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Research Article

Predictability of biomarkers in diagnosis of neonatal sepsis: a cross sectional study

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ABSTRACT

Background: There is lack of sensitive and specific markers for diagnosis of early neonatal sepsis. Procalcitonin and CRP are known sensitive marker of sepsis and CRP is known to be affected by other independent variables. Hence this study was conducted to compare their predictability for diagnosis of early neonatal sepsis and to assess the effect of variables on them.
Methods: It is a cross sectional study. Study group comprised of neonates with definitive (n=09) and probable (n=24) signs, symptoms and laboratory markers of sepsis compared with no sepsis (n=67). Procalcitonin and CRP were measured by immunofluoroscence and immunoturbidometric methods respectively in serum of neonates admitted to intensive care unit on their first day of admission. Fishers test, median test, ROC curve and multiple regressions were used for statistical analysis.
Results: Procalcitonin ($p=0.007$) and CRP ($p<0.001$) levels were high in sepsis. Procalcitonin was not significantly affected by independent variables in asymptomatic neonates ($r^2=0.12$). ROC curve analysis revealed the predictability of procalcitonin (area under curve - 0.92) and CRP (area under curve-0.74) for the diagnosis of neonatal sepsis.
Conclusions: Procalcitonin found to have better predictability than CRP in the diagnosis of neonatal sepsis.

Keywords: Procalcitonin, C reactive protein, Neonatal sepsis

INTRODUCTION

Neonatal sepsis is one of the commonest causes of morbidity and mortality in the neonates in India compared to the developed countries. Conventional sepsis evaluation parameters have low sensitivity and are nonspecific; often demonstrating increased level response to various other neonatal conditions. The definitive conventional diagnosis of sepsis rests upon isolation of pathogenic bacteria in blood cultures, which has low sensitivity and lacks timeliness to influence initiation of antibiotic therapy. Without specific diagnostic predictors that remain abnormal for a significant time to allow detection of neonatal sepsis, delay in therapy increases mortality and morbidity risk.[1]

CRP can be considered as a "specific" but "late" marker of neonatal infection and hence serial measurement of CRP may be useful in treatment of neonatal sepsis especially in the first 48 hours.[2,3] PCT has been shown to be useful not only in the early diagnosis but also monitoring prognosis and response to treatment of patients with neonatal sepsis.[4,5] Studies have shown that multiple independent variables can affect the procalcitonin synthesis.[6] Hence this study was conducted to compare the predictability of procalcitonin and CRP for the diagnosis of neonatal sepsis and to determine the effect of multiple independent variables on procalcitonin levels.

METHODS

The cross sectional observational study was conducted in neonatal division of Department of Pediatrics, Sapthagiri Institute of Medical Sciences over a period of one year. Informed oral and written consent were taken from the parents. Neonates born to mothers with at least one of the following risk factors are included (i) premature rupture of membranes (PROM) >12 hours, (ii) >3 vaginal examinations after rupture of membranes, (iii) Intrapartum fever (>38 ^{o}C), (iv) foul-smelling liquor/meconium stained liquor, (v) maternal UTI within 2 weeks prior to delivery, (vi) prolonged and difficult delivery with instrumentation. New born babies with gestational age less than 28 weeks, birth weight less than 1000gm, lethal congenital anomalies, still born and fetal deaths, postdated neonates were excluded from the study. Detailed birth events, Apgar score, sex of the baby, weight of baby will be recorded on the precoded proforma made available. Gestational age was assessed by using modified Ballard scoring system. Neonates were divided into definite sepsis i.e. neonate with signs and symptoms suggestive of sepsis with a positive blood culture, probable sepsis i.e. neonates with two or more signs suggestive of sepsis with at least one abnormal laboratory parameters, or one or more signs suggestive of sepsis with two or more abnormal laboratory parameters and no sepsis i.e. neonates with no signs of sepsis or abnormal lab parameter. Blood samples from neonates were collected and procalcitonin and CRP levels were assayed in serum using enzyme linked immunofluorescence assay and immune turbidometry method respectively. Haematological parameters were analyzed by coulter counter.

Statistical analysis

Fishers exact and chi square test were used to analyses the difference between categorical variables amongst different groups. Parameters were not normally distributed and did not show homogeneity of variance. Median test was used for multiple group comparison. Mann-Whitney U test was used for between group comparisons. PCT was log transformed since it was not normally distributed and extreme values were removed for regression analysis. Multiple linear regressions were done to find the independent effects of perinatal variables on procalcitonin. ROC analysis was done to determine the predictability of PCT and CRP to diagnose neonatal sepsis. SPSS version 21 was used for analysis.

RESULTS

100 samples were collected from neonates. The signs and symptoms considered for diagnosis of neonatal sepsis are included in Table 1. Demographic features of the neonates and risk factor distribution of neonatal sepsis in different groups are elaborated in Table 2. PCT (p=0.049) and CRP (p=0.008) were positive, significantly in neonates with foul smelling liquor compared to those without it (Table 2).

Table 1: Signs and symptoms in neonatal sepsis.

General	Hypothermia/ poor feeding/ sclerema/ mottling/ lethargy
CVS	Bradycardia/ tachycardia/ CFT>2 seconds
RS	Apnea/ RDS/ chest retractions/ cyanosis/ grunting
CNS	Hypotonia/ irritability/ seizures/ high pitched cry
GIT	Vomiting/ abdominal distension/hepatomegaly
Hematology	Jaundice with serum bilirubin <15 mg% in the absence of blood group abnormality/pallor/ petichiae/bleeding diathesis
Total leukocyte count	<5000/cu mm
Absolute neutrophil count	>5000/cu mm
Band cell count	>20%

Findings of the comparison of birth weight, procalcitonin, CRP, total count, absolute neutrophilic count and band cell ratio between the groups are elaborated in Table 3. PCT (p<0.001), CRP (p<0.001), TC (p=0.019) and ANC (p=0.024) differed significantly amongst the groups. PCT was significantly (p<0.001) high in probable sepsis and definite sepsis compared to no sepsis (Table 3). PCT was significantly (p=0.007) high in probable sepsis compared to definite sepsis. CRP was significantly (p<0.001) high in Probable sepsis compared to no sepsis. Total count was significantly (p=0.012) in definite sepsis compared to probable sepsis (Table 3).

Independent effects of gender, gestational age, birth weight, PROM, vaginal examination, intrapartum fever, meconium stained liquor, maternal urinary tract infection and prolonged or instant labor on variation in procalcitonin is assessed in non-sepsis patients by multiple regression. None of the variables are correlated significantly with procalcitonin. Only 12% of variation in procalcitonin in non-sepsis neonates was explainable due to the independent effect of perinatal variables and this further decreased to 2% when adjusted for collinearity (Table 4).

Sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio of procalcitonin as well as CRP at cutoff values selected to achieve 90% sensitivity and maximum you dens index possible are elaborated in Table 5.

Table 2: Demographic and risk factor characteristics of patients included in the study.

	All	All Sepsis (n:33)	Definite Sepsis (n:9)	Probable sepsis (n:24)	No sepsis (n:67)	PCT+n; (% in all)	CRP+n; (% in all)
Gender	110					34	22
Male	55	16	06	10	39	18	12
Female	45	17	03	14	28	16	10
Birth weight <2.5 kg	28	12	03	09	16	12	9
Birth weight >2.5 kg	72	25	10	15	50	22	13
Gestational age <37 wk	23	12	04	08	11	10; (43.5%)	8; (34.8%)
Gestational age >37 wk	77	22	06	16	54	24; (31.2%)	14; (18.2%)
Meconium stained liquor	60	20	7	13	40	20; (33.3%)	13; (21.7%)
PROM	25	8	01	07	17	9; (36.0%)	5; (20%)
Prolonged or Inst Del	12	2	1	1	10	4; (33.3%)	2; (16.7%)
Maternal UTI	5	1	00	01	04	1; (20.0%)	0; (0%)
> 3 Vaginal examination	5	3	1	2	2	3; (60.0%)	2; (40%)
Foul smelling liquor	2	02	0	02	0	2; (100.0%) *	2 (100%) **
Intrapartum fever(>38*C)	2	0	0	0	02	0 (0%)	0 (0%)
Maternal infections	0	0	0	0	0	0 (0%)	0 (0%)
General	27	11	01	10	16	11	07
RS	53	22	6	16	31	23	12
GIT	25	12	4	8	13	11	8
CNS	13	6	2	4	7	7	6
CVS	7	3	1	2	4	3	1
Hematology	4	1	0	1	3	1	0
Procalcitonin (ng/ml)							
Negative (<0.50)	66	4	4	0	62	NA	0
Positive (>0.50)	34	29	5	24	5	NA	22
CRP (mg/dl) negative (<5.0)	78	13	4	9	65	12	NA
CRP (mg/dl) positive (>5.0)	22	20	5	15	2	22	NA
Blood culture negative	91	24	0	24	67	36	17
Blood culture positive	9	9	9	0	0	5	5
Coag Neg staph	1	1	1	0	0	0	0
E. Coli	2	2	2	0	0	2	2
Klebsiella	4	4	4	0	0	2	2
Pseudomonas	2	2	2	0	0	1	1
Total count >5000	90	8	8	24	58	32	21
Total count <5000	10	1	1	0	9	02	1
Absolute neutrophil count >1000	100		9	24	67	34	22
Absolute neutrophil count <1000	0	0	0	0	0	0	0
Band cell ratio <20%	96		6	24	66	31	20
Band cell ratio >20%	4		3		1	3	2

Legend to Table 2: PCT and CRP were positive significantly in neonates with foul smelling liquor. *p=0.049;**p=0.008. Fishers exact test was used to analyses the difference between groups.

Predictability of procalcitonin and CRP for the diagnosis of neonatal sepsis analyzed by ROC curve revealed, better predictability of procalcitonin (area under curve - 0.92) compared to CRP (area under curve - 0.74) (Figure 1).

DISCUSSION

The study showed significantly high procalcitonin and CRP levels in sepsis when compared to neonates with no sepsis. In healthy people, plasma PCT concentrations are found to be below 0.05 ng/ml.

Table 3: Comparison of parameters in definite, probable and no sepsis groups.

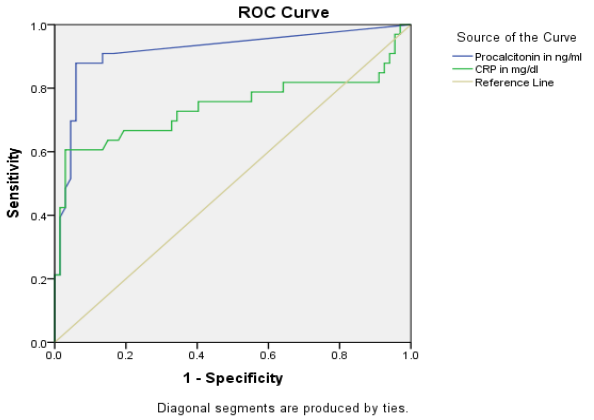
Median test	No sepsis median (IQR)	Probable sepsis median (IQR)	Definite sepsis median (IQR)
Birth weight in kg	2.8 (0.59)	2.6 (2.12)	2.7 (0.74)
Procalcitonin (PCT) in ng/ml	0.05 (0)	2.85 (3.68)	1.2 (1.98)
CRP in mg/dl	3.11 (1.94)	12.1 (23)	6.18 (9.16)
Total count (TC) in per cubic mm	7400 (5200)	9650 (6550)	15000 (14150)
Absolute neutrophilic count (ANC)	51 (12)	56.5 (17.5)	60 (19)
Band cell ratio (BCR)	4 (2)	6 (4.75)	6 (20)
Legend to Table 3: PCT ($p<0.001$), CRP ($p<0.001$), TC ($p=0.019$) and ANC ($p=0.024$) differed significantly amongst the groups. Median test was used for multiple group comparison. PCT was significantly ($p<0.001$) high in probable sepsis and definite sepsis compared to no sepsis. PCT was significantly ($p=0.007$) high in probable sepsis compared to definite sepsis. CRP was significantly ($p<0.001$) high in Probable sepsis compared to no sepsis. Total count was significantly ($p=0.012$) in definite sepsis compared to probable sepsis. Mann-Whitney U test was used for between group comparisons.			

Table 4: Independent effects of perinatal variables on procalcitonin in non-sepsis neonates.

	Mean	SE	Correlation factor	b	β
Sex (Male)	58%	5%	0.16	0.192	0.203
Gestational age (<37 week)	18%	4%	0.04	0.026	0.022
Birth weight in kg	2.7	0.56	0.05	-0.013	0.292
Premature rupture of membrane	25%	44%	0.01	0.313	0.292
Vaginal examination >3 time	3%	17%	-0.07	0.159	0.058
Intrapartem fever	3%	17%	0.1	0.367	0.134
Meconium stained liquor	6%	5%	0.09	0.286	0.302
Maternal urinary tract infection	3%	17%	-0.07	0.062	0.023
Prolonged or instant labour	15%	36%	0.18	0.334	0.255
Legend to Table 4: Multiple linear regressions were done to find the independent effects of perinatal variables on procalcitonin. Procalcitonin was transformed to log (procalcitonin). None of the variables are correlated significantly with procalcitonin. Only 12% of variation in procalcitonin in non-sepsis neonates was explainable due to the independent effect of perinatal variables and this further decreased to 2% when adjusted for collinearity. This model cannot explain the variation 47% of the time. The findings of independent effects are not significant. [$r^2 = 0.12$; adjusted $r^2 = -0.02$; SE (standard error) = 0.47; p for F test =0.57].					

Table 5: Performance of procalcitonin and CRP for the diagnosis of neonatal sepsis.

	Procalcitonin in ng/ml cut off points selected to achieve 90% sensitivity	Procalcitonin in ng/ml cut off points selected to achieve maximum you den's index	CRP in mg/dl cut off points selected to achieve 90% sensitivity	CRP in mg/dl cut off points selected to achieve maximum you den's index
Cutoff	0.16	1.04	1.18	5.52
Sensitivity in %	90.9	88	90.9	60.6
Specificity in %	86.6	94	6	97
You den's index	0.775	0.819	-0.031	0.576
Positive predictive value in %	76	87	33	91
Negative predictive value in %	95	94	57	83
Positive likelihood ratio	6.7	14.6	18.18	0.63
Negative likelihood ratio	0.105	0.127	1.51	0.4
Legend to Table 5: You dens index (sensitivity + specificity) -1.				



Legend to Figure 1: Area under curve for Procalcitonin and CRP for the diagnosis of neonatal sepsis were 0.92 (95% CI;0.85-0.99) and 0.74 (95% CI;0.61-0.87) respectively; p<0.001 for both.

Figure 1: ROC curve procalcitonin and CRP for the diagnosis of neonatal sepsis.

PCT concentrations can increase up to 1,000 ng/ml in patients with sepsis, severe sepsis or septic shock. Usually, PCT concentrations exceeding 0.5 ng/ml are interpreted as abnormal values suggestive of a sepsis syndrome. Concentrations above 10 ng/ml are almost exclusively found in patients with severe sepsis or septic shock.[7] Several studies have shown that PCT concentration increases in the serum within 2-3 hours of beginning of infection peaking by 6-12 hours and returning to normal concentrations in 2 days. This short half-life of PCT i.e. 20-24 hours enables not only rapid detection but also response to treatment.[8]

PCT can increase physiologically in asymptomatic uninfected neonates. It has been suggested that postnatal physiological surge of PCT most likely represents endogenous synthesis and this phenomenon was attributed to direct stress on the baby during perinatal period or to adaptation to extra uterine environment. Even with physiological peak PCT is useful in the diagnosis and monitoring of neonates at risk of infection.[9]

It has been shown that a great amount of PCT is produced in human liver cells after TNF-α and IL-6 stimulation. The PCT level remains continuously high despite a decrease in TNF-α and IL-6 levels in parallel with the severity of the ongoing infection.[2] PCT has been shown to be useful not only in the diagnosis but also monitoring prognosis and response to treatment of patients with neonatal sepsis.[10] The return to baseline is usually rapid and a second increase of PCT can be interpreted as the development of a new sepsis episode.

Procalcitonin levels were not significantly affected by multiple independent risk factor variables in neonates with no sepsis. However some of the variables showed minor degree of influence on the procalcitonin variability. The effect of prolonged labor on procalcitonin levels could be due to perinatal asphyxia and hypoxia causing stress to the neonate during the labour.[11] In our study independent variables like low birth weight, gestational age <37 weeks, maternal urinary tract infections, meconium stained liquor affected the level of procalcitonin in asymptomatic neonates. However the findings were of no significance. This could be due to the fact that asymptomatic patients were all early neonates and PCT is a more reliable marker for early onset sepsis within the first 12 hours of life than CRP or IL6.[10]

Such patients might end up with neonatal sepsis on follow up. Other markers of neonatal sepsis, signs and symptoms and blood culture are affected by various other factors, whereas procalcitonin is the earliest one to be elevated.[11]

Several studies have shown sensitivity of procalcitonin for early diagnosis of neonatal sepsis varied from 83-100% while the specificity varied from 73-100%, our study also showed comparable performance characteristics of procalcitonin. However, less than 100% specificity of procalcitonin shown in our study could be due to the effect of other independent variables. Several studies have reported the increased levels of procalcitonin in asymptomatic neonates with perinatal asphyxia, prolonged labor, PROM and maternal infection have negatively affected procalcitonin specificity.[12] Procalcitonin showed good predictability for diagnosing neonatal sepsis compared to CRP which showed moderate predictability (Figure 1). Several studies have shown similar results.[13-15]

The sample size taken in our study is not sufficient to achieve 80% statistical power. Since we have compared asymptomatic neonates without evidence of infection with the symptomatic sepsis group, which may increase the unreliability of procalcitonin as a diagnostic test when used in population of probable sepsis.[16]

In our study we have not compared the effect of independent variables on CRP variability. Metacentric comparison of procalcitonin with other markers of sepsis, including the study of effect of other variables on its variability in a large size population is needed, to overcome these limitations.

CONCLUSION

Procalcitonin is found to be a better predictor of neonatal sepsis compared to CRP and is not affected by independent variable significantly, which needs to be confirmed by large population studies.

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