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Evaluation of C-Reactive Protein and Procalcitonin in Sepsis

Medical Science

KEYWORDS : Procalcitonin, C-Reactive protein, sequential quantitative estimation, sepsis

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ABSTRACT

Early diagnosis and appropriate therapy of sepsis is a daily challenge in the emergency room and in intensive care unit. In addition non infectious clinical syndromes can exhibit sepsis like symptoms, thereby making a diagnosis of sepsis more difficult . Today various therapeutic strategies are known to improve survival in patients with sepsis, therefore rapid and accurate diagnosis is essential . In sepsis there is lack of specific clinical symptoms and signs. Microbiological cultures require time , do not reflect the host response of systemic inflammation and may not be positive in sepsis patients. For a number of reasons even if cultures are positive , it is not enough evidence to discriminate infection and colonization. Procalcitonin and CRP are valuable markers for the diagnosis and prognosis of sepsis. Sequential quantitative estimation and comparison of CRP and Procalcitonin in serum by ELISA method in 30 patients suspected to be suffering from septicaemia with out any history of parenteral antibiotics were included in the study. Serum Procalcitonin and CRP levels were estimated sequentially i.e. on day of admission , after 48 hours and on fourth day , as sequential measurement have greater diagnostic impact than single measurement. The overall corelation between Procalcitonin and CRP is 83.3%. Thus both serological markers are complimentary to each other. Sequential testing of procalcitonin and CRP are helpful in diagnosis and prognosis of sepsis.

INTRODUCTION:

Procalcitonin (PCT) and C-reactive protein (CRP) are biomarkers that have been studied for their ability to identify bacterial infections. There are several types of cells and organs which produce Procalcitonin. The various substances which induce Procalcitonin production are bacterial endotoxins and pro-inflammatory cytokines. Thus Procalcitonin is helpful in the diagnosis of sepsis and clinical conditions with systemic inflammatory response. In Europe Procalcitonin is used as an important tool in the diagnosis of sepsis. In healthy individuals the Procalcitonin levels are <0.05ng/ml. Procalcitonin level of <0.5ng/ml denote strong suspicion for sepsis, levels 0.5-2ng/ml denote uncertain sepsis and levels ≥ 2ng/ml denote sepsis confirmed. In other conditions such as viral infection, auto immune diseases etc, the procalcitonin levels are < 0.5ng/ml. Procalcitonin levels return to normal levels rapidly and thus it is help full in prognosis. Sequential estimation is helpful in diagnosis and in progonosis[1].

C-reactive protein is an important Acute Phase Protein. Cytokines induce the production of C-reactive protein by hepatocytes. C-reactive protein gene is present on chromosome no.1. C-reactive protein concentration is highest in patients with severe bacterial infections and C-reactive protein levels are also elevated in auto immune diseases[2]. In healthy individuals the mean C-reactive protein serum levels is <1mg/lit. C-reactive protein release may require 6-12 hours. Sequential C-reactive protein measurements have greater diagnostic impact than single measurement and also improves prognostic value of this serological parameter. [2, 3]

OBJECTIVES:

Objective of this study is Sequential Quantitative estimation and comparison of C-reactive protein and Procalcitonin in serum by ELISA method in patients suspected to be suffering from septicemia.

Emphasise the clinical applicability of C-reactive protein and Procalcitonin measurements in patients with septicemia.

Use of C-reactive protein and Procalcitonin in discriminating septicemia from other inflammatory causes of fever, thus prevention of antibiotic abuse and ultimate development of drug resistance.

MATERIAL AND METHODS:

In total 33 adults patients were included in this study. This study was done in the Microbiology Department, Kamineni Institute of Medical Sciences, Narketpally from June 2014 - June 2015. All the patients with signs and symptoms of septic shock, without any history of parenteral antibiotics were included in this study. Samples were collected within 24hours of hospital admission. Three patients were excluded from the study as the test sample was insufficient. Thus final total number of cases included in the study are 30.

The age of the patients varied from 11years to 80years with mean age of 40.16. Of these, 15(50%) were males and 15(50%) were females. Nearly all the patients in the study had rural background and agriculture as their main occupation.

Sucilathangam Get al conducted study to determine the diagnostic performance of Procalcitonin and C-reactive protein as early diagnostic markers in detection of neonatal sepsis. Authors had drawn the inference that a procalcitonin level of ≥0.5ng/ml was considered pathological. Procalcitonin levels of 0.5-2ng/ml, 2-10ng/ml and more than 10ng/ml were considered as weakly positive, positive and strongly positive respectively. The sensitivity of C-reactive protein for predicting sepsis was 6mg/lit[3].

Kinetics of Procalcitonin follow similar pattern in children and adults[5]

COLLECTION OF SAMPLES:-
Blood sample was collected after observing aseptic precaution.

Samples were collected on the day of admission, after 48hours and on fourth day in 21 patients. In 9 patients we could not collect sample on 4th day due to administrative reasons that is they took discharge against medical advice. Sequential estimation of serum concentration of C-reactive protein and Procalcitonin is more significant.

All the samples were properly labelled and clinical history recorded.

Sample was allowed to clot at room temperature and serum was separated and preserved at a temperature -20°C.

Before processing, all the serum samples were thawed. Repeated freezing and thawing cycles were avoided.

For quantitative determination of C-reactive protein and Procalcitonin, serum samples were processed by ELISA (Enzyme-linked immunosorbent assay) method.

Measurement of Biomarkers:
Procalcitonin and CRP were measured in serum samples using solid phase direct sandwich ELISA method according to the manufacturer's instructions (Sincer Biotech, China and Calbiotech, USA respectively).

Enzyme-linked immunosorbent assay is considered to be highly sensitive and specific for the quantitative estimation of antigens and antibodies [11]

Calculation of CRP results:
Standard curve was plotted by plotting the absorbance of the CRP standards on vertical axis and CRP standard concentrations on horizontal axis on a liner graph paper.

The value of CRP of patient samples and controls were recorded by plotting the absorbance repeatedly on the standard curve.

The obtained values of the patient sample and controls were multiplied by dilution factor to obtain CRP result in mg/l.

CALCULATION OF PCT RESULTS
Standard curve was prepared by plotting the absorbance of the PCT standards on vertical axis and PCT standard concentrations on horizontal axis on a liner graph paper.

The value of PCT of test samples was recorded by plotting the absorbance of test samples repeatedly on the standard curve.

The obtained values of the patient sample were multiplied by dilution factor to obtain PCT result in pg/ml. The values of PCT calculated in pg/ml were converted to ng/ml by following the standard unit conversation table i.e. 1pg = 0.001ng.

Following the Sucilathangam G et al[3] guidelines we have concluded our results as follows:

CRP levels and PCT levels in 30 patients samples on 0 day, 2nd day, 4th day is depicted in Table nos. 2&3.

Study group constituted of 30 patients. In 21 patients out of 30 patients we could procure the samples on 0day, 2nd day and 4th day. In remaining 9 patients we could procure the sample on 0day and 2nd day only because of administrative reasons that is they took discharge against medical advice, hence we failed to procure sample on 4th day. For the aforesaid reason we divided the study groups into two groups that is group I constituting of 21 patients and group II constituting of 9 patients. For both the group's results were analysed separately,(Table nos. 2&3)

PROCALCITONIN AND SEPSIS
As reflected in the Table. nos.2&3, all the 30 patients included in study had Procalcitonin levels of more than 0.5ng/ml or ≥0.5ng/ml.

Analysis of PCT Results of group I Patients (21):-
In 10 patients (47.6%) Procalcitonin levels were in range of 2-10ng/ml on 0 day, 2nd day and 4th day. 2) 8 patients (38.08%) had procalcitonin level of 1-2ng/ml on all three days that is 0 day, 2nd day and 4th day. 3) The remaining 3 patients (14.28%) had Procalcitonin levels of 0.5-1ng/ml but <2ng/ml on 0day, 2nd day and 4th day.(Table no.2)

Analysis of PCT Results of group II Patients (9):-
1) In one patient (11.1%) Procalcitonin levels were in the range of 2-10 ng/ml on sequential testing (0 day & 2nd day). 2) 7 patients (77.7%) had procalcitonin levels of 1-2 ng/ml on sequential testing. 3) In remaining one patient (11.1%) Procalcitonin levels were in the range of 0.5-1 ng/ml on sequential testing. (Table no.3)

C-REACTIVEPROTEIN AND SEPSIS
In septicemia there is acute phase reaction and one of the proteins which levels increase is CRP. The normal CRP level in serum is less than 1 mg/L with an upper reference limit of 6mg/L.[3]

Analysis of CRP Results of group I Patients (21):-
In 16 (76.16%) patients C-reactive protein levels were more than 6mg/l on all three days that is 0 day, 2nd day and 4th day.

In 3 (14.28%) patients C-reactive protein levels were more than 6mg/l on 0 day and 2nd day. On 4th day the CRP one was <6mg/l

In 1 (4.76%) patients on 0 day CRP level was marginally higher than 6mg/l but subsequently on 2nd and 4th day the conc. of CRP was less than 3mg/l.

In 1 patient(4.76%) on 0 day CRP levels was less than 3mg/l but subsequently in 2nd and 4th day the conc. of CRP was >6mg/l. (Table no.2)

Analysis of CRP Results of group II Patients (9):-
In all 9 patients (100%) the CRP levels were >6mg/l on 0day and 2nd day. (Table no.3)

COMPARISION OF CRP AND PCT RESULTS-(Table no.1)
In group I (21patients)

In 10 patients the procalcitonin values were more than 2ng/ml on 0 day, 2nd day and 4th day. The corresponding C-reactive protein level in these patients was more than 6mg/l on all the aforesaid three days. Thus results show 100% correlation between PCT & CRP and further suggest that these patients are positive for sepsis.[3]

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In 8 patients the procalcitonin values were 1-2ng/ml on 0 day, 2nd day and 4th day. The corresponding C - reactive protein level in 5 patients out of 8 patients was more than 6mg/l on all the aforesaid 3days. Of the remaining 3 patients, in 2 patients CRP levels were >6mg/l on 0 day and 2nd day where as in remaining one patient the CRP level was >6mg/l on 0 day only. The correlation between CRP and PCT was 62.5%.The results suggest that 5 patients were weakly positive for sepsis.[3]

In 3 patients PCT values were 0.5-1ng/ml on 0 day, 2nd day and 4th day. The corresponding CRP level in one patient out of 3 patients was more than 6mg/ml on all the aforesaid three days. In remaining two patients the corresponding levels of CRP were more than 6mg/l on 2 day only not all 3 days. The correlation between CRP and PCT was 33.3%. The result suggests that health status in one patient is pathological. (Fig.no.1)

In group II (9patients)

In one patient the Procalcitonin Value was 2-10 ng/ml on 0 day and 2nd day. The corresponding CRP levels were >6mg/l on both the aforesaid days. The correlation between CRP and PCT was 100%. Thus result suggest that the patients are positive for sepsis.[3]

In 7 patients the Procalcitonin values were 1-2 ng/ml on 0day and 2nd day. The corresponding C-reactive proteins levels in all the 7 patients were >6mg/l, thus the correlation was 100% .The results suggest that 7 patients were weakly positive for sepsis.[3]

The remaining 1 patient the procalcitonin values were 0.5-1ng/ml on 0 day and 2nd day. The corresponding C-reactive protein levels were >6mg/l on both the days. The correlation between PCT and CRP was 100%. The results suggest that the health status of the patient is Pathological.[3] (Fig.no.2)

In the present study if the results of group I and group II are viewed together, the overall correlation between PCT and CRP is 83.3%.Thus both these serological markers are complimentary to each other Fig.no.3. The study comprises of small group of 30 patients and the health status in the group is reflected in Fig.no.4. Further study is warranted to arrive at an inference. The scarcity of kits in market, high cost of kits and short shelf life compelled us to restrict to a modest group.

Fig.no.1

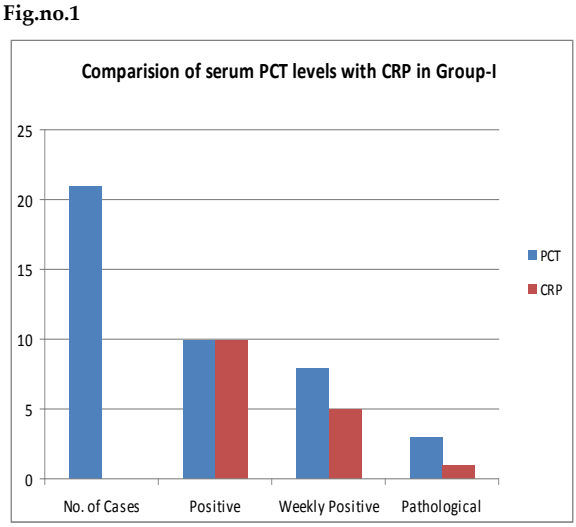

Fig no: 2

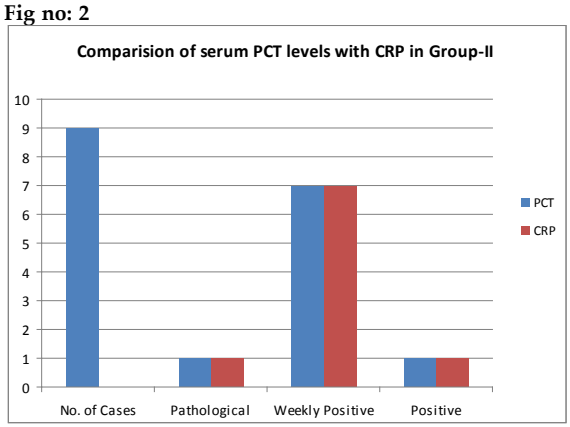

Fig no:3

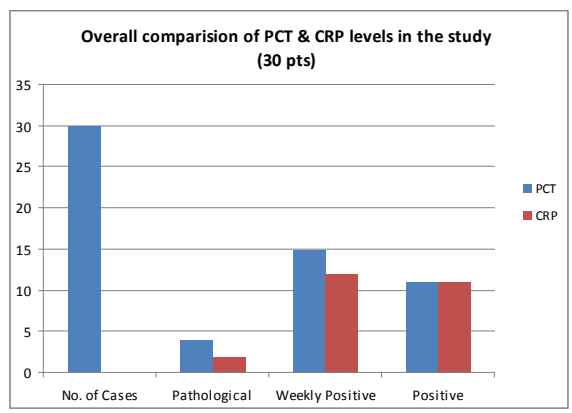

Fig no:-4

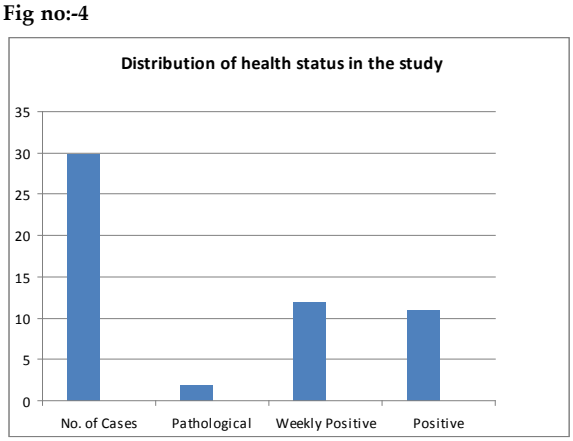

Table No :1
of PCT &CRP Values

PCT values	No. Of Patients	Corresponding levels of CRP on 0day, 2nd day and 4th day >6mg/L for Gr. I. For Gr. II the corresponding level of CRP >6mg/L on 0day& 2nd day only.	Correlation Between PCT&CRP	Inference
Group I (21patients)				
PCT Values >2ng/ml on 0day,2nd day & 4th day	10	10	100%	Positive for sepsis*

PCT Value 1-2ng/ml on 0 day, 2nd day & 4th day	8	In 5 patients >6mg/L on all 3days. In two patients >6mg/L on 0&2nd day only, in one patient > 6mg/L on 0 day only.	62.5%	Weakly positive for sepsis *
PCT Value 0.5-1ng/ml on 0day, 2nd day and 4th day.	3	In 1 patients >6mg/L on all 3 days. In two patients >6mg/L on two days only.	33.3%	Pathological*
Group II (9 Patients)				
PCT Values >2ng/ml on 0 day and 2nd day	1	1	100%	Positive for sepsis*
PCT Value 1-2ng/ml on 0day and 2nd day	7	7	100%	Weakly positive for sepsis *
PCT Value 0.5-1ng/ml on 0 day & 2nd day.	1	1	100%	Pathological*

*Sucilathangam G et al[3] Guidelines:
Normal CRP levels as per kit insert: < 3mg/L
PCT level of ≥0.5ng/ml - Pathological
PCT levels 0.5-2ng/ml - Weakly positive
PCT level 2-10 ng/ml - Positive.

Table no.2

Group-I(21patients)								
PATIENT PATICULAR			CRP Levels (mg/l)			PCT Levels (ng/ml)		
S no	Age	Sex	0 day	2nd day	4th day	0 day	2nd day	4th day
1	27	F	7	8	7	1.26	2.1	1.45
2	24	F	8	7	3	3.7	1	1.9
3	38	F	7	7	7.2	1.8	4	2.1
4	11	F	8	7	3	.8	0.5	0.6
5	22	M	8	8.6	7.8	2.4	3.8	4.8
6	35	M	7	7.2	8	4.7	2.2	2.4
7	80	M	8	8	8	1.8	2.9	2.5
8	75	F	8.6	7	7	2.9	1.9	3.6
9	61	F	7	8	8	3.5	2	3.5
10	22	F	8	8	8.6	4.2	2.6	2.2
11	42	M	7	7	7.2	2.3	1.8	1.2
12	20	M	8	8	8	2.5	2.4	3.6
13	22	M	8	9.6	7.8	3.8	3.6	4.3
14	40	M	7	7.2	8	0.9	1.5	1.7
15	18	M	8	8	8	2.2	2.2	2.2
16	65	F	8	7	7	4.6	4.7	3.8
17	14	F	6.4	2.6	2.4	.85	1.2	1.2
18	58	F	7	7	7	2.1	1.4	0.4
19	28	M	8	7	3.6	0.9	1.1	1.8
20	38	F	8	8.6	7	2.1	0.6	0.5
21	70	M	2.2	7.2	8	0.6	0.9	1.3

Table .no.3

Group-II(9 Patients)								
PATIENT PATICULAR			CRP Levels (mg/l)			PCT Levels (ng/ml)		
S no	Age	Sex	0 day	2nd day	4th day	0 day	2nd day	4th day
22	22	F	7	7.2	-	1	1.5	-
23	50	M	8	8.6	-	1.3	2.2	-
24	46	F	7.8	7	-	1.6	1	-
25	14	F	7	8	-	0.9	1.15	-
26	18	F	8	8.6	-	1.2	4	-
27	60	M	7	7	-	3.7	1.9	-
28	55	M	7	8	-	0.5	1	-
29	50	M	8	8.6	-	1	2.3	-
30	80	M	7	7	-	1	2.5	

DISCUSSION:

There is no single reliable test for the early definite diagnosis of sepsis and therefore there is a continuing search for a new infection marker. C-reactive protein has been the most analysed parameter for the detection of bacterial infection for years. Procalcitonin has been proposed as a marker of bacterial sepsis. The adventures of Procalcitonin as compared to C-reactive protein is that the increase in concentration of Procalcitonin in bacterial infection and its restoration to normal is more rapid.[3]

Sucilathangam G et al[3] concluded that serum Procalcitonin level was superior to serum C-reactive protein level for early diagnosis of neonatal sepsis.

Mark Hatheril et al[4] share their opinion.

Early identification of infection is a challenge for clinician therefore markers specific for bacterial infection will be most helpful. In sepsis Procalcitonin and C-reactive protein levels rise with in 6 hours. Thus detection of procalcitonin levels and C-reactive protein levels helps in the early diagnosis of sepsis.[5]

Meisner. M et al[6] recommended the measurement of PCT concentration in patients with multiple organ dysfunction syndrome and sepsis as it provides more information about the severity and the course of the disease than that of CRP.

Chauhan S et al[7] analyzed that CRP levels are very low in normal infants whereas there is a rapid rise of CRP with in 12-24 hours of sepsis.

Hong-Xiang Li et al[8] in their study concluded that there is a significant correlation between Procalcitonin and C-reactive protein when sepsis occurs. They further suggested that Procalcitonin and C-reactive protein could be used together.

Jerome Cornillon et al[9] have evaluated PCT and CRP as serological markers for sepsis in patients with neutropenia, concluded that CRP is a sensitive marker for inflammation and PCT decreases very soon after bacterial resolution .

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Gian Paolo Castelli et al[10] hypothesised that of the two serological markers, PCT is a better parameter to estimate severity and prognosis of sepsis.

In present study the sequential results reflect that in 11 patients procalcitonin levels were more than 2ng/ml, in 15 patients Procalcitonin levels were 1-2ng/ml and in remaining 4 patients the Procalcitonin levels were in the range of 0.5-1ng/ml.

In 83.3% of patients the corresponding C-reactive protein levels were more than 6mg/L sequentially. Thus there is a correlation between C-reactive protein and Procalcitonin for the diagnosis of sepsis.

Monitoring the levels of both serological markers Procalcitonin and C-reactive protein has been proposed to improve the accuracy of diagnosis of sepsis.

CONCLUSION:

Present study suggests that sequential testing of Procalcitonin and C-reactive protein are useful serological markers. These not only helps in early diagnosis and prognosis of sepsis but consequentially it also helps to shorten duration of antibiotic treatment.

All the 30 patients had rural back ground with agriculture as their main occupation in the present study. These serological markers can be included as first line of investigation in the cases of suspected sepsis. The ELISA kit for C-reactive protein and procalcitonin are not easily available in the market, especially Procalcitonin ELISA kit is forbiddenly expensive and thus not cost effective for patients with rural, agricultural back ground with humble socioeconomic status.

There are no sepsis markers available which can diagnose 100% of infected cases. Procalcitonin and C-reactive protein are useful adjunct test in the diagnosis of sepsis.

Very scanty reference material is available where the workers adopted ELISA method for the estimation of Procalcitonin and C-reactive protein levels.

As is evident from the literature, the methods adopted for estimation of CRP are Immuno-turbidimetric[3], Semi quantitative latex agglutination[7],Turbi-qunti[6],Vitros 250 dry Chemistry system[8] and Nephelometric[9,10]. For PCT estimation Immuno-luminometric[3,4,5,6,9,10] and Immuno chromatography[8]methods were adopted. None of the workers have used ELISA test, as such more studies are warranted for the estimation of CRP and PCT by aforesaid method. For this the primary requirement is easily availability of the ELISA kits in the market at an affordable cost. The present study confirmed the findings of other investigators that Procalcitonin and C reactive protein are helpful in detection of Sepsis.

References

1. Pugin J, Meisenar M, Leon A, Gendrel D, Anna Ferndez lopez. Guide for the clinical use of PCT in Diagnosis and monitoring of sepsis. 4th ed. B.R.A.H.M.S Aktiengesellschaft,Germany. 2005:4-11.
2. Husebekk A, Lars-Ol of Hansson. C-reactive protein in clinical practice. 2nd ed. Axis-Shield poc As. Norway;2003:5-49.
3. Sucilathangam G, Amuthavalli K, Velvizhi G, Asihabegum M.A, Jeyamurugan T, Palaniappan N. Early Diagnostic Markers for Neonatal Sepsis: Comparing Procalcitonin (PCT) and C-Reactive Protein (CRP). Journal of Clinical and Diagnostic Research 2012 ;vol-6(4):627-631.
4. Hatherill M, Shane MT, Sykes. K Turner .C, Murdoch. IA Diagnostic markers of infection: comparison of procalcitonin with C- reactive protein and leucocyte count. Arch Dis Child 1999 ; 81:417-421.
5. Simon.L France Gauvin, Amre.D, saint-Louis. P, Lacroix. J. Serum Procalcitonin and C-Reactive Protein Levels as Markers of Bacterial Infection: A Systematic Review and Meta-analysis. Clinical Infectious Diseases 2004;39:206-217.
6. Meisner.M, Tschaikowsky.K Palmaers.K and Joachim Schmidt. Comparison of Procalcitonin(PCT) and C-reactive protein (CRP) plasma concentrations at different SOFA scores during the course of sepsis and MODS. Critical care 1999, 3:45-50.
7. Chauhan Setal B, Vaghasia , Chauhan Bimal B. C-reactive protein (CRP) In Early Diagnosis Of Neonatal Septicemia. National Journal of Medical Research Issue 2012;2(3):276-278.
8. Hong-Xing Li, Zhong-Min Liu, Shu-jie Zhao, Dong Zhang, Shi-ji Wang and Yu-Shan Wang. Measuring both Procalcitonin and C-reactive protein for a diagnosis of sepsis in critically ill patients. Journal of International Medical Research 2014; 42(4) 1050-1059.
9. Jerome Cornillon, Marie Bouteloup, Claude Lambert. Evaluation of Procalcitonin and CRP as sepsis markers in 74 consecutive patients admitted with prolonged febrile neutropenia. Journal of Infection 2011;63(1):93-95.
10. Gian Paolo Castelli, Claudio Pognani, Michael Meisner. Anonio Stuani, Daniela Bellomi and Laura Sgarbi. Procalcitonin and C-reactive protein during systemic inflammatory response syndrome, sepsis and organ dysfunction. Critical Care 2004, 8:R234-R242.
11. A. Voller, A. Bartlett, and D.E.Bidwell, Enzyme immunoassays with special reference to ELISA techniques. Journal of Clinical Pathology, 1978,31(June 1978):507-520.