International Journal of Medical Science and Dental Research

Comparison of Serum Markers Procalcitonin, IL-6, IL-8 and CRP in Diagnosing Cases of Sepsis.

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Abstract

Introduction: Availability of diagnostic assays is highly variable in India, making diagnosis of sepsis even more problematic. Though many biomarkers are valuable for diagnosis, recently few of the biomarkers like Procalcitonin, IL-6, IL-8 and CRP have been found to be useful in the diagnosis and prognosis of sepsis.

Materials and methods: Ours was a prospective study which included 60 consecutive patients admitted with a suspected diagnosis of infection and hospitalized in the ICU which fulfilled at least two of the 4 criteria of SIRS. Samples for Hematology, Cultures and Sepsis biomarkers (Procalcitonin, IL6, IL8 and CRP) were collected at admission, 24 and 48 hrs.

Results: Blood cultures were positive in 19 (31.66%) of the total patients enrolled in the study. The cut off level of Procalcitonin was 4.45 ng/ml (Sensitivity 93%, Specificity 81%); CRP was 26 mg/dl (Sensitivity 83.6%, Specificity 91%); IL6 was 132.5 pg/ml (Sensitivity 84.5%, Specificity 72%) and IL8 was 113 pg/ml (Sensitivity 83%, Specificity 67.5%).

Conclusion: We concluded that using Procalcitonin (cut off of 4.45 ng/ml) is the single biomarker which can be used to diagnose sepsis (AUROC 0.904 to 0.913).

Keywords: Biomarkers, Cytokines, cultures, sensitivity, specificity and SIRS.

I.   Introduction:

In the patients admitted with critical illness, bacterial infections and sepsis are common problems; both as a cause of admission in intensive care units and healthcare-associated infection subsequent to admission. Now it is unequivocally accepted that initiating effective antibiotic therapy initially in the course of illness decreases the morbidity and mortality in these group of patients [1]. But the use of antibiotics is a double-edged sword as on one hand it reduces the complications and length of stay; while on the other hand indiscriminate use may lead to the ever-increasing challenge and burden of Antibiotic resistance.

India is a developing nation with one of the highest infectious disease loads in the world [2]. Availability of diagnostic assays is variable in India [3], making diagnosis of sepsis even more problematic. Recently, many biomarkers have been evaluated as a marker for sepsis. Several studies evaluating various biomarkers as an adjunct to clinical features in detecting sepsis have been done, though most of these studies have been conducted in the developed world. Recently few of the biomarkers like Procalcitonin (PCT), IL-6, IL-8 and CRP have been found to be useful in the diagnosis and prognosis of sepsis. In this study we compared serum markers Procalcitonin, IL-6, IL-8 and CRP to detect the best diagnostic and prognostic marker in cases of sepsis.

II.   Materials and Methods

This study was conducted in a zonal level army hospital in North India, a 700 bedded army hospital. Sixty patients who presented to ICU were selected. The study was conducted from Aug 2014 to Feb 2017. The patients who fulfilled two of the four criteria of SIRS {fever (oral temperature >38°C) or hypothermia (<36°C); tachypnea (>24 breaths/min); tachycardia (heart rate >90 beats/min); leukocytosis (>12,000/L), leukopenia (<4,000/L), or >10% bands} along with evidence of infection or clinical suspicion of bacterial infection were included in our study. The patients who were admitted to the ICU without any sign of clinical infection or not fulfilling 2 of the 4 criteria of Sepsis were excluded.

Samples were then drawn from patients for Complete Blood Count (CBC), Peripheral smear, blood culture and urine culture. Serum samples for procalcitonin, IL-6, IL-8 and CRP were collected within 6 hrs of admission, at 24 and 48 hrs respectively. These samples were evaluated by Quantitaive Enzyme Linked Immuno Sorbent Assay (ELISA). The data was analysed by the Statistical Package for the Social Sciences software package (version 17).

Descriptive statistics were used to analyse the following:

1. Calculation of cut off values for different markers.
2. ROC curve preparation. The more the area under the curve the better is the diagnostic accuracy of the said test.
3. Calculation of mean and SD for various markers of sepsis.
4. Youden's index (sensitivity+ specificity -1) calculation.

III.   Results

During the study period, a total of 60 patients were recruited into the study. Of these, 40 (66.7%) were males and 20 (33.3%) were females. Most of the patients were more than 50 years of age, with the mean age being 54.7 years. Area under ROC calculation as shown in Table 1. Age and sex distribution is depicted in the tables and figures below (Table 2, Table 3 and Figure1):

Table 1: Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
procal1st	.904	.039	.000	.828	.980
procal24	.913	.037	.000	.841	.986
procal48	.905	.039	.000	.829	.981
crp1st	.899	.047	.000	.807	.990
crp24	.876	.059	.000	.761	.990
crp48	.905	.036	.000	.854	.965
il61st	.809	.058	.001	.696	.921
il624	.736	.078	.010	.583	.888
il648	.838	.050	.000	.739	.937
il81st	.750	.074	.006	.604	.895
il824	.753	.065	.006	.626	.879
il848	.753	.066	.006	.624	.882

Table 2:- Sex distribution of the patients

Sex	Frequency	Percentage
Female	20	33.3%
Male	40	66.7%
Total	60	100.0%

Comparison of serum markers procalcitonin, IL-6, IL-8 and CRP in predicting prognosis in cases of sepsis.

Table 3:- Age distribution of the patients

Age (years)	Numbers
11-20	01
21-30	05
31-40	09
41-50	07
51-60	16
61-70	14
71-80	06
81-90	02

Figure 1:- Sex distribution of the patients

Profile of the markers of sepsis

Procalcitonin: The mean levels of Procalcitonin at the time of admission was 20.55 ng/ml , while at 24 and 48 hrs were 22.51 and 20.90 ng/ml respectively. Thus, procalcitonin showed a trend with a peak at 24 hrs and remaining static to slight decline at 48 hrs. The cut off for Procalcitonin was 6, 5.9 and 4.45 ng/ml respectively at the time of admission, 24 and 48 hrs. The area under the curve was maximum at 24 hrs(Figure 2, Figure 3).
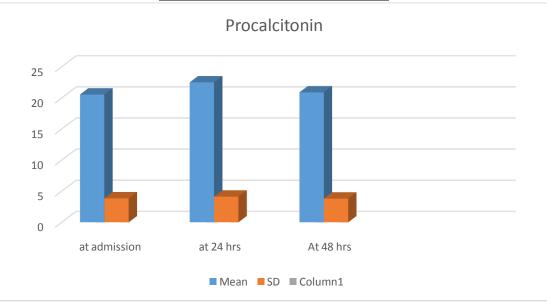
Figure 2: Mean and SD for Procacitonin

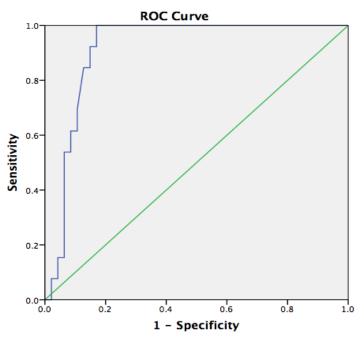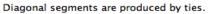*Comparison of serum markers procalcitonin, IL-6, IL-8 and CRP in predicting prognosis in cases of sepsis.*

Figure 3: Procalcitonin at 24 hrs

Diagonal segments are produced by ties.

C Reactive Protein: The mean values of CRP were 112.33, 129.64 and 129.24 mg/dl respectively at the time of admission, at 24 and 48 hrs. The AUROC for CRP varied from .899 to .905 with the maximum area shown at 48 hrs (Table 4, Figure 4).

Table 4: Mean, SD for CRP

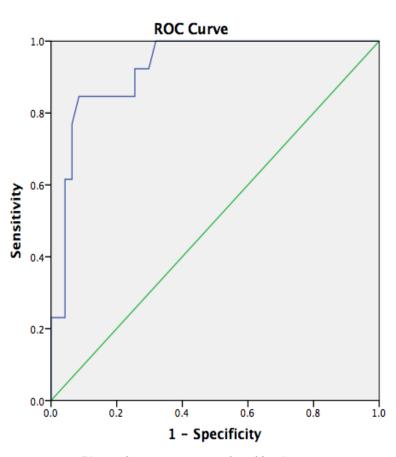|	At admission	At 24 hrs	At 48 hrs
Mean	112.33	129.64	129.24
SD	7.35	8.38	10.75

Figure 4: CRP at 48 hrs

Diagonal segments are produced by ties.

Interleukin 6: The mean levels of IL-6 were 146.5, 152.3 and 144.55 pg/ml respectively at the time of admission, at 24 hrs and at 48 hrs; demonstrating a peak at 24 hrs. The AUROC varied from 0.736 to 0.838 at the different intervals. The cut off for IL-6 was 137.5, 132.5 and 156.5 pg/ml at the time of admission, at 24 and 48 hrs respectively.

Interleukin -8: The mean for IL-8 were 152.85, 162.08 and 169.69 pg/ml respectively at the time of admission, at 24 hrs and at 48 hrs. The cut-off for IL-8 were 113, 201 and 142.5 pg/ml at the time of admission, at 24 and 48 hrs respectively.

Our study found that the highest sensitivity was for Procalcitonin (93%) while the highest specificity was seen in CRP (91%). The following table shows sensitivity, specificity and Positive Predictive Value (PPV) of each biomarker evaluated in our study (Table 5):

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Table 5: Sensitivity, specificity and PPV of Procalcitonin, IL6, IL8 and CRP in Sepsis

	Sensitivity	Specificity	PPV
Procalcitonin	93%	81%	0.92
IL-6	84.5%	72%	0.78
IL-8	83%	67.5%	0.76
CRP	83.6%	91%	0.77

IV.   Discussion

This part of the study was an observational study done in 60 patients who were admitted in ICU with clinical diagnosis of sepsis and who fulfilled two out of the four criteria for SIRS. Most of the patients were more than 50 years of age, with the mean age being 54.7 years.Leukocytosis was consistent to all the patients in the study. However, blood cultures were positive in 19 (31.66%) of the total patients enrolled in the study. Among these Gram-positive bacteria constituted 31.57 % and Gram-negative bacteria were responsible in 68.43 % of the cases.

In one study termed ANZICS, Gram positive infection constituted 48.3% and Gram-negative 38.5% of all infections [4]. An epidemiologic study of sepsis in the period from 1979 to 2000 revealed that Gram positive infections have overtaken Gram-negative cases [5]. Though in a more recent study which involved 14,000 ICU patients in 75 nations, Gram negative bacteria were found in 62% of patients with severe sepsis who had positive cultures, Gram positive bacteria in 47% and fungi in 19% [6].

Procalcitonin in sepsis: The PCT levels start to rise in approximately 2 to 4 hrs after the inception of sepsis, with a peak at 24 to 48 hrs after the onset of sepsis. Many studies have established the fact that survival rate of patients with sepsis can be markedly improved if Antibiotics are initiated instantaneously using the correct antibiotics [1]. Many meta-analyses have been done assessing PCT as a marker in sepsis [7], [8], [9], [10]. Though the previous meta-analyses had contradictory results and were limited by various patients studied and sepsis classifications, the latest meta-analysis [10] which evaluated 30 studies with 3244 patients produced a sensitivity of 77% (95% confidence interval (CI): 72–81%) and specificity of 79% (CI: 74–84%) indicating that it was a valuable biomarker for identification of early sepsis. Our study showed a cut off level of Procalcitonin to be 4.45 ng/ml. In a study by Sinha et al, PCT above 10 ng/ml was observed in 12 patients; 2-10 ng/ml, in 7 patients; 0.5-2 ng/ml, in 3 patients; and <0.5 ng/ml, in 18 patients. This study showed that the PCTassayhad amoderatesensitivity (86%)andhighspecificity(95%)atacut-off≥2ng/ ml [11].

A single CRP concentration obtained early in the course of illness has a low sensitivity between 35% and 96% in identifying the presence or absence of infection as the sampling time may herald a measurable increase in CRP levels; this upsurge may lag 12 to 24 hours after the beginning of symptoms [12], [13], [14], [15]. In another study by Shaaban *et al.* revealed that CRP (cut-off=70 mg/L) and PCT (cut-off=1.5 ng/mL) outdid eosinopenia as a marker of sepsis in a critical care unit (negative predictive value=94%, 87% and 80%, respectively) in North America [16].

IL-6 and IL-8 in sepsis:

The normal values of IL-6 serum concentration is less than 5.9 pg/mL. After inflammation sets in, serum levels of IL-6 have been demonstrated to rise within a hour, prior to CRP levels and even before the onset of fever. IL-6 values greater than 500 pg/mL were seen in patients with sepsis [17].

IL-8 has been shown to have chemoattractant activity [18], [19], [20] and is able to trigger neutrophils and leading to their degranulation [19], [21]. Also, it inhibits adhesion of neutrophils to the endothelial cells [22], although augmentation of adhesion has been defined too [23]. In vitro IL-8 is synthesised by several cells, including macrophages, monocytes and endothelial cells [24],[25].

Erik Hack et al showed that the median IL8 in patients of sepsis (survivors) was 271 pg/ml while in non-survivors was 379 pg/ml [26]. Danner et al. found that in patients with septic shock IL-8 levels on admission were lower in non-survivors than in survivors [27]. Out of the 60 patients enrolled in our study, only 2 patients succumbed to their illness within 48 hrs of observation. In these two patients, the levels of IL-8 were considerably higher that the survivors (365 vs 189 pg/ml).

V.   Conclusions

We concluded that Procalcitonin (cut off of 4.45 ng/ml) as the single biomarker can be used to diagnose sepsis (AUROC 0.904 to 0.913). This was higher than other studies which revelaed cut off levels of approx 2 ng/ml. Further studies are required using a larger sample size possibly multicentric studies, with longer folllow up to assess the role of these biomarkers.

Conflicts of interest: None.

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