A study on biomarkers of sepsis and potential role of procalcitonin and ferritin marker in diagnosis, prognosis and treatment

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Abstract

Objectives: To evaluate the potential value of serum procalcitonin and serum ferritin levels in patients with clinically suspected and proven sepsis and their comparison with established inflammatory markers like C-reactive protein (CRP) and total leukocyte count. Materials and Methods: A total of 60 clinically suspected cases of sepsis were included in this study and each patient was investigated for serum S. ferritin, procalcitonin, and CRP and blood cultures using the BacT/Alert system. Results: Serum procalcitonin at a cut-off value of >2 ng/ml is a valuable biomarker for early diagnosis in sepsis patients due to bacterial infection and has a greater predictive value than serum ferritin, CRP, or any other biomarkers.

Keywords: CRP, ferritin, inflammatory biomarkers, procalcitonin, sepsis

Introduction

Bacterial sepsis is one of the common causes of morbidity and mortality in patients admitted to the intensive care unit. Sepsis can be difficult to differentiate from non-infectious conditions in critically ill patients in the early stages; and diagnosis, treatment, and outcomes greatly differ between patients with and without sepsis.[1] Biomarkers can indicate the presence, absence, or severity of sepsis. They also have roles in prognosis, guiding antibiotic therapy, evaluating the response of therapy and recovery from sepsis predicting complications, and the development of organ dysfunction.[2] There are several markers of sepsis, like C-reactive protein (CRP), serum procalcitonin (PCT), Interleukin-6, Interleukin-8, ferritin, lactate, etc.

Early diagnosis of sepsis is a challenge to the clinician and the laboratory. Though blood culture is the gold standard method for the diagnosis of bacterial sepsis, isolating the bacteria and determining its antibiotic susceptibility takes a minimum of 48 hours and traditional markers of infection, such as body temperature and white blood cell count, may not be specific. Furthermore, there are apprehensions about negative blood culture results in clinical sepsis, particularly in the case of increased prophylactic and empirical antibiotic usage.[3]

Recently PCT has emerged as a biomarker for the diagnosis of bacterial infection because higher levels are found in severe bacterial infections relative to viral infections and non-specific inflammatory diseases.[3] PCT-guided antibiotic therapy can be
used by primary care physicians for initiation and discontinuation of antibiotics.

Another acute phase reactant- Ferritin has emerged as a diagnostic biomarker for neonatal sepsis. But its role in diagnosing early sepsis has not been studied in detail in adults so far. Elevated levels of serum ferritin reflect the clinical response to deprive microorganisms of serum iron. Since early identification of infections and sepsis is vital for patient management, an effective marker specific for bacterial infection is very valuable in critical care settings.

To the best of our knowledge, there are very few comparative studies of serum PCT and serum ferritin in sepsis diagnosis and prognosis from Pondicherry.

**Aims and Objectives**

To evaluate the potential value of serum procalcitonin and serum ferritin levels in patients with clinically suspected and proven sepsis and to compare it with established inflammatory markers like CRP and total leukocyte count and the role of procalcitonin in differentiating viral and bacterial infections.

**Materials and Methods**

This case-control study was done in our 740 bedded tertiary care hospital in collaboration with the Dept of Microbiology and General Medicine. The study included patients admitted with suspected sepsis in the Medicine and Intensive Care Unit of our hospital. Informed consent was taken from patients and the study was approved by the institutional ethical committee.

Patients: All adult patients aged above 18 years with suspected cases of fever with sepsis (temperature >38.0°C) were included in this study. Patients with cardiogenic shock, major trauma, major surgical interventions, and severe burns were excluded from the study as PCT is non-specifically elevated in these conditions.

**Case definition- Identification of sepsis patients- Sepsis clinical criteria**

Organ dysfunction is defined as an increase of 2 points or more in the Sequential Organ Failure Assessment (SOFA) score for patients with suspected infections, an increase of 2 SOFA points gives a mortality rate of 10%. Patients with suspected infection admitted in ICU can be easily identified for sepsis at the bedside using a simple qSOFA score comprising the following three items-

- Having Systolic Blood Pressure <100 mmHg
- Respiratory rate ≥22 breaths per minute
- Altered mentation with Glasgow Coma Scale (GCS) less than 15.

The clinical condition, signs, and symptoms of sepsis, antibiotics used, blood culture, serological results of procalcitonin and ferritin, and outcome of patients were recorded for all patients. Patients’ demographic profile was collected which included the clinical data, associated symptoms, vital signs, and the general, and systemic examination findings and recorded and cross evaluated.

The patients were divided into three groups according to their diagnosis. Patients with bacterial infection were divided into two groups (Group I and Group II) according to the result of the blood culture. Group I represented patients with bacteremia and defined by positive blood culture. Group II represented patients with bacterial infection but negative for growth. Patients in Group III had no bacterial infection. Following laboratory investigations were also done- bacterial culture and antibiotic sensitivity test- Blood, urine as per standard protocols. The following serological tests were done- C-Reactive protein (CRP), Serum Procalcitonin, Serum ferritin tests. Under haematology investigations- Complete blood count and Erythrocyte Sedimentation Rate (ESR) were done. Within 12 hours of ICU admission, under aseptic conditions, blood was collected from patients clinically suspected of sepsis. Serum was separated by centrifugation (2500 rpm for 20 minutes) - Procalcitonin test (immunochromatography), CRP test, serum ferritin test (Enzyme-Linked Immunosorbent Assay (ELISA)) were performed as per standard procedures along with other investigations.

Statistical analysis was carried out using the Chi-square test to evaluate the correlation between PCT levels, Ferritin, and sepsis. A P value < .05 was considered statistically significant.

**Results**

In this study, which spanned over a period of 2 months, a total of 484 fever cases were screened. Clinically suspected cases of sepsis were 60. Thirty-eight patients (63.3%) in the age group of 41–60 years were more commonly involved and males (66.67%) were more commonly seen than females [Table 1]. Out of 60 patients, 11 (18.3%) were blood culture positive and among those 8 (72.7%) grew gram-negative organisms. The most common gram-negative organism grown was *Escherichia coli* (45.5%), *Klebsiella pneumonia* (18%) followed by *Acinetobacter baumannii* (9%) while the most common gram positive organism grown was *Staphylococcus aureus* (27.5%) [Table 2]. The most commonly identified source of sepsis was respiratory tract infection (55%), followed by urinary tract infection (15%).

| Table 1: Age and Gender distribution in sepsis cases |
|-----------------------------------------------------|
| Age group (Yrs) | Group I (n=11) (Blood culture positive) | Group II (n=29) (Blood culture negative) | Group III (n=20) (Non-Bacterial infections) |
|-----------------|----------------------------------------|-----------------------------------------|-------------------------------------------|
| 20-40           | M (%) 10 (F 0)                         | M (%) 2 (F 7)                          | M (%) 4 (F 20)                            |
| 41-60           | M (%) 05 (F 45)                        | M (%) 10 (F 34.5)                      | M (%) 6 (F 30)                            |
| 61-80           | M (%) -                                | M (%) 8 (F 27.5)                       | M (%) 3 (F 15)                            |
| Total           | M (%) 11                               | M (%) 29                               | M (%) 20                                 |

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The following laboratory parameters were measured in all the patients- PCT, S. Ferritin, CRP test and leucocyte count.

**Procalcitonin**

Serum PCT cut offs of >0.5 ng/ml and 1.5 ng/ml were analyzed separately for their sensitivity and specificity as biomarkers for sepsis among 40 patients with bacterial sepsis and 20 patients without sepsis. There was a statistically significant correlation with the presence of bacterial sepsis determined using either PCT ≥0.5 ng/ml or ≥2 ng/ml

Using a cutoff of 0.5 ng/ml or more as cut-off, 36 of the 40 patients with sepsis could be detected, but 3 out of the 20 patients without sepsis showed PCT above 0.5 ng/ml (sensitivity- 90%; and specificity- 85%).

Using PCT cut-off of 1.5 ng/ml or more as a marker of sepsis, 31 of the 40 patients with sepsis could be detected by PCT and only 1 out of the 19 patients without sepsis showed PCT above 2 ng/ml (sensitivity- 77.5% and specificity- 95%) [Table 3].

All culture positive cases (Group I) had PCT levels of >7.0 ng/ml. Culture negative cases of sepsis (Group II) 69% patients had PCT levels between 4.0 – 12.0 ng/ml. Even in culture-negative sepsis patients, high PCT levels were evident and consistent with the severity of sepsis, and in Group III- Nonbacterial infections the PCT levels were <0.4 ng/ml and was evident in 85% of patients. An increase in the level of PCT was clearly evident in bacterial sepsis.

**Ferritin**

A ferritin assay was also performed. Using a cutoff of 300 ng/ml or more as a marker of sepsis, 24 of the 40 patients (60%) with sepsis could be detected and 6 out of 20 without sepsis showed S. Ferritin above 300 ng/ml. (sensitivity- 60% and specificity- 70%) [Table 4].

Using S. Ferritin cut-off of 600 ng/ml or more as a marker of sepsis, 11 of the 40 patients with sepsis could be detected using this assay and only 2 out of the 20 patients without sepsis showed PCT above 2 ng/ml (sensitivity-35% and specificity- 90%).

**C-Reactive Protein**

About 65% of the patients had a CRP level of ≥200 µg/ml. The correlation of PCT to CRP in all the included subjects demonstrated that although CRP was high in patients with sepsis, it did not correlate well with the severity of sepsis [Table 5].

**Total leucocyte count and ESR**

Almost all patients with sepsis had leukocytosis (88%) and high ESR (82%).

There was a significant difference in procalcitonin concentrations between patients with bacterial sepsis and non-bacterial infection patients.

**Discussion**

The aim of our study was to evaluate the usefulness of PCT and S. Ferritin as an early indicator for the marker of sepsis in critically ill patients and guide them in antimicrobial therapy. Our study evaluated the potential value of measuring acute phase reactants like PCT, serum ferritin in patients with clinically suspected and proven sepsis and correlated it with the outcome of the patients and compared it with other established inflammatory markers like C- reactive protein (CRP) and total leucocyte count. Our study reported a male preponderance with sepsis. This is similar to a study done by other authors.[9,10] E. coli and *Staphylococcus aureus* were the main causes for bloodstream infection which is in accordance with another study done by Gagliotti et al.[11] In our study, higher concentration levels of PCT were found in gram negative bacteremia patients as compared to Gram positive bacteremia. This is in accordance with the studies done by Li et al.[12] and Guo et al.[13] To date no gold standard test has been identified for diagnosis of sepsis.
Microbiological culture reports take at least 48 hours and sometimes blood culture is not positive in clinically proven sepsis despite improved blood culture methods.\textsuperscript{[10]}

At a serum PCT cutoff of 0.5 ng/ml, our study has reported a sensitivity and specificity of 90% and 85%, respectively. And at 2 ng/ml serum, PCT had a sensitivity and specificity of 77.5% and 95%, respectively. This cutoff had a better specificity as compared to a cutoff of 0.5 ng/ml. This finding is similar to studies done by various authors.\textsuperscript{[11,16]} Therefore, we recommend that for differentiating bacterial sepsis from other causes of sepsis a cut-off of 2 ng/ml should be utilized. De Werra et al.\textsuperscript{[17]} observed that patients with procalcitonin values of more than 1.5 ng/ml were most likely to undergo septic shock with a sensitivity of 100% and a specificity of 72%, and Suprine et al.\textsuperscript{[18]} reported that the best cut-off values of 2 ng/ml with 65% sensitivity and 70% specificity in diagnosing infection in medical ICU patients. In a study, Muller et al.\textsuperscript{[19]} reported that the PCT concentrations of >1 ng/ml had a sensitivity of 89% and specificity of 94% for the diagnosis of sepsis in 101 medical ICU patients.

In our study, serum ferritin results showed low sensitivity in sepsis patients [Table 4]. In infectious diseases, highly elevated serum ferritin has been reported in viral hepatitis causing liver injury and viral hemorrhagic infections. Serum ferritin is increased as an acute phase reactant in extracellular bacterial sepsis, but the level is usually not high.\textsuperscript{[18]}

Though CRP is a highly sensitive biomarker of inflammation, it is not specific to infection. Its serum levels increase in both infectious and non-infectious causes of inflammation.\textsuperscript{[19,20]}

Higher WBC counts were found in bacterial sepsis (especially in culture-proven) is in agreement with other studies.\textsuperscript{[21‑23]} Though CRP, Erythrocyte Sedimentation Rate (ESR), and leukocyte counts is known inflammatory marker, but they have less value for early diagnosis.

**Summary**

The observation and results from our study confirm that serum PCT at a cutoff of >2 ng/ml is a valuable biomarker for early diagnosis in sepsis patients due to bacterial infection and has a greater predictive value than serum ferritin, CRP, or any other biomarkers. PCT is also an excellent marker for antibiotic stewardship and a valuable diagnostic tool in the diagnosis of sepsis. Ferritin might not be a valuable biomarker in identifying bacterial infections in febrile patients. Easy availability and lesser cost of CRP make it the most commonly used inflammatory marker in the ICU.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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