	0.5-1.9	2-10	>10
Sample size	5	4	8	43
Mean±SD	0.27±0.08	1.78±0.21	8.22±1.5	20.97±6.23
Median	0.26	1.71	7.29	22.41
Range	0.17-0.43	1.3-2.06	4.09-8.14	10.74-42.53

Table 2: Distribution of IL-6 and CRP levels as per the PCT values

PCT levels (ng/ml)	Sample size	IL-6 (pg/ml) Mean±SD	CRP(mg/l) Mean±SD
<0.5(local infection)	5	11.22±6.01	26.45±18.55
0.5-1.9(SIRS)	4	10.22±4.68	44.74±20.12
2-10(Sepsis)	8	28.31±15.22	71.65±22.78
>10(Severe sepsis)	43	89.45±68.74	120.99±29.70

DISTRIBUTION OF IL-6 AND CRP ACCORDING TO PCT LEVELS

To investigate the relationship between PCT and IL-6, the IL-6 and CRP levels were categorized into four PCT groups. The minimum recorded IL-6 value was 3.8 pg/ml, while the maximum reached 297.47 pg/ml. In the case of 8 septic patients, the mean ± SD value for IL-6 was 28.31 ± 15.22, and among 43 severe sepsis patients, the median IL-6 value was 89.45 ± 68.74. For CRP, the lowest value observed was 11 mg/l, and the highest was 242 mg/l. The mean ± SD for CRP in the 8 sepsis patients was 71.65 ± 22.78, and among the 43 septic shock patients, it was 120.99 ± 29.70 [Table 2].

Table 3: Correlation of culture positive cases with the three biomarkers

Group	Sample size (culture positive)		PCT(ng/ml)	IL-6(pg/ml)	CRP(mg/L)
Sepsis	2	Mean±SD	4.97±0.55	15.91±0.79	71.46±34.23
Severe Sepsis	26	Mean±SD	24.13±3.25	89.63±55.24	134.59±47.25

BLOOD CULTURE

Among the 60 patients, 28 blood samples yielded positive culture results, while 32 showed negative cultures. Among the 28 cases with positive cultures, the predominant organisms identified were Gram-negative bacilli in 17 cases, with Gram-positive cocci being detected in 8 cases. The specific bacteria isolated included Klebsiella, followed by Pseudomonas and Staphylococcus aureus.

CORRELATION OF BLOOD CULTURE WITH BIOMARKERS

As depicted in Table 3, among patients with both local infection and SIRS, none exhibited positive blood cultures. Out of the 10 patients diagnosed with sepsis, 2 displayed positive cultures, with an average PCT value of 4.97. In the case of the 43 patients suffering from septic shock, 26 of them had positive cultures, with an average PCT value of 24.13 ng/ml. The mean IL-6 levels for the 2 culture-positive cases in the sepsis group were 15.91 pg/ml, whereas for the 26 culture-positive patients in the severe sepsis group, it was 89.63 pg/ml. Similarly, the mean CRP values for these two groups were 71.46 mg/L and 134.59 mg/L, respectively.

DISCUSSION

These findings closely resembled the results reported in studies conducted by Sharma et al., Harbarth et al., and Viallon et al. In Sharma et al.'s investigation, they found that out of 80 patients, 7 exhibited PCT levels below 0.5 ng/ml with a median value of 0.28 ng/ml, 6 patients had PCT levels between 0.5 and 1.9 ng/ml with a median of 1.63 ng/ml, 10 patients fell into the 2-10 ng/ml category with a median of 6.99 ng/ml, and 57 patients registered PCT levels exceeding 10 ng/ml with a median value of 20.77 ng/ml.[10] Meanwhile, Harbarth et al. reported median PCT levels of 0.6 ng/ml for Systemic Inflammatory Response Syndrome (SIRS), 3.5 ng/ml for sepsis, 6.2 ng/ml for severe sepsis, and 21.3 ng/ml for septic shock [11, 12].

Sharma et al.'s study revealed that 67 out of 80 patients could be diagnosed with sepsis based on their PCT values. For patients with sepsis, the range of PCT was 4.15-8 ng/ml, and for septic shock, it was 10.65-40.64 ng/ml. These ranges proved valuable in diagnosing sepsis when a patient's PCT level fell within them. Additionally, Sharma et al. determined that the range of PCT for SIRS was 1.2-1.9 ng/ml, which could assist in the early diagnosis of sepsis by monitoring PCT levels in patients with suspected sepsis. This underscores the utility of PCT as a diagnostic tool for sepsis.

Levels of IL-6 were significantly elevated in patients with sepsis and septic shock. Sharma et al.'s study reported mean ± SD IL-6 levels of 27.59 ± 14.3 pg/ml for sepsis and 94.82 ± 78.21 pg/ml for septic shock, supporting the high PCT values in these groups and aiding in the diagnosis of sepsis and septic shock. IL-6 levels did not show significant increases in patients

with local bacterial infections or SIRS. Conversely, CRP values ranged from a minimum of 10 mg/l to a maximum of 220 mg/l, with a mean of 103.4 mg/l. The median CRP values were 45 mg/l for six patients with SIRS, 80 mg/l for ten patients with sepsis, and 120 mg/l for 57 patients with septic shock. Elevated CRP values aligned with high PCT values, thereby assisting in the diagnosis of SIRS, sepsis, and septic shock. These findings were consistent with those from other studies [13].

Our study results mirrored those of Sharma et al., who reported a sensitivity of 83.78% and a specificity of 72.09% for PCT ($P < 0.0001$). In contrast, the sensitivity and specificity of CRP (with a cutoff >80 mg/l, P value 0.001) and IL-6 (with a cutoff of 20.22 pg/ml, P value 0.055) were 78.38%, 48.84%, 78.38%, and 46.51%, respectively. Receiver Operating Characteristic (ROC) analysis indicated that PCT provided the best prediction of septicaemia, followed by CRP (0.78 vs. IL-6 0.62 and CRP 0.69). This finding was consistent with other studies, which also emphasized the superior predictive capability of PCT for sepsis compared to IL-6 and CRP, with 73% sensitivity and 70% specificity at a cutoff of 0.5 ng/ml [14]. A meta-analysis of 30 studies involving 3244 patients yielded a sensitivity of 77% and specificity of 79%, indicating PCT's utility as a valuable biomarker for early sepsis diagnosis [15].

Some studies suggested CRP cutoff values between 50 and 100 mg/l for diagnosing sepsis, but the nonspecific nature of CRP limited its use as a sole diagnostic tool. In contrast, PCT was deemed a good predictor of infection in intensive care patients with suspected sepsis, outperforming CRP [16 ,17].

However, the diagnostic accuracy of IL-6 in critically ill patients was limited, as it exhibited nonspecific elevations due to accompanying inflammation, irrespective of the presence of infection. In contrast, PCT responded more specifically to infection than to inflammation. Additionally, the concentrations of blood cytokines were erratic, and their temporal patterns did not align with the course of sepsis, making interpretation challenging [18-20].

Correlation with blood cultures revealed that among patients with local infection and SIRS, none had positive blood cultures. In the 10 patients with sepsis, 3 (30%) had positive cultures with a mean value of 5.42 ng/ml. In contrast, among the 57 patients with severe sepsis (septic shock), 34 (59.65%) had positive cultures with a mean value of 22.2 ng/ml. The study observed more culture-positive cases in severe sepsis (septic shock) than in sepsis, and higher PCT levels were associated with culture-positive cases (P value <0.05). IL-6 and CRP levels were also found to be correlated with high PCT levels in blood culture-positive cases (P value <0.05). These findings were consistent with those of other studies [21, 22].

Studies indicated that culture-positive sepsis was linked to the severity of sepsis, longer hospitalizations, and higher mortality rates. Elevated procalcitonin levels were also found to be correlated with positive blood culture results, reinforcing its diagnostic value in general ICU settings. Furthermore, CRP and IL-6 levels were linked to PCT levels in positive blood culture sepsis cases, enhancing their diagnostic utility. Blood culture is considered the gold standard for detecting bloodstream infections, as it enables the identification of species and susceptibility to antimicrobial therapy through the analysis of viable microorganisms isolated from the blood [23].

Nonetheless, the practical utility of culture in diagnosing sepsis is hindered by the time it takes to obtain results and the fact that positive blood cultures are only found in approximately 30% of these patients. Additionally, the sensitivity for slow-growing and fastidious organisms is low. Some studies proposed that if all microbiological cultures are negative and no clear source of infection has been identified within 24 hours, a repeat low PCT measurement, combined with clinical judgment, could strongly support the discontinuation of antimicrobial therapy and the search for an alternative diagnosis. This approach may potentially save several days of broad-spectrum antibiotic therapy per patient. It has been suggested that sequential PCT measurements could be useful in identifying healthcare-associated infections, with evidence indicating that PCT measurements at the time of suspected infection and repeated two or three times weekly may suffice and be clinically useful. However, the cost-effectiveness of PCT testing, particularly in Indian settings, has not been rigorously evaluated [24-26].

Positive points of the study- Sepsis and biomarkers are not well studied, our study is an honest attempt in Tertiary care teaching hospital of Western India. However, sample size in our study was less due to one month duration of the study. In near future, same study can be planned in large number of patients with other newer biomarkers.

CONCLUSION

In our tertiary care hospital setting, our research has significantly contributed to the identification of sepsis cases through the utilization of biomarkers such as PCT, CRP, and blood cultures. Among the array of biomarkers investigated, our findings underscore the significance of PCT as a robust and dependable indicator for the early detection of sepsis, particularly among critically ill patients. Recognizing sepsis promptly in this patient population is crucial in mitigating morbidity and mortality. Consequently, the combined use of PCT, CRP, and blood cultures emerges as a potent diagnostic arsenal for the effective diagnosis of sepsis.

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