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Significance of Interleukin-6, Interleukin-8, Procalcitonin and C-reactive protein as early diagnostic markers of sepsis

Immunology

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ABSTRACT

Background: Sepsis is a leading cause of death among critically ill patients in Intensive Care Unit (ICU). Rapid diagnosis and timely initiation of effective therapy are the major challenges. Interleukin-6 (IL-6), Interleukin- 8 (IL-8), Procalcitonin (PCT) and C reactive protein (CRP) are the informative laboratory markers in this regard.

Objectives: To determine serum levels of IL-6, IL-8, PCT and CRP in patients with sepsis and to see the relation of these markers with various stages of sepsis, also to determine the diagnostic performance of these markers in patients with sepsis.

Methods: In this prospective cross sectional study, a total of 80 subjects was enrolled. 60 of them were patients with at least 2 systemic inflammatory response syndrome (SIRS) criteria and 20 age matched people without SIRS criteria. All patients had been selected from the ICU of BIRDEM General Hospital with known clinicopathological parameters. Serum levels of IL-6, IL-8, and PCT were assessed using Emzyme Linked Immunosorbent assay (ELISA) method.

Results: Significant difference was found in IL-6, IL-8 and PCT values in the patients with bacteriological culture positive and negative group ($p<0.05$), but CRP could not draw any differences. There was increasing trend of serum IL-6 and PCT with the developing stages of sepsis. In diagnostic performance test, PCT yields highest diagnostic value than IL-6 and receiver operating characteristics (ROC) curve shows area under the curve (AUC) for PCT was 0.785 (95% CI; 0.654-0.915), sensitivity 89.47%, specificity 50%, positive predictive value (PPV) 75.55% and negative predictive value (NPV) 73.33% with cutoff value >753pg/ml.

Conclusion: Our study revealed elevated levels of serum IL-6, IL-8 and PCT in the patients with sepsis. Therefore, combinatorial use of these biomarkers may serve a potential role in early diagnosis of sepsis. However, multi-centric prospective study on large cohort may be undertaken to evaluate the exact influence.

KEYWORDS:

Interleukin – 6; Interleukin – 8; Procalcitonin; C-reactive protein; Systemic inflammatory response syndrome (SIRS); Sepsis; Intensive care unit (ICU).

Introduction:

The term 'sepsis' is used to define the systemic inflammatory response syndrome (SIRS) to an infectious agent. It is complicated to differentiate sepsis from other noninfectious conditions in critically ill patients admitted with systemic inflammatory response syndrome. Though we have new treatment modalities, advanced laboratory technique and clinical experience, mortality rates in sepsis are still higher [1]. Hospital mortality in ICU patients of Bangladesh suffering from severe sepsis was 49·2% which is very higher than other country [2]. The critically ill patient frequently presents with similar clinical pictures in infection, SIRS, various severities of sepsis and organ dysfunction. This issue is of paramount importance given that therapies and outcomes greatly differ between patients with and those without sepsis. Thus, there is an unmet need for laboratory tools to determine SIRS and the various forms of sepsis early.

The incidence of sepsis and the number of sepsis-related deaths are increasing day by day and among hospitalized patients by 8.7% per year [3]. Early diagnosis and appropriate therapy of sepsis is a daily challenge in intensive care units (ICU). An international prospective cohort study done among patients admitted to the 28 participating units in eight countries between May 1997 and May 1998 were followed until hospital discharge and overall 3034 infectious episodes (incidence: 21.1%) were recorded at ICU admission [4]. In a cross-sectional point-prevalence study done over 454 ICUs in Germany in 2003 prevalence was 12.4% (95% CI, 10.9–13.8%) for sepsis and 11.0% (95% CI, 9.7–12.2%) for severe sepsis including septic shock [5]. Despite the enormous investment in critical care resources, sepsis mortality ranges from 25% to 80% [6]. Moreover, cases of severe sepsis are expected to rise for several reasons, including: increasing numbers of immunocompromised patients and aged population; wider use of invasive procedures; more resistant microorganisms. In point prevalence study 32.8% of 895 patients in ICUs had sepsis on a single day [7].

Physicians use various clinical and laboratory data to differentiate infectious from noninfectious conditions in newly admitted patients. But no single clinical or biological indicator of sepsis has gained unanimous acceptance. Though blood culture is the gold standard and has higher sensitivity and specificity over the hematological value and cytokine levels but this highly sensitive microbiological parameter is not available in our community health situation and it is time consuming also. In these cases, several indicators have been proposed as new diagnostic tests to assess various stages of sepsis in critically ill patients.

Several attempts have been made to correlate cytokine levels with sepsis and patient prognosis. Among the cytokines, IL-6 and IL-8 is important mediator of host response to bacterial infection and has important role in inflammatory pathogenesis. These cytokines also stimulate release of acute-phase reactants such as CRP and PCT. These cytokines and acute phase proteins have been proposed to be the most promising candidates for diagnosis of sepsis in early stage. Few studies abroad showed combinatorial use of these markers as early diagnosis and prognosis of sepsis in ICU patients. In view of these conflicting findings and the utmost importance of the timely diagnosis of sepsis at time of admission in ICU, the present study was undertaken to prospectively investigate the diagnostic value of PCT, CRP, IL-6, and IL-8 in a group of severely ill Bangladeshi patients admitted with sign of systemic inflammatory response syndrome in ICU.

Materials and Methods:

This prospective cross sectional study was conducted at the Department of Immunology in collaboration with Intensive Care Unit, BIRDEM General Hospital, Dhaka for a period of one year from 1st January 2015 to 31st December 2015. The study includes 80 subjects and random sampling done. Here 60 patients were randomly chosen with at least 2 SIRS criteria and 20 age matched randomly selected subjects without SIRS criteria taken from same hospital outpatient department. All of them were above 18 years and after taking full medical history all of them examined properly and all medical data were reviewed. Selected patients under study were diagnosed as

various stages of sepsis patient with different age group. Basic haematological, biochemical, microbiological laboratory data were recorded from laboratory report. IL-6 (DRG Inc International, USA), IL-8 (DRG Inc International, USA) and PCT (Bio-Vendor, Germany) was measured by sandwich enzyme immunoassay. All patients were classified into SIRS, sepsis, severe sepsis, septic shock and MODS according to ACCP/SCCM definition which is now widely used.

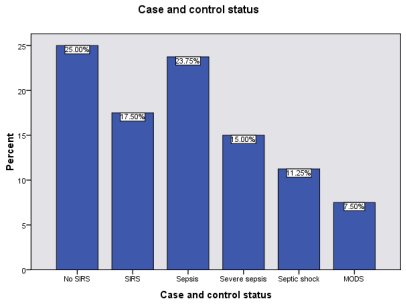
All data was checked and edited after collection. From the primary data obtained, tables and graph were made and interpreted. Levels of IL-6, IL-8, PCT and CRP were expressed as the means ± SD or median ± inter quartile range. A 'p' value of <0.05 was considered to be statistically significant. A nonparametric levene's test was used to verify the equality of variance in the samples data. The quantitative variables were analyzed by Mann Whitney U test. Data was applied in the SPSS version 16 for statistical analysis. Their diagnostic utilities were compared using ROC curves.

Results:

A total of 80 subjects were include in the study, 60 of them were patients with at least 2 SIRS criteria and their mean age was 51.90±9.89 years and 20 healthy subjects as controls with mean age of 49.65±9.58 years. Among study population male were 51.2% (41) and female were 48.8% (39). Commonest age range in both groups was 51-60 years (40%).

All of the patients diagnosed clinically at various stages of sepsis. But SIRS criteria may appear in non-inflammatory condition and without bacterial infection also. The gold standard method to prove sepsis is positive bacteriological culture. In this study 38 (63.33%) patients were bacteriological culture positive and 22 (36.67%) patients were bacteriological culture negative. In this study, 20 (25%) healthy individual were taken as control (No SIRS) and 60 (75%) patients were categorized into SIRS, sepsis, severe sepsis, septic shock and MODS according to ACCP/SCCM guideline (figure-1).

Figure 1: Healthy control and staging of sepsis according to ACCP guideline.*The difference of IL-6, IL-8, PCT and CRP values between the patients and control:*



The sample data were not approximately normally distributed. A nonparametric levene's test was used to verify the equality of variance in the samples data (p<0.05) which confirmed the non-homogeneity of the distribution. Because of the nonhomogeneity, nonparametric Mann-Whitney U test was used to see the difference between the groups. On Mann-Whitney U test, statistically significant difference was found in between the IL-6, IL-8, PCT and CRP values in the study subject and control population where p<0.05 in all four biomarkers (table-1).

Table 1: Significance of serum IL-6, IL-8, PCT and CRP values in patient and control

Biomarkers	Study group	Median	Interquartile range	P Sig. (2-tailed)
Il6 level (pg/ml)	Case	202.55	957.93	.000
	Control	4.43	8.03	
IL-8 level (pg/ml)	Case	29.31	168.10	.002
	Control	16.34	15.39	
PCT level (pg/ml)	Case	2873.26	3847.9	.000
	Control	22.5	32.22	
CRP level (mg/L)	Case	48	84	.010
	Control	6	20	

The difference of IL-6, IL-8, PCT and CRP values in between bacteriological culture positive and negative cases:

The sample data were also not approximately normally distributed in bacteriological culture positive and negative patients. Because of no homogeneity, nonparametric Mann-Whitney U test was used to see the difference between the groups. On Mann-Whitney U test, statistically significant difference was found in the IL-6, IL-8 and PCT values in the bacteriological culture positive and negative group (p<0.05), but there was no significant difference found in CRP values (p>0.05) (Table- 2).

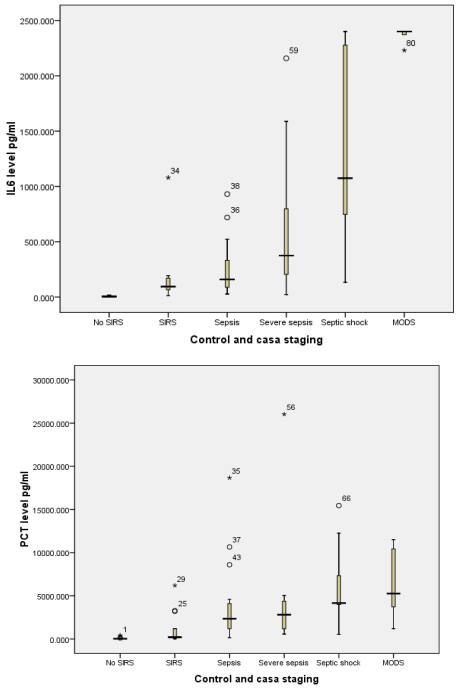
Table 2: Significance of serum IL-6, IL-8, PCT and CRP values in culture positive and negative cases

Bio markers	Group	Median	Interquartile range	P
Il6 level pg/ml	Positive	374.19	2027.84	0.007
	Negative	114.21	362.57	
IL-8 level pg/ml	Positive	43.71	289.07	0.021
	Negative	18.41	105.48	
PCT level pg/ml	Positive	3703.65	3195.69	0.000
	Negative	688.08	2219.11	
CRP level mg/L	Positive	48	84	0.712

Clinical observational relation of IL-6, IL-8, PCT and CRP with various stages of sepsis:

There was increasing trend of serum IL-6 and PCT with the developing stages of sepsis and control. Median and interquartile ranges of values for IL-6 was (No SIRS 6.5±13.95, SIRS 94.04±121.56, Sepsis 159.01±359.56, Severe sepsis 374.19±757.33, Septic shock 1074±1870.67, MODS 2400±64.35) and for PCT was (No SIRS 31.4±41.5, SIRS 208.5±1632.55, Sepsis 2347.07±3244.73, Severe sepsis 2810.4±3449.33, Septic shock 4162.33±6148.215, MODS 5262.3±4606.41) respectively (figure- 2). But IL-8 and CRP did not show any gradual increasing trend with progression of diseases.

Figure 2: Relation of IL-6 and PCT level with developing stages of sepsis



Performance of diagnostic tests of IL-6, IL-8, PCT and CRP in sepsis:

Receiver operating characteristics (ROC) curve of IL-6, IL-8, PCT and CRP was made according to the sensitivity and specificity of serum values of these markers using data from all study subjects (figure- 3). PCT showed highest accuracy and sensitivity to determine sepsis than other bio-markers (table-4).

Figure 3: Receiver operating characteristics curve (ROC) of serum biomarkers.

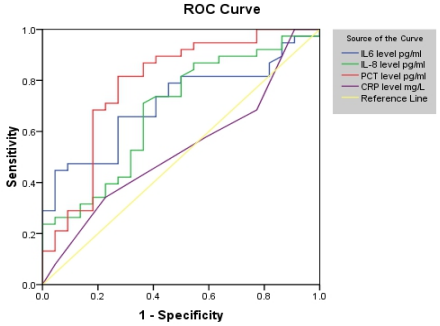


Table 3: Diagnostic performance of different sepsis indicator.

Validity test	IL-6	IL-8	PCT	CRP
Cutoff value	177pg/ml	20pg/ml	753 pg/ml	12 mg/L
Sensitivity	54.16%	76%	89.47%	68.42%
Specificity	59.09%	50%	50%	77.27%
PPV	74.28%	72.5%	75.55%	83.87%
NPV	52%	55%	73.33%	58.62%
Accuracy	65%	66.66%	75%	71.66%
AUC	.710	.681	.785	.528

Discussion:

In this study our results indicate that all biomarkers made difference between healthy individual and patient. But significant difference was found in IL-6, IL-8 and PCT values in the patients with bacteriological culture positive and negative group. IL-6 and PCT proved to be best indicator of sepsis and PCT yielded the highest discriminative value than others in diagnostic performance test.

Rapid diagnosis and timely initiation of effective therapy of sepsis is a daily challenge in the ICU. Identification of sepsis has a major impact on the clinical course, management, and outcome of critically ill patients. There is no multicenter based study in our country to determine incidence of sepsis in our country. But in a prospective cross sectional study done in BIRDEM General Hospital ICU, 95 (41%) sepsis patients isolated within 228 patients [9].

In this study total of 80 subjects were enrolled, 60 of them were patients with at least 2 SIRS criteria, their mean age was 51.90±9.89 years and 20 healthy controls were with mean age of 49.65±9.58 years. The commonest age range was between 51 to 60 years in both patients and healthy control. Among study population male were 51.2% (41) and female were 48.8% (39). Clinically 23.75% patients diagnosed with sepsis following severe sepsis (15%), septic shock (11.25%) and multiple organ dysfunctions (7.5%). In a prospective cross sectional study in Bangladesh within BIRDEM General Hospital ICU showed 41% of all admitted patients were sufferings from sepsis, mean age of patient suspecting sepsis in ICU were 59.56 ±14.3 years and commonest age range was 61-70 (28%) [9]. It's indicated that elderly populations were at risk group to develop sepsis and may be more prone to developed sepsis.

Inflammation or infection leads to the activation of the inflammatory cascade and various cells are able to produce a multitude of pro-inflammatory cytokines such as IL-6, IL-8, CRP and PCT. IL-6, CRP and PCT can draw statistically significant differences between sepsis group and non-sepsis group [12]. IL-8 concentration in patients with septic syndrome was significantly higher than in controls [13]. In our study statistically significant difference was found between the IL-6, IL-8, PCT and CRP values in the study subject and healthy control group amongst all four biomarkers ($p<0.05$).

But systemic inflammatory response syndrome may appear in non-inflammatory condition and without bacterial infection also. Systemic response to infection is known as sepsis and proceeds to severe sepsis, shock and organ dysfunction if untreated, so early diagnosis of sepsis is very important to make effective decision about treatment plan. Bacteriological culture is the gold standard method to isolate infection. But early diagnosis of sepsis can be difficult because positive bacteriological samples may be late or absent, the clinical interpretation of infections may be ambiguous, and traditional markers of infection may be nonspecific. So if any biomarker can predict bacterial infection in earlier phase of sepsis in the patient with inflammatory response that will help in treatment procedure and minimize progression of diseases.

In our study IL-6, IL-8 and PCT values are statistically significant to predict bacterial infection and draw difference between patients with and without bacterial infection. Luzzani and his colleagues done a prospective study over ICU patients and found PCT is better marker than CRP to diagnosis sepsis [14]. PCT also showed better and closer correlation than that of CRP with the severity of sepsis and organ dysfunction and rise in sepsis-related organ failure assessment score was related to a higher median value of PCT [15]. Klaus and his colleagues identify PCT and IL-6 is better than CRP to predict survival rate in patients with severe sepsis [16]. PCT is valuable for the early diagnosis of bloodstream infection and concentration in patients with Gm (-ve) bacterial bloodstream infection were significantly higher than those of Gm (+ve) bacterial bloodstream infection group [17]. A single-centre prospective follow-up study proved that PCT and IL-6 superior to CRP in detecting patients with severe sepsis and PCT and IL-6 is significant independent predictors to diagnose sepsis [18].

Zhao and Li did a prospective study in Beijing on the patients with SIRS to determine use combination of biomarkers including IL 6, PCT, and CRP for diagnosis of sepsis and severe sepsis [19]. They found PCT and IL-6 were independent factors for diagnosis of sepsis and severe sepsis and enhances the diagnostic ability. In our study, PCT yields highest diagnostic value than IL-6 and ROC curve shows AUC for PCT was 0.785 (95% CI; 0.654-0.915), sensitivity 89.47%, specificity 50%, PPV 75.55% and NPV 73.33% with the best cut-off value >753pg/ml. PCT emerged as the best marker for sepsis, but the difference in AUC was not significant between PCT and IL-6 and both proved superior to CRP in detecting patients with severe sepsis. The findings of our study support the use of PCT and IL-6 as early tool to diagnose sepsis. IL-6, IL-8 and CRP are closely related cytokines and produced during inflammation in cascade induction process. In our study, IL-6 showed positive significant correlation with IL-8 and CRP. There was no significant relation between PCT and other markers [13].

In our study serum IL-6 and PCT shows increasing trend with the developing stages of sepsis and control (figure-2). In another study, median PCT serum levels and interquartile ranges were 0.6 (0 to 5.3), 3.5 (0.4 to 6.7), 6.2 (2.2 to 85), 21.3 (1.2 to 645) ng/ml in patients with SIRS, sepsis, severe sepsis and septic shock respectively [15]. Therefore, IL6- and PCT appeared to be helpful in differentiating patients with various stages of sepsis from those with SIRS.

Conclusion:

It is highly unlikely that a single parameter will ever be able to diagnosis sepsis in critically ill patients. Rapid diagnosis and timely initiation of effective therapy mainly depends on appropriate clinical observation along with suitable and useful laboratory tools. Our results suggest that serum IL-6, IL-8 and PCT may be an early diagnostic marker of sepsis than other traditional markers. Combinatorial use of these biomarkers will help in early diagnosis and also greatly improve outcome. These will help clinicians to make their decision in diagnosis of sepsis along with other systemic manifestation. However more prospective, large scale and multicenter based studies are required to validate the role of IL-6, IL-8 and PCT in depth as diagnostic markers of sepsis.

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