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ORIGINAL ARTICLE

Evaluation of Triple Biomarker Algorithm for Identification of Bacterial Sepsis in Critical Care Patients of a Tertiary Care Hospital

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ABSTRACT

Introduction: Early and accurate identification of bacterial infection is crucial for the improved clinical outcome of a patient with sepsis, a diagnostic challenge in the intensive care unit (ICU). Virtually, all patients in the ICU have some inflammatory response associated with the fever which does not at all require antibiotics, thus differentiating between sepsis and non-infectious systemic inflammatory response syndrome (SIRS) is imperative. A study was therefore done to evaluate triple biomarker algorithm for identification of bacterial sepsis in critical care patients.

Materials and methods: One hundred and ninety-seven immunocompetent adult patients with presumed bacterial sepsis admitted in various ICUs of Sri Guru Ram Das University of Medical Sciences and Research were consecutively enrolled from November 2016 to October 2017. Blood samples obtained from these were subjected to culture and sensitivity as per clinical and laboratory standards institute (CLSI) guidelines in Department of Microbiology after approval by the ethical committee. Serial concentrations of C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6) of 39 patients who fulfilled the inclusion criteria were determined at baseline, 24 hours, 48 hours and 72 hours in the Department of Biochemistry. The performance characteristic of various biomarkers individually and in combination was studied.

Results: Of the total 2831 adult indoor febrile patients, ICU admissions were 197/2831(6.9%) of which only 58/197(29.44%) were culture positive, yielding bacteria and *Candida* species in 52/58 (89.65%) and 6/58 (10.34%) respectively. There were significant ($p < 0.05$) difference in the levels of PCT and IL-6 among the bacteremic group.

Conclusion: Procalcitonin (PCT) and IL-6 are superior to CRP in the early identification of bacterial infection. However, more perspective and large-scale studies are warranted to confirm these findings.

Keywords: CRP, IL-6, CT, Infection, SIRS.

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INTRODUCTION

Sepsis refers to a SIRS caused by infections, and the incidence of severe sepsis around the world is up to 18 million cases annually.[1] It is associated with marked morbidity and mortality continues to be a serious complication of infection in the ICU patients. Prompt identification of bacterial infection in fever is very important, since appropriate etiological treatment and avoidance of unnecessary antimicrobial therapy could not only reduce the morbidity, mortality, and costs to patients, but also can reduce the emergence of antibiotic-resistant bacteria.[2] Virtually all patients in the ICU have some inflammatory response associated with fever which does not at all require antibiotics, thus differentiating between sepsis and non-infectious SIRS is imperative although challenging. The traditional diagnostic tools, such as C-reactive protein (CRP) and leukocyte count, are not specific enough for differentiating bacterial infections from viral infections and systemic inflammation. Microbiologic culture requires at least 24 to 48 hours, and negative cultures do not exclude the presence of infection.

Moreover, only 5 to 10% of blood cultures performed in hospitals show microorganisms. Therefore, there is an obvious need for more specific biomarkers of bacterial infections in febrile patients. Procalcitonin (PCT), the precursor of the hormone calcitonin, is produced by C-cells of the thyroid gland or neuroendocrine cells in the lung or intestine, and its level in blood of healthy people is less than 0.01 ng/mL. Levels of PCT rise dramatically during bacterial infections, whereas low levels were detected during viral infections or non-infectious febrile conditions. Many studies showed the diagnostic property of PCT superior to CRP, despite many studies, it remains unclear whether PCT adds significantly to the discriminative properties of the already used set of diagnostic biomarkers, and further studies need to be done to determine the specific

diagnostic cut-off value. Though the negative cut-off value for PCT is generally believed to be <0.5 ng/mL, it is not consistent since "negative" PCT value has been observed in some patients with bacterial infection. Interleukin-6 (IL-6) is an important proinflammatory cytokine in the early phase of inflammation, which increases in local and blood circulation after stimulus such as infection, surgery and trauma. Therefore, the detection of PCT, CRP and IL-6 may be a better combination to identify early bacterial infection in febrile patients.[2] Procalcitonin (PCT) has been investigated in several studies in India, though most have focused on case reports or series looking at specific diagnoses such as scrub typhus, septic arthritis and osteomyelitis, H1N1, pancreatitis, pyelonephritis, and meningitis.[3-9]

While sepsis is not interchangeable with bloodstream infections, the majority of research has been done on sepsis as a syndrome.[10] India, with a population of 1.32 billion, has one of the highest infectious disease burdens in the world. While systemic data on presentations of acute febrile illness are lacking, 12% of adults (range 1 to 51%) of those presenting with acute febrile illness will have bacteremia. Availability of diagnostic assays is variable in India, making the diagnosis of these common infections even more difficult. There is considerable interest in developing decision tools that utilize biomarkers to help aid the rapid diagnosis of bacterial infections.

Additionally, due to rising antibiotic resistance on the Indian subcontinent, biomarkers that help with antibiotic stewardship are equally needed. There have been numerous studies evaluating PCT in different clinical scenarios, including sepsis, though the majority of these studies have been in the United States and Europe; there is the great opportunity for well-designed studies evaluating biomarkers for sepsis in India. Because of its ability to help differentiate between viral and bacterial infections, PCT has been evaluated to guide decisions for appropriate antibiotic therapy all over the world. PCT measurements have also been found to be statistically significantly higher in patients with true bacteremia when compared to patients deemed to have contaminants with coagulase-negative staphylococci which certainly have implications for decreasing inappropriate antibiotic use. With highest rates of infectious diseases and alarmingly high rates of resistant bacteria, utilization of PCT-based diagnostics that help indicate when unnecessary antibiotics can be avoided is a prime goal in India.[11]

Kaur et al. published a review in 2013 evaluating PCT to reduce inappropriate antibiotic use in India, but no studies assessing PCT for antibiotic stewardship in India have been published to date.[12]

We, therefore, undertook this study to compare the diagnostic properties and evaluate the optimum cut-off values of PCT, CRP, IL-6 to detect bacterial infections early in ICU patients.

MATERIALS AND METHODS

Study Design and Settings

The study was conducted from November 2016 to October 2017, in the Department of Microbiology and Biochemistry of Sri Guru Ram Das University of Medical Sciences and Research after hospital ethical committee approved the protocol for this research. Written informed consent was obtained from every enrolled patient.

Study Population

Immunocompetent adult patients (18 to 85 years) with presumed bacterial sepsis (temperature >38°C) admitted in various ICU's of Sri Guru Ram Das University of Medical Sciences and Research were enrolled.

Specifically, patients were required to meet the established criteria for SIRS, as well as new empiric antibiotic therapy initiated and blood cultures ordered, thereby indicating the suspicion of bacterial infection. SIRS would be considered present if patients meet at least two of the following criteria within 4 hours of enrolment of which one must be temperature or leukocyte count changes.[13]

- Body temperature >38°C,
- Heart rate >90/min,
- Respiratory rate >20/min
- WBC count >12000 cells/µL or less than 4000/µL.

New empiric antibiotic therapy was defined as either the initiation of new antibiotic therapy in a patient previously not on any antibiotics or broadening of antibiotic therapy in a patient who has already receiving at least one antibiotic.[13]

Exclusion Criteria

- Initiation of board spectrum antibiotic therapy for a documented bacterial infection in the 5 days prior to enrolment.
- Presence of immunocompromising condition, eg., HIV
- Antibacterial therapy within the last 48 hours.
- The absence of informed consent and pregnancy.

Blood Culture and Sensitivity Testing

A 5 ml of blood collected from each patient by all aseptic and antiseptic precautions was inoculated into BacT/alert standard bottles which were loaded into the BacT/alert incubator and incubated for the maximum period of 5 days. Standard BacT/alert software was used for recording the results. Bottles flagged as positive by the BacT/alert instrument were subcultured on a plate each of blood and

MacConkey agar. Cultures were declared sterile if there was no growth on the plates after 48 hours of incubation at 37° C. Positive cultures were processed in the Vitek-2 compact as per the following protocol.

Suspension Preparation

A sterile swab/applicator stick was used to transfer a sufficient number of colonies of pure culture into 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 × 75 mm clear plastic (polystyrene) test tube to make a uniform suspension, the turbidity of which was adjusted using a turbidity meter called the DensiChekTM.

Identification with VITEK 2 Compact

Identification cards were automatically filled with bacterial suspension by a vacuum device, sealed and inserted into the VITEK 2 reader-incubator module (incubation temperature, 35.5° C), subjected to a kinetic fluorescence measurement every 15 minutes and interpreted by the ID-GPC/GNB database. Final results were obtained automatically, and all cards used were discarded into a waste container.[14]

Biomarkers Measurement

Candidate biomarkers were measured at the time when patients meet all eligibility criteria (baseline), then daily for 3 days (24, 48, and 72 hours) as per manufacturer's protocol. PCT levels were measured using biovendor human procalcitonin elisa (BHPE). The CRP levels were measured using Vitros CRP slides. Interleukin 6 levels were measured by ELISA using Human IL-6 ELISA kit Daclone SASF-25026 Besancon Cedex, France.

Statistical Analysis

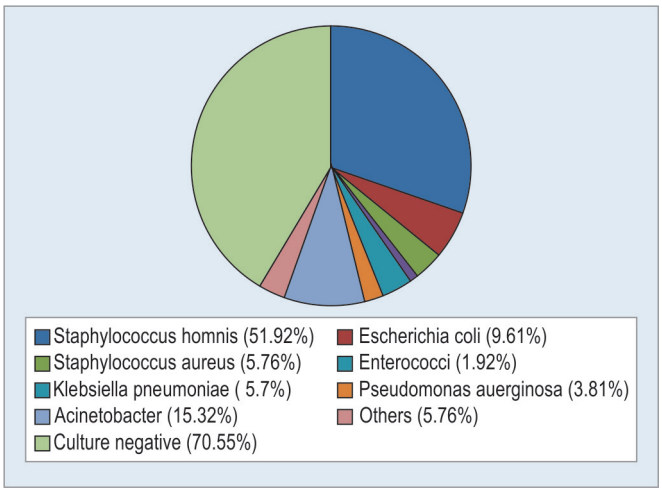
The data was analyzed using statistical package for the social sciences (SPSS) version 20.0. Repeated measure analysis of variance (ANOVA) was applied to see the significance of CRP, PCT and IL-6 levels with time. Post-hoc Bonferroni test was also applied for pairwise comparison.

RESULTS

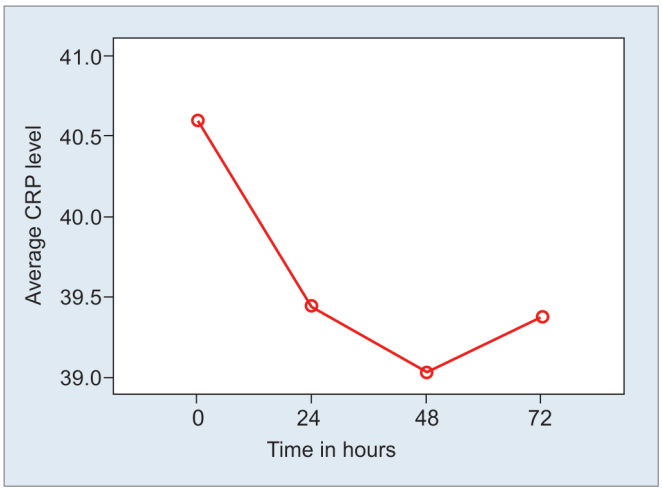
Of the total 2831 adult febrile patients admitted in various wards of our hospital during the study period, ICU admissions were 197/2831(6.9%). Further, only 58/197(29.44%) were culture positive. Bacteria and Candida species were isolated in 52/58(89.65%) and 6/58 (10.34%) respectively. Various bacteria in decreasing order of prevalence among ICU patients were *Acinetobacter* species 8/52 (15.32%), *Escherichia coli* 5/52 (9.61%), *Klebsiella pneumonia*, *Staphylococcus aureus*, and others 3/52 each (5.76%). *Pseudomonas aeruginosa* 2/52 (3.8%) and *Enterococcus* species 1/52 (1.92%) as shown in Graph 1.

Of the 58 culture positive samples, only 39/58 (67.24%) fulfilled all study inclusion criteria. Reasons for exclusion included the following: Antibiotics received for more than 4 hours past the baseline biomarkers measurement time point (n = 7), current receipt of board spectrum antibiotic therapy for a recently documented bacterial infection (n = 5), inability to obtain residual blood sample for all time point measurements (n = 7).

- Residual samples from all 39 patients were subjected to CRP, PCT and IL-6 estimation at baseline, 24 hours, 48 hours, and 72 hours time points.
- CRP levels did not show a significant difference compared with time (p = 0.602) as shown in Graph 2 and Table 1.
- PCT showed an initial steep rise up to 24 hours followed by slight increase till 48 hours after which there is a sharp decline. The decline is significant at 72 hours compared to 24 hours (p = 0.015) and 48 hours (p = 0.017) as shown in Graph 3 and Table 1.



Graph 1: Blood culture results of ICU febrile patients



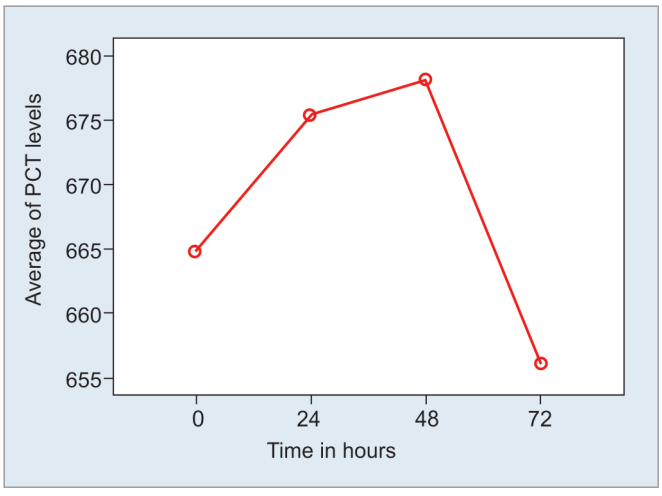
Graph. 2: Blood culture results of ICU febrile patients

Table 1: Comparative performance of biomarkers at serial time points

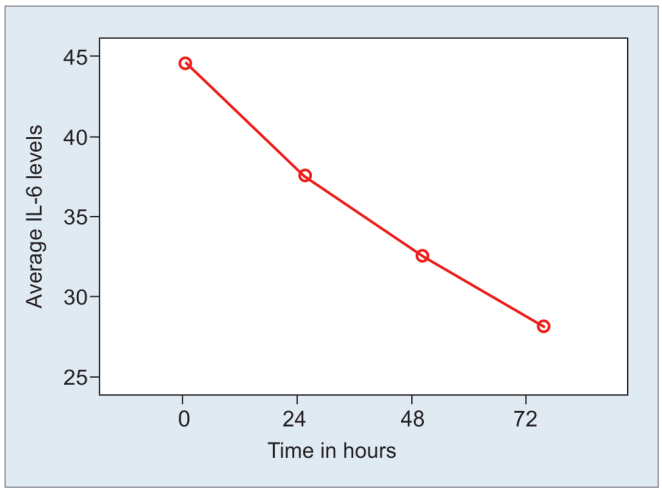
	Time point(mean ± SD)					
Biomarkers	0 hr	24 hours	48 hours	72 hours	*F*	*p value*
PCT	664.82 ± 1489. 44	675.49 ± 1504. 13	678.13 ± 1501.77	656.00 ± 1473. 20	3.126	0.039*
IL-6	44.70 ± 38.19	37.52 ± 37.11	32.57 ± 32.52	28.17 ± 30.62	20.115	0.000**
CRP	40.6 ± 52.73	39.44 ± 52.97	39.04 ± 49.58	39.37 ± 50.27	0.433	0.602

*significant at p < 0.05

**highly significant



Graph 3: Average PCT level with respect to time



Graph 4: Average IL-6 level with respect to time

Although IL-6 showed a steady fall which is highly significant at 24 hours (p = 0.000), 48 hours (p = 0.000) and 72 hours (p = 0.000) compared to the baseline, however, decline at 48 hours as compared to that at 24 hours is not significant (p = 0.082) although decline at 72 hours as compared to 24 hours (p = 0.004) and 48 hours (p = 0.027) is significant as shown in Graph 4 and Table 1.

DISCUSSION

Ours was a prospective cohort study for evaluating the utility of three biomarkers to differentiate bacterial sepsis from other causes of SIRS in the ICU patient population. We found that in all bacteremic patients there was an increase in PCT levels from 24 to 48 hours followed by the decline at 72 hours in all cases except three who died indicating both diagnostic and prognostic value of PCT. Although high CRP values were observed initially in all samples, its variation, later on, was not conclusive. IL-6 values which were high at baseline, later on, decreased in all cases.Thus IL-6 had no prognostic value in our study. Many previous studies have shown that PCT level can be used to identify bacterial infections in patients with sepsis admitted to intensive care units (ICUs).[2] Our study suggested PCT as a valuable biomarker in detecting bacterial infection in febrile patients. This observation is consistent with the findings of previous researches.[2,15-18] Previous studies have also used CRP level to identify bacterial infections in febrile patients and PCT was superior to CRP in identifying bacterial infection. Though CRP is the most commonly measured acute parameter in infection and sepsis, it is not a reliable marker in identifying bacterial infection because of its low sensitivity and specificity.[2]

Contrary to our study a previous study showed IL-6 as a better prognostic marker of bacterial infection than CRP in patients with febrile neutropenia.[19] In addition, there was no significant association between PCT and CRP or IL-6, which also might be related to the different peak time and the plasma half-life of different biomarkers after a stimulus. In bacterial infections, IL-6 levels rise 2 hours after endotoxin administration, and then gradually decline. PCT is probably synthesized in the liver or monocytes, in response to cytokines such as IL-6 and TNF-α. PCT increases in blood six hours after a stimulus reach a plateau between 12 hours and 48 hours and then decreases if the stimulus stops. CRP increased four hours later than PCT. In general, biomarker measurement has some disadvantages. Probably the combination of biomarkers would lead to better sensitivity and specificity to predict bacterial infections.[2] In our study Acinetobacter species were isolated from eight patients, as reported in previous studies.[20,21] Our study also showed that PCT levels in Gram-negative bacterial infections were significantly higher than that in Gram-positive bacterial infections, consistent with results of previous studies.[22,23]

In spite of having an extremely high mortality rate, sepsis has no established death risk prediction model with high specificity and sensitivity and simple procedures.

Biochemical markers have been increasingly used in early diagnosis, management, and prognosis of sepsis. The ideal biochemical marker prognostic model should meet the following characteristics: has a simple method for measurement that can be widely used in clinical practice, enables clinicians to intervene to improve prognosis quickly, and can provide even better prognostic information than critically ill disease score. Recent studies have shown that various serological proteins, such as serum PCT, highly sensitive CRP, and IL, have high levels in the sera of sepsis patients and are correlated with the severity of sepsis.[24]

Suhua et al. found that on the day of hospitalization, the PCT, hs-CRP, and IL-6 levels in the death group patients were significantly higher than those in the survival group similar to our study.[24] This is consistent also with the findings of Li Zhenyu et al. who detected levels of serum procalcitonin, C-reactive protein, lactic acid, interleukin-6, and interleukin-10 in patients with sepsis and found that the levels of these substances in patients who died within 28 days were significantly higher than those who survived within 28 days.[25]

Systemic inflammatory response syndrome (SIRS) is common in ICU population and given the association with a reduction in mortality, patients with SIRS often receive empiric board spectrum antibiotic therapy. Further, prolonged courses of antibiotics often used to treat ICU patients with a non-bacterial etiology of SIRS, contribute to overuse of antibiotics.[13] Thus there is a need for reliable diagnostic biomarkers as an adjunct to PCT in the overall clinical decision-making process for differentiating bacterial sepsis from other causes of SIRS.

CONCLUSION

In conclusion, PCT may be a valuable biomarker of bacterial infections in febrile patients, with higher predictive value than CRP and other biomarkers of infection. However, the small sample size and patient inclusion from a single hospital, the two main limitations of our study may have lead to weak statistical power and patient selection bias. Also, specific exclusion of significantly immunocompromised patients because it is unlikely that empiric antibiotics would be discontinued in this population (regardless of biomarker results), decreasing the utility of such an algorithm in this group. Therefore, more perspective and large-scale studies are needed on the development of algorithms using a combination of biomarkers to reduce unnecessary antibiotic use in the ICU setting.

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