

Role of serum procalcitonin and C-Reactive Protein in the diagnosis of neonatal sepsis

Naher BS¹, Mannan MA², Noor K², Shahiddullah M²

¹Department of Neonatology, Sir Salumullah Medical College & Mitford Hospital, Dhaka,

²Department of Neonatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka

Abstract

This cross sectional observational study was done in the division of neonatology, department of pediatrics, Bangabandhu Sheikh Mujib Medical University (BSMMU) in the year 2007. The study population was 50 newborns in total who needed evaluation of sepsis on clinical suspicion. The main objective of this study was to assess serum procalcitonin (PCT) as a better diagnostic marker than C-Reactive Protein (CRP) in neonatal sepsis. The total study populations were classified into 4 groups like highly probable, probable, and possible and no sepsis group according to the clinical and blood parameters. PCT and CRP were assessed and compared by statistical analysis. For the estimation of PCT and CRP, venous blood was drawn and centrifuged and stored at – 20°C in the refrigerator. Later on PCT was measured by rapid semi quantitative immunochromatographic test. Level of CRP was determined by semi quantitative method (latex). All data were analyzed by SPSS version 10 windows. For statistical analysis appropriate tests were done. In all observations sepsis was found to be more common in male newborns and in those who were delivered by caesarean section. In low birth weight and preterm newborns sepsis was more prevalent. Premature rupture of membrane (PROM) was found to be the commonest maternal clinical condition as a risk factor of sepsis. There was positive correlation between serum PCT and CRP and values of serum PCT as well as CRP differed significantly in the different categories of sepsis indicating relation to the severity of sepsis. PCT is a useful, sensitive and independent biomarker of neonatal sepsis. CRP measurement along with PCT measurement may increase the specificity. Though PCT measurement is comparatively expensive but an easy bed side prompt convenient procedure for sick neonates in addition to CRP for rapid evaluation of neonatal sepsis rather than waiting for the report of blood culture.

Introduction

Neonatal sepsis is a major cause of neonatal mortality and morbidity throughout the world. Neonatal septicemia is a clinical syndrome of systemic illness accompanied by bacterium occurring in the 1st 28 days of life¹.

Neonatal sepsis may be categorized as early onset and late onset sepsis. Eighty five percent of newborns with early onset infection present within 24 hours, five percent present at 24-48 hours and smaller percentage of patients between 48 hours and 6 days of life². These infants have a history of one or more significant obstetric complications and many of the infants are preterm and of low birth weight. Bacteria responsible for early onset sepsis are acquired from infected amniotic fluid or from the birth canal during delivery and the mortality rate is high. On the other hand late onset sepsis occurs at 7-28 days of life and it is more insidious in presentation with lower mortality and etiologic agents are acquired from the care giving environment.

In the global perspective, the microorganisms most commonly associated with early onset infection include group B streptococcus (GBS), Escherichia Coli, Haemophilus influenzae and Listeria monocytogenes³. In case of late onset infection causative organisms are coagulase negative staphylococci, staphylococcus aureus, E. coli, Klebsiella, Pseudomonas, Enterobacter, Candida, GBS, Serratia, Acinetobacter and anaerobes⁴.

The infants skin, respiratory tract, conjunctiva, gastrointestinal tract and umbilicus may become colonized from the environment, leading to the possibility of late onset sepsis from the invasive microorganisms. Vectors for such colonization may include vascular or urinary catheters, other indwelling lines or contact from caregivers with bacterial colonization.

The reported incidence of neonatal sepsis varies from 7.1 to 38 per 1000 live births in Asia^{5,6}.

In Bangladesh, there is scarcity of studies regarding incidence. There is no population based study report but a study conducted in Institute of

Postgraduate Medicine & Research (IPGM&R) showed that 9.21% of admissions in neonatal unit was attributable to neonatal sepsis⁷.

Overall mortality from septicemia varies between 26-40 percent⁸. It varies between 15-50 percent for early onset septicemia (EOS) and 12-20 percent for late onset septicemia (LOS) with highest mortality of 70 percent when sepsis occurs within first 48 hours of life³. It is one of the commonest cause of perinatal mortality in the developing world.

Infection in neonates is difficult to identify solely on the basis of physical finding, because signs are not specific and may be absent when the infection is identified just before delivery¹. Definitive diagnosis of neonatal sepsis is based on blood or CSF culture, both of which take at least 24 to 48 hrs and are often falsely negative. This results in the over treatment of large number of neonates who present with clinical suspicion of sepsis.

The major problem in neonatal infection is the identification of the infected infant. Identifying the noninfected infant is equally important which is often overlooked. It is desirable to administer appropriate therapy as early as possible to the infected infant and to avoid such therapy in the others⁹. Diagnosis of neonatal septicemia is one of the most difficult task in clinical medicine¹⁰.

The clinical findings of sepsis are uncertain in newborn infants and these findings may be associated with multiple condition besides infection. Therefore antibiotics are started immediately in newborn who have nonspecific finding of infection and are continued until the final result of the blood culture is obtained^{11,12}. Blood culture can remain negative despite bacterial sepsis.

The difficulty in making early diagnosis of neonatal sepsis, despite improved bacteriologic techniques, is attested to by recent reviews¹¹. Therefore a group of tests were studied to assess their usefulness either singly or in combination in predicting neonatal sepsis. Determination of procalcitonin (PCT) is another laboratory study which supports the diagnosis¹³.

In 1993 PCT was first described as a marker of the extent and course of systemic inflammatory response to bacterial and fungal infections¹⁴. Procalcitonin (PCT) propeptide is the precursor protein of calcitonin and has no hormonal activity. It is a glycoprotein having 116 amino acid protein with a molecular mass of 14.5k Da¹⁵. Normally it is produced by the C cells of the thyroid gland. In healthy persons procalcitonin levels are undetectably low. But in severe bacterial, fungal, parasitic infections with systemic manifestations a significant rise in procalcitonin levels are seen. In this condition the production site is the extra thyroid tissues^{16,17}.

It was shown in healthy volunteers that PCT is detectable in the plasma two hours after the injection of a small amount of bacterial endotoxins, increasing rapidly in 6-8 hours, and reaching a plateau between 12 and 48 hours¹⁸. PCT levels increase in severe sepsis and its plasma concentration is related to the patient's clinical condition. Serum PCT levels appeared to correlate with the severity of microbial invasion¹⁹.

CRP is one of the acute phase proteins. Although it is a classical and sensitive marker of inflammation; it cannot be used to differentiate between bacterial and other infection. It is a disadvantage that CRP increases after PCT. This is why, several authors have opined that it is important to be cautious with the interpretation of CRP values in children with fever lasting less than 12 hours because at that time it may remain negative although there is presence of sepsis.¹⁵

Chiesa et al stated that an increase in PCT levels in early and late onset of neonatal sepsis is quite reliable¹². Monneret et al reported that elevated PCT level correlate with sepsis¹⁹. They also found that CRP did not show a similar correlation. Noninfectious condition, as perinatal asphyxia, respiratory distress syndrome, brain haemorrhage and meconium aspiration syndrome and post surgical period can induce abnormal values of CRP^{20,21}. In contrast localized bacterial infections, severe viral infections and inflammatory reactions of noninfectious origin do not or only slightly increase PCT level²². The increase of PCT has been observed before the rise in CRP¹⁵. The unique feature that PCT levels increase in bacterial and fungal infections, but remain unchanged even in severe viral infections and other inflammatory diseases, makes PCT attractive as a potential diagnostic variable for the diagnosis of bacterial infection.

Materials and Methods

This cross sectional observational study was carried out in neonatal care unit (NCU) of Bangabandhu Sheikh Mujib Medical University (BSMMU). A total of 60 neonates with suspected sepsis who needed sepsis evaluation on clinical ground were considered as cases.

Inclusion criteria: Any suspected case of neonatal sepsis with maternal risk factors for sepsis e.g. prolonged labour, premature rupture of membrane (PROM) or prolonged PROM >18 hours, maternal intrapartum fever, urinary tract infection (UTI), chorioamnionitis and clinical signs and symptoms of the newborn having sepsis related clinical signs: temperature instability, apnea, need for supplemental oxygen, need for ventilation,

bradycardia, tachycardia, hypotension/hypoperfusion, feeding intolerance, abdominal distension, necrotizing enterocolitis.

Criteria Employed for defining the sepsis score

1. Sepsis score

Groups	Criteria
Group 1 (Highly probable sepsis)	<ul style="list-style-type: none"> ▪ At least 3 sepsis related clinical signs ▪ CRP>6 mg/dl ▪ At least 2 other altered blood parameters* in addition to CRP ▪ Blood culture: Positive or negative
Group 2 (probable sepsis)	<ul style="list-style-type: none"> ▪ Less than 3 sepsis related clinical signs ▪ CRP>6 mg/dl ▪ At least 2 other altered blood parameters* in addition to CRP ▪ Blood culture: Negative
Group 3 (possible sepsis)	<ul style="list-style-type: none"> ▪ At least 3 sepsis related clinical signs ▪ CRP<6 mg/dl ▪ Less than 2 other altered blood parameters* in addition to CRP ▪ Blood culture: Negative
Group 4 (no sepsis)	<ul style="list-style-type: none"> ▪ no sepsis related clinical signs ▪ CRP<6 mg/dl ▪ No altered blood parameters* ▪ Blood culture: Negative

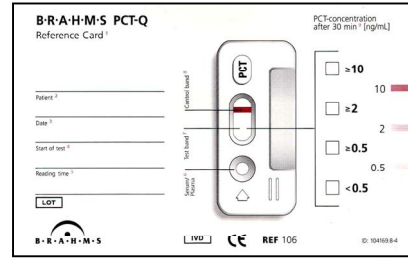
* Blood parameters other than CRP: white blood cell count, absolute neutrophil count, platelet count.

Categories of infection: Babies were grouped into different categories of infection before obtaining the PCT result.

Exclusion criteria: Baby of diabetic mother.

For PCT and CRP estimation, 2 ml of venous blood was collected from the peripheral vein. Blood sample was centrifuged at 5000 rpm for 15 minutes to separate the serum.

After that, serum was stored at -20°C in the refrigerator of the laboratory of Immunology department of BIRDEM. PCT was measured by a rapid semi quantitative immunochromatographic test (Brahms PCTQ; Brahms diagnostic Berlin Germany) in 20 min. (range result: <0.5ng/ml, more than 0.5ng/ml, more than 2ng/ml and more than 10ng/ml.). Briefly 200 microlitre of serum was applied onto the test strip. PCT in the sample is bound by mouse anti calcitonin anti bodies conjugated with colloidal gold to form a complex. This complex moves by means of capillarity through an area containing fixed anti calcitonin anti bodies to form a sandwich complex that can be seen as a reddish band. The colour intensity of the band directly proportional to the PCT concentration of the sample. Normal serum and plasma levels of PCT are less than 0.5 ng/ml. Levels above this value was accepted as pathological. PCT level was compared between the categories of infection. CRP was determined by latex serology test.



PCT- Q reference card

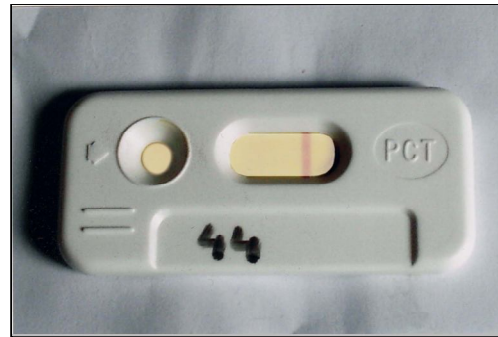


Photo shows Negative PCT test

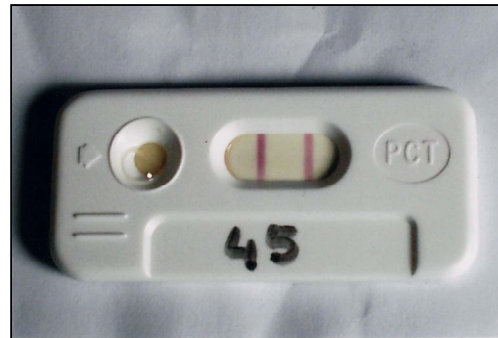


Photo shows Positive PCT test.

Laboratory investigations: Serum PCT and CRP in addition to total and differential count of WBC, platelet count, blood film, blood for culture and sensitivity were measured when a bacterial sepsis was suspected. Leukopenia was defined as leukocyte count <5000/mm³. Leukocytosis was defined as leukocyte count >25000/mm³ at birth and >21000/mm³ after the second day. Thrombocytopenia was accepted as platelet count <150,000/mm³. Normal absolute neutrophil count was accepted as 7,800–14,500/mm³ in the first 60 hours and 1750–5400/mm³ after 60 hours.

Data analysis: The collected data was analyzed using the statistical package for social science (SPSS) version 10 for window. ANOVA, Chi square test and diagnostic test for (sensitivity, specificity, positive predictive value and negative predictive value) were done. Area under the Receiver operating characteristic (ROC) curve was evaluated.

Data presentation: Data and result is presented in the form of table and diagram where applicable.

Results

The study included a total of 60 newborns who met the inclusion criteria. 10 were excluded because of incomplete data (e.g. missing blood culture report and haemolysed blood sample) by which estimation of CRP and PCT could not be done. Therefore 50 newborns were included in the final statistical analysis. Newborns were classified into 4 groups according to the study protocol.

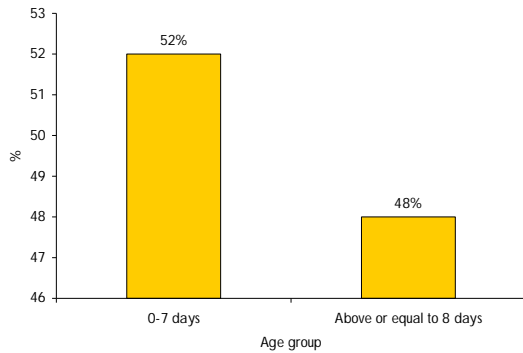


Figure 1: Shows incidence of sepsis is more in newborns of < 7 days age (early onset sepsis).

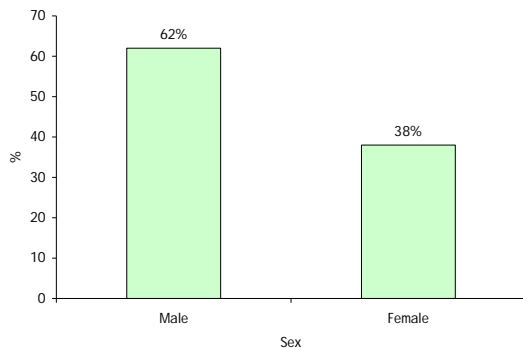


Figure 2: Shows sepsis to be more common in male newborns than female.

Table I shows sepsis score was more among LBW than normal birth weight newborn.

Table I: Distribution of newborn by birth weight and sepsis score

Groups	Birth weight (gm)		Total
	<2500 gm	≥2500 gm	
Group 1 (Highly probable sepsis)	7 (21.2)*	3 (17.6)	10 (20.0)
Group 2 (Probable sepsis)	5 (15.2)	6 (35.3)	11 (22.0)
Group 3 (Possible sepsis)	15 (45.5)	4 (23.5)	19 (38.0)
Group 4 (No sepsis)	6 (18.2)	4 (23.5)	10 (20.0)
Total	33 (100.0)	17 (100.0)	50 (100.0)

Chi square value=3.72, df=3, p value= 0.293

*Figure in parenthesis denoted corresponding percentage

Table II shows sepsis is more seen in preterm with gestational age of <37 weeks in comparison to term newborn.

Table II: Distribution of newborn by gestational age & sepsis score

Groups	Gestational age		Total
	<37 weeks	≥37 weeks	
Group 1 (Highly probable sepsis)	5 (17.9)	5 (22.7)	10 (20.0)
Group 2 (Probable sepsis)	6 (21.4)	5 (22.7)	11 (22.0)
Group 3 (Possible sepsis)	12 (42.9)	7 (31.8)	19 (38.0)
Group 4 (No sepsis)	5 (17.9)	5 (22.7)	10 (20.0)
Total	33 (100.0)	17 (100.0)	50 (100.0)

Chi square value=0.697, df=3, p value= 0.874

*Figure in parenthesis denoted corresponding percentage

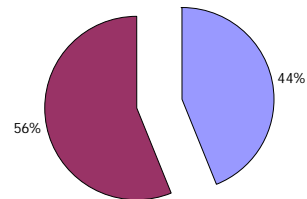


Figure 3: Shows newborn with suspected sepsis are delivered more by caesarean section.

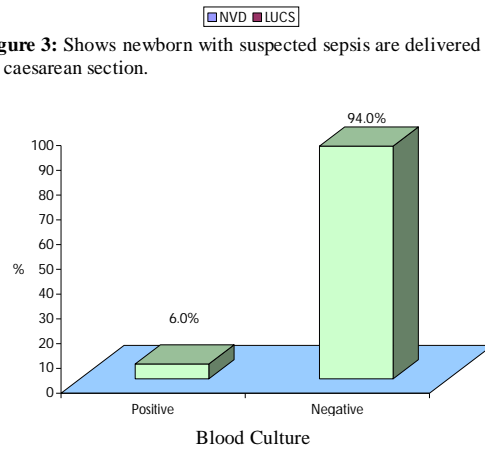


Figure 4: Shows most of the clinically suspected sepsis were not culture positive in blood

Table III: Distribution of the CRP and serum PCT level by different sepsis score

Groups	Mean ±SD (Range)	
	CRP mg/L	Serum PCT (ng/ml)
Group 1 (n=10) (Highly probable sepsis)	28.8±8.24 (12-96)	5.85±1.39 (0.5-10)
Group 2 (n=11) (Probable sepsis)	20.73±3.27 (12.00-48)	5.3636±1.35 (.5-10.)
Group 3 (n=19) (Possible sepsis)	6.95±.95 (6.0-24.0)	1.55±0.5 (.50-10.0)
Group 4 (n= 10) (No sepsis)	6.0±0 (6-6)	.65±.15 (.50-2.0)
F value*	8.629	7.898
p value	0.001	0.001

*ANOVA test was done

Table IV: Significant level of PCT(ng/ml) level in different groups

Sepsis score	Sepsis score	p value
Group 1	Group 2	0.727
	Group 3	0.001
	Group 4	0.001
	Group 4	0.001
Group 2	Group 3	0.003
	Group 4	0.001
Group 3	Group 4	0.469

In multiple comparison test (LSD; least significant difference) of serum PCT significant differences have been found between group-I and group-III as well as group-I and group-IV (p value .001) Comparison between group-II and group-III and also group-II and group-IV shows statistically significant difference.

Table V: Significant value of CRP (mg/L) level in different groups

Sepsis score	Sepsis score	p value*
Group 1	Group 2	0.157
	Group 3	0.000
	Group 4	0.000
	Group 4	0.000
Group 2	Group 3	0.007
	Group 4	0.012
	Group 4	0.851
Group 3	Group 4	0.851

The above table shows by comparing between group-I and group-III/group- IV as well as group-II and group- III/group-IV that CRP seems to be a valid independent marker. CRP differs significantly with the degree of invasion.

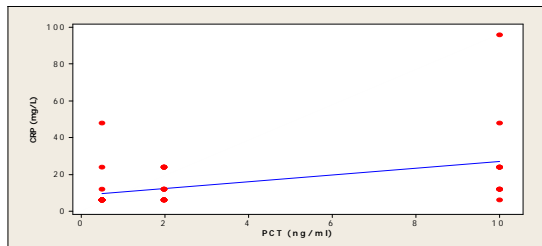


Figure 5: Scatter plot of correlation of CRP (mg/L) and PCT (ng/ml) level of patients.

The above scatter plot shows the positive correlation between the CRP (mg/L) and PCT (ng/ml) level of the patients in this study. Table showed Pearson correlation =0.448, which indicates positive correlation between this two variables and it is highly significant (p value <0.01).

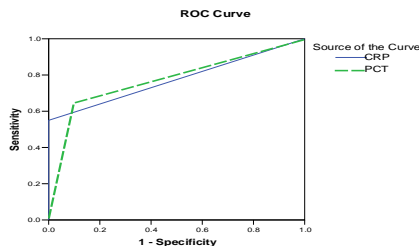


Figure 6: Comparison of AUC in the diagnosis of overall sepsis

Receiver operating characteristic (ROC) analysis was made according to the sensitivity and specificity of PCT and CRP values in the groups and the best results were determined for CRP and PCT for a cut off value of <6 mg/l and <0.5ngm/ml respectively). Values of area under the curve (AUC) PCT and (AUC) CRP were same (0.775) on the ROC curve.

Table VI: Distribution of sepsis in relation to PCT cut off level.

PCT (cut-off value<0.5ng/ml)	Sepsis		Total
	Present	Absent	
Normal	1 (10.0)*	22 (55.0)	23 (46.0)
Abnormal	9 (90.0)	18 (45.0)	27 (54.0)
Total	10 (100.0)	40 (100.0)	50 (100.0)

Chi square value=6.52, df=1, p value=0.01, *Figure within parenthesis denoted corresponding percentage (P value < .05 is considered to be significant)

The above table shows level of procalcitonin (PCT) was above normal in most of the newborns with suspected sepsis.

Table VII: Distribution of sepsis in relation to CRP cut off level.

CRP (cut-off value<6mg/L)	Sepsis		Total
	Present	Absent	
Normal	0 (.0)*	28 (70.0)	28 (56.0)
Abnormal	10 (100.0)	12 (30.0)	22 (44.0)
Total	10 (100.0)	40 (100.0)	50 (100.0)

Chi square value=15.91, df=1, p value=0.001, *Figure within parenthesis denoted corresponding percentage (P value < .05 is considered to be significant).

The above table shows the level of C- reactive protein (CRP) was normal in most of the newborns with clinically suspected sepsis.

Table VIII: Comparison of validity tests of PCT (ng/ml) and CRP (mg/L) in the diagnosis of high to any possibility of sepsis (overall).

Validity tests	High to possibility of sepsis	
	PCT	CRP
Sensitivity	65.0%	55.0%
Specificity	90.0%	100.0%
PPV	96.3%	100.0%
NPV	39.1%	35.7%
Accuracy	70.0%	64.0%

This table of validity test shows sensitivity 65% and negative predictive value (NPV) (39.1%) were higher for PCT where as specificity (90%) and positive predictive value (PPV) were lower in comparison to CRP.

Table IX: ROC (Receiver operating characteristic) curve.

Test Result Variable(s)	AUC*	p value	95% Confidence Interval	
			Lower Bound	Upper Bound
CRP	0.775	0.008	0.646	0.904
PCT	0.775	0.008	0.627	0.923

*Area under curve. Null hypothesis: true area = 0.5

Discussion

New and efficacious laboratory tests are needed in the diagnosis of neonatal sepsis. Acute phase reactants have been used frequently as an early marker of bacterial sepsis. Previous studies have shown CRP to be a useful marker of bacterial sepsis in the neonate. There is no single reliable test for the early confirmation of definite neonatal sepsis. Therefore, there is a continuing search for a new infection marker, including investigation of PCT and the other cytokines. In this study sepsis was more common in male (62%) than female

(38%), which is consistent with the findings of Washburn et al., Mannan^{23,24}. This is probably due to the attitude of the parents who seek medical services more for their male babies than female babies in this region.

It was seen that infection was more common in the low birth weight baby compared to babies of normal birth weight in studies reported from India²⁵, Bangladesh²⁴ as is also in our study.

One of the important risk factors was premature rupture of the membrane (PROM) which was more prevalent in 24% cases. The fact lying here is that PROM poses risk of ascending infection to the fetus.

Out of 50 cases of suspected sepsis, caesarean section was found in 56% and normal delivery in 44% cases. This is probably due to increased number of high risk pregnancies admitted in this hospital resulting into increased caesarean section.

Out of 50 cases of suspected neonatal sepsis, blood culture was found positive only in 3 cases (6%) and negative in 47 cases (94%). In many studies the incidence of culture positive sepsis was not more than 10%. In our study less number of culture proven sepsis may be due to late arrival, sample collection after giving antibiotic and faulty technique in collection procedure. This is similar to the study of Magudumana et al.²⁶.

It is important to note that there seems to be no significant differences of lab parameters between group 1 & group 2 as culture proven sepsis found in our study is only 6%. In multiple comparison test (LSD; least significant difference) of serum PCT significant differences has been found between group 1 & group 3 as well as group 1 & group 4 (p value 0.001). Comparison between group II & group III as well as group II & group IV shows statistically significant difference (p value 0.001). So PCT may be helpful as an independent biomarker and it seems to be a better differentiating biomarker. For this reason, PCT may be used not just as a marker of infection, but more importantly as a good marker of the severity of infection.

CRP also may be a valid independent marker while comparing between group 1 & group 3 / group 4 as well as group 2 & group 3/group 4. CRP also differs significantly with the degree of invasion. But it is to be noted that PCT seems to be statistically a better marker (p-.001) than CRP (p-.01) while comparing group 2 & group 4. PCT correlated significantly with CRP at presentation of suspected neonatal sepsis. Correlation coefficient is 0.448 p<.01. It is consistent with the study of Monneret et al.¹⁹.

In this study sensitivity of PCT in the diagnosis of neonatal sepsis was found to be higher 65% than CRP 55%. Specificity of PCT was lower (90%) than that of CRP (100%). These findings are similar to (Janota et al. 2001)'s study who found the sensitivity and specificity of PCT in the diagnosis of early onset neonatal sepsis as 75% and 85% respectively using a cut off value of 2 ng/ml²⁷. In the same study they reported the sensitivity and specificity of CRP as 25% and 90% respectively. They concluded that lower specificity of PCT can be related to the multi organ dysfunction of the infants who did not have sepsis.

Bonac et al. compared the levels of CRP, PCT and IL-6 in the diagnosis of early onset neonatal sepsis in 58 infants. They found that the sensitivity, specificity, PPV and NPV of PCT was 59%, 82%, 36% and 96% respectively using cut off value of 0.99 ug/ml. They also reported that the sensitivity specificity, PPV and NPV of CRP at the time of diagnosis was 36%, 92%, 43% and 89% respectively using a cut off value of 14 mg/l²⁸. They reported the higher sensitivity and lower specificity of PCT over CRP.

To evaluate the test performance ROC curve using sensitivity and specificity of two test like PCT & CRP was made for cut off value of <0.05 ng/ml and <6 mg/l respectively. Area under the curve was found to be equal (0.775). So finally both the markers seem to be similar in the diagnosis of neonatal sepsis. This is consistent with the study of Enguix et al²⁹.

Blommendahl et al. reported that although the PCT test appeared to be useful for the diagnosis of neonatal sepsis in his small study, it did not offer any significant advantages over traditional test like CRP for the diagnosis of sepsis³⁰. Our study is consistent with their study. So, PCT test can be done as an additional test with CRP for the diagnosis of neonatal sepsis.

PCT- Q kit had been made available in our institute from abroad. At present this test is available only in private hospital, Dhaka. Each test costs Tk 1800. In future as it is a new method of diagnosis and if it is widely used the cost will comedown and more and more diagnostic laboratory will acquire this kit.

Conclusions and Recommendations:

Our data confirm the data of other studies indicating the low incidence of culture proven neonatal sepsis. PCT is a sensitive, independent and useful biomarker of neonatal sepsis. It correlates with the severity of sepsis. Additional measurement of CRP may increase the specificity. In patients with elevated serum CRP level, PCT may be used as a measure to support further the diagnosis of

neonatal sepsis. We conclude that clinical evaluation seems to be the most reliable method in diagnosis, although all of the markers including PCT help us as supportive evidence.

As this is a study in small group of population, we recommend further studies in a large number of populations to confirm the role of PCT in the diagnosis of neonatal sepsis.

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