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C-reactive protein: Unveiling its reliability as a sepsis biomarker

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Abstract

Background: The term "sepsis" is primarily used to distinguish between a microbiologically-based sickness and a similar clinical state that can occur in a number of non-microbial disorders. Traditionally blood cultures are used to aid in the diagnosis of patients with suspected sepsis secondary to either a fungemia or bacteremia. But accurate microbiological identification may be delayed due to the lengthy nature of conventional standard culture techniques. C reactive protein which is an acute phase protein synthesized by liver as an immunological response to any inflammation, injury or infection. Its level in blood may be regularly checked and used as a marker for the improvement or worse of inflammation and infection.

Aim and Objective: To study the C- reactive protein in Unveiling its reliability as a sepsis biomarker at a tertiary care centre.

Material and method: This was a Cross sectional study carried out in the Department of Microbiology for a period of 12 months (December 2022 to December 2023) at School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar pradesh. In the present study the serum samples were received in the plain vial or serum separating tubes and were subjected in the centrifuge for 15 minutes to separate serum from the whole blood and was further processed for the quantitative analyses of C-reactive protein using Ichroma II system.

Results: Out of the 120 blood samples, 70 were culture positive and 50 came out to be sterile. 71.42% had an elevated CRP level and 28.57% had CRP levels within normal range. It was observed that Positive culture isolation and elevated CRP levels were found to be significantly correlated with their respective wards with a p value of 0.002 ($p<0.005$ is considered significant).

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Keywords: C-reactive protein, Sepsis, Blood culture, Ichroma II system, level

Introduction

Presence of infection (probable or documented) along with systemic manifestations of infection is defined as sepsis.[1] It is one of the most serious and urgent events in the clinical practice. The term "sepsis" is primarily used to distinguish between a microbiologically-based sickness and a similar clinical state that can occur in a number of non-microbial disorders. Systemic inflammatory response is a variety of severe clinical insults including, Temperature >38°C or <36°C; heart rate > 90 beats per min; respiratory rate > 20 breaths per min or PaCO2 < 32 mmHg; and white blood cell count > 12,000/cu mm, <4000/cu mm, or >10% immature (band) forms. Sepsis is a systemic response to infection, manifested by two or more of the SIRS criteria as a result of infection.

Sepsis originates from a breach of integrity of the host barrier, either physical or immunological, and direct penetration of the pathogen into the bloodstream, creating the septic state.[2]

Traditionally blood cultures are used to aid in the diagnosis of patients with suspected sepsis secondary to either a fungemia or bacteremia. But accurate microbiological identification may be delayed due to the lengthy nature of conventional standard culture

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techniques. The work of creating sepsis biomarkers that may be used to track therapy responses and anticipate the diagnosis and prognosis of sepsis is still continuing.[3]

One such potential biomarker is C reactive protein which is an acute phase protein like procalcitonin, calprotectin, proadrenomedulin and pentraxin13.

CRP is synthesized by liver as an immunological response to any inflammation, injury or infection. It is possible to detect the blood's concentration of CRP, which might increase as soon as two hours after the triggering event and peak in 48 hours. When someone has sepsis, CRP is a sensitive measure. Its level in blood may be regularly checked and used as a marker for the improvement or worse of inflammation and infection.This study aimed to monitor the levels of C-Reactive protein as a potential biomarker in screening of sepsis and it comparison with blood culture positivity.[4]

Materials and methods

This was a 12 months (December 2022 to December 2023) Cross-sectional study, conducted in the Department of Microbiology, at School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar pradesh.

The study was approved by Institutional Ethics committee with Ref. No. SU/SMS&R/76-A/ 2024/13.

Samples

Quantitative analysis of CRP was done for the all the samples received for blood culture. The samples were subjected to centrifugation for 15 minutes to separate serum from the whole blood, after which they were processed for the quantitative analyses of C-reactive protein using Ichroma II system.

Ichroma II system

Ichroma CRP is a flourescence Immunoassay (FIA) that determines levels of CRP in human whole blood/serum/plasma quantitatively.

Ichroma CRP kit consists of cartridge and detection buffer. The cartridge contains membrane called test strip which has anti human CRP at the test line, and streptavidin at the control line. The detection buffer contains anti-human CRP fluorescence conjugate, Bovine Serum Albumin- biotin fluorescence conjugate, bovine serum

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albumin as a stabilizer and sodium azide in phosphate buffered saline as a preservative. The detection buffer is pre-dispensed in a tube.

Two drops of the test serum were subjected to the ichroma II specialized cartridge. The buffer vial was poked open after shaking well and the initial one drop was discarded. 2-3 drops of the remaining buffer was added to the cartridge followed by loading it into the Ichroma II machine.[5]

A recorded value greater than 10 mg/dL was considered as an elevated CRP level and value lower than that was considered negative according to the kit literature.

Results

In the present study a total of 120 blood cultures were included in the study, of which 70 (58%) were culture positive and 50 (42%) came out to be sterile (Fig 1). Quantitative analysis of CRP was done using the serum samples of the same patients. Among the 70 culture positive samples, 50 had an elevated CRP i.e, value higher than 10 mg/dL and rest 20 had CRP value lower than 10 mg/dL.

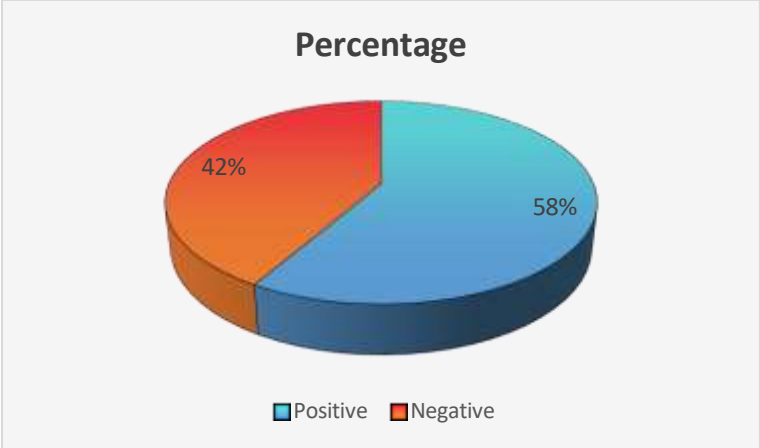

Fig 1: Percentage of Blood culture Positive and negative samples

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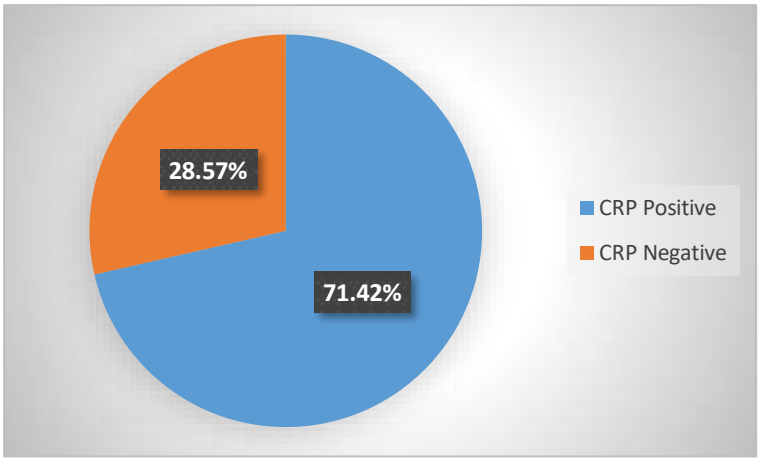

Fig 2: Percentage of CRP Positives and negatives among total 120 samples

Table 1: Number of samples received from different wards and the percentage.

Wards	Total no. of samples n (%)
NICU	28 (23.33)
MICU	30 (25.00%)
SICU	8 (6.66%)
RICU	5 (4.16%)
PICU	11 (9.16%)
ICCU	8 (6.66%)
Orthopedics	4 (3.33%)
Respiratory Medicine	4 (3.33%)
General Medicine	10 (8.33%)
General Surgery	8 (6.66%)
Pediatrics	4 (3.33%)

Positive culture isolation and elevated CRP levels were found to be significantly correlated with their respective wards (Table 1) with a p value of 0.002 ($p<0.05$ is considered significant).

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Among the total blood culture positives (n= 70), 46 were male and 24 were female. CRP was elevated 34 out of the 46 males and 12 had a normal CRP value.
Also among the 24 females, 16 had an elevated CRP level and 8 had normal value.

Table 2: Gender wise distribution of the CRP in blood culture positive samples.

Blood Culture Positive (n= 70)	CRP raised	CRP normal
Male	34	12
Female	16	8

Similarly, out of 50 blood culture negative patients, 29 were male and 21 were female. Among these 29 blood culture positive males, 10 had an elevated CRP values and 18 had a normal CRP value. And out of the 21 females, 9 had raised CRP and 13 had normal CRP values(Table 2 and 3).

Table 3: Gender wise distribution of the CRP levels in blood culture negative samples.

Blood Culture negative (n= 50)	CRP raised	CRP normal
Male	10	18
Female	9	13

No correlation between gender and the C-reactive protein levels in the serum of the patients was found as the *p* value came out to be 0.277 ($p<0.05$ is considered significant).

From 70 blood culture positive samples, gram-negative bacilli were grown in 40 (57.14%) samples. Gram-positive cocci in 27 (38.57%) and *Candida spp.* in 3 (4.28%) samples. (Fig 3)

Among the Gram-negative bacilli, *E. coli* was the most commonly isolated organism (20%) followed by *Klebsiella pneumoniae* (12.85%) and *Acinetobacter* spp. (10.00%). Other isolates included *Pseudomonas* spp., Salmonella Typhi and *Enterobacter* spp. Among the Gram-positive cocci, *Coagulase negative staphylococcus* spp. was the most common isolate (21.42%), followed by *Methicillin resistant staphylococcus aureus*

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(8.57%), *Methicillin sensitive staphylococcus aureus* (4.28%) and *Enterococcus* spp. (4.28%).

Also in total 70 isolates, 3 were found to be *Candida* spp. (4.28%). (Fig 4)

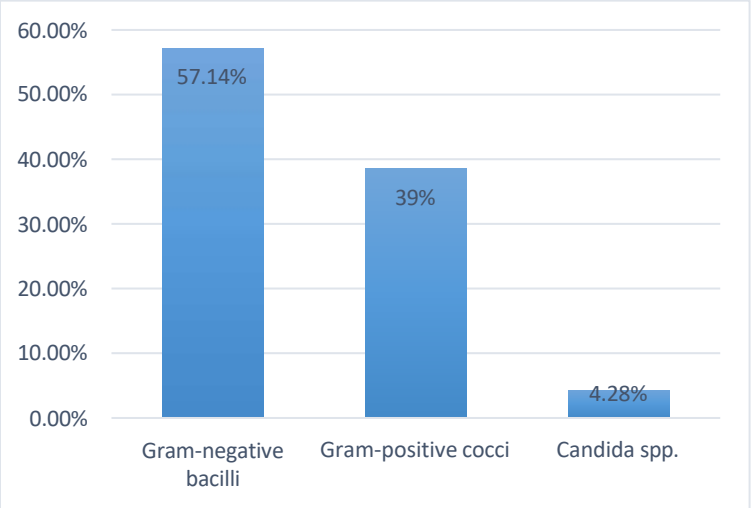

Fig 3: Distribution of positive blood culture isolates in the total study population.

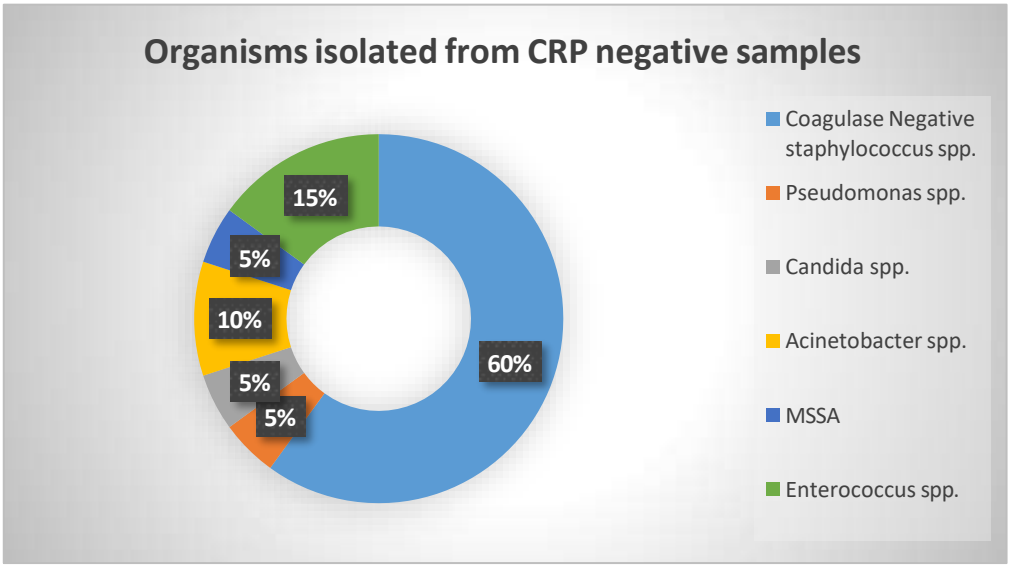

Fig 4: Chart showing organism distribution in the positive blood cultures which were CRP negative.

Among the organisms isolated from 20 CRP negative samples which were blood culture positive were *Coagulase negative staphylococcus* spp. Were the highest (60%),

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followed by *Enterococcus* spp. (15%), *Acinetobacter* spp.(10%), *Pseudomonas* spp. (5%), *Candida* spp. (5%) and *Methicillin sensitive staphylococcus* spp. (5%). (Fig 4).
Organisms wise correlation with the value of C-reactive proteins in patient's serum was found to be significant with a *p* value = 0.00 ($p<0.05$ is considered significant).
An overall association of the CRP value and sepsis in this study was found to be statistically significant (p=0.00, chi square test).

Discussion

This study was undertaken to examine a correlation, if any, between the blood culture proven sepsis and values of C- reactive protein in the serum samples. A total of 120 samples were included and their culture and CRP levels were analysed.
Seventy among the 120 samples came out to be culture positive out of which 50 had a raised CRP level and 20 had a normal value.
Organisms isolated from positive cultures which had a raised CRP value were *E.coli, Klebsiella pneumoniae, Acinetobacter, Psuedomonas,* S. Typhi *and Enterobacter.* Whereas among the 20 culture positives which had a normal CRP value, most frequently isolated organism were Coagulase-negative staphylococci. The normal CRP value in such samples could be indicative of CONS being the skin commensals and not a pathogen to have caused sepsis. A significant impact of the culture isolate on the CRP value was found in this study. A significant association between CRP levels and culture proven sepsis was found in the present study. However Levels of CRP in sepsis patients has no correlation with sex or age of the patients. Hence it is safe to state that CRP levels are not associated with the differences of hormones in males and females. Culture proven sepsis patients need intensive care due to the rapid regression of their medical state, which is why majority of the samples in this study were from different ICUs.
Devran et.al, in their study showed that CRP levels greater than 100 mg/dL on the third day in the ICU may be as useful of a predictor of mortality as high SOFA scores. [6]
Oliveira et al., in their study determined that CRP was as useful as PCT in reducing antibiotic use in septic patients, causing no apparent harm. In their investigation, a treatment based on serum CRP levels outperformed a PCT-based protocol in terms of lowering the usage of antibiotics. Surprisingly, the CRP group's antibiotic medication lasted for a shorter period of time than the maximum suggested duration.[7]

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C reactive protein (CRP) is a central component of the innate immune inflammatory response; by binding to the cell surface of dead or dying cells and some bacteria it leads to the activation of the complement system. The synthesis of CRP is mediated by factors released by macrophages and adipocytes [8-10]. CRP also leads to the promotion of pro-inflammatory cytokines, which increases the inflammatory response. CRP was elevated in patients with acute heart failure, and has been associated with the risk of developing coronary artery disease, head and neck squamous cell carcinoma mortality, and inflammatory bowel disease[11,12].

Despite the enormous investment in critical care resources, severe sepsis mortality ranges from 28% to 50% or greater. Moreover, cases of severe sepsis are expected to rise in the future for several reasons, including: Increased awareness and sensitivity for the diagnosis; increasing numbers of immunocompromised patients; wider use of invasive procedures; more resistant microorganisms; and an aging population [13-15].
Thus, C reactive protein has been identified as a promising biomarker that may provide added value to the clinical decision process, i.e. assist in diagnosis, assess prognosis, and assist in treatment selection and monitoring and should be included in diagnostic guidelines for sepsis and in clinical practice in intensive care units in our country.

Conclusion

Sepsis is a medical emergency; the treatment has to be started even on suspicion basis without waiting for the blood culture report. But sepsis screening should be done to get a proper prognosis and to make a good choice of antibiotics for treatment in the respective clinical case.
CRP is an economical test that is consistent and reproducible and available in most hospital settings.As such, quantitative analysis of C- reactive protein can be done before a blood culture report, which may take up to 5 days, is available. This measure will help in arriving at a reasonable prognosis about the patient.
CRP analysis can also be useful for critically ill patients as factor to monitor the course of disease. It can also be used as a valuable indicator to determine success or failure of the treatment. It can possibly spare patients from toxicity caused by drugs and decrease the risk of antimicrobial resistance.

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Declarations:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: We have consent to participate.

Consent for publication: We have consent for the publication of this paper.

Authors' contributions: All the authors equally contributed the work.

References

1. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med. 2013;41 Suppl 2:580–637.Lever A, Mackenzie I. Sepsis: definition, epidemiology, and diagnosis. BMJ. 2007; 335(7625):879-83.
2. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. *Crit Care Med* 2003; 31:1250-6.
3. F.R. Cockerill 3rd, J.W. Wilson, E.A. Vetter, et al.Optimal testing parameters for blood cultures.
4. Larsen FF, Petersen JA. Novel biomarkers for sepsis: A narrative review. *Eur J Intern Med.* 2017; 45:46–50.

5. https://www.boditech.co.kr/en

6. Devran O, Karakurt Z, Adiguzel N, Gungor G, Mocin OY, Balci MK, et al. C-reactive protein as a predictor of mortality in patients affected with severe sepsis in intensive care unit. Multidiscip Respir Med. 2012; 7

7. Oliveira CF, Botoni FA, Oliveira CR, Silva CB, Pereira HA, Serufo JC, et al. Procalcitonin versus C-reactive protein for guiding antibiotic therapy in sepsis: a randomized trial. Crit Care Med. 2013; 41: 2336–2343.

8. Naher BS, Mannan MA, Noor K, Shahiddullah M. Role of serum procalcitonin and C-Reactive Protein in the diagnosis of neonatal sepsis. *Bangladesh Med Res Counc Bull.* 2011; 37:40–46

9. Hong Zhang et al. Unveiling promising sepsis biomarkers: a clinical perspective. All life. 2024; 17(1).

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10. Lundberg OHM, Rosenqvist M, Bronton K, Schulte J, Friberg H, Melander O. Bioactive adrenomedullin in sepsis patients in the emergency department is associated with mortality, organ failure and admission to intensive care. . 2022; 17(4):e0267497.

11. Mahemuti N, Jing X, Zhang N, Liu C, Li C, Cui Z, Liu Y, Chen J. Association between systemic immunityinflammation index and hyperlipidemia: a populationbased study from the NHANES (2015-2020). Nutrients. 2023; 15(5):1177.

12. Scozzi D, Liao F, Krupnick AS, Kreisel D, Gelman AE.. The role of neutrophil extracellular traps in acute lung injury. Front Immunol. 13:953195. doi:10.3389/fimmu.2022; 953195.

13. Wang G, Lam WKJ, Ling L, Ma M-JL, Ramakrishnan S, Chan DCT, Lee W-S, Cheng SH, Chan RWY, Yu SCY, et al. Fragment ends of circulating microbial DNA as signatures for pathogen detection in sepsis. Clin Chem. 2023; 69(2):189–201.

14. Xu W-J, Wu Q, He W-N, Wang S, Zhao Y-L, Huang J-X, Yan X-S, Jiang R. Interleukin-6 and pulmonary hypertension: from physiopathology to therapy. Front Immunol. 2023; 14:1181987.

15. A, Ling L, Qin H, Arabi YM, Myatra SN, Egi M, Kim JH, Nor MBM, Son DN, Fang W-F, et al. Prognostic evaluation of quick sequential organ failure assessment score in ICU patients with sepsis across different income setting Crit Care. 2024; 28(1):30.