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PROCALCITONIN AND CRP MARKERS IN NEONATAL SEPSIS

Neonatology

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ABSTRACT

Introduction: Neonatal sepsis is a major cause of mortality in developing countries. Accurate and quick diagnosis IS difficult because clinical presentation is non-specific, bacterial cultures are time-consuming and other laboratory tests lack sensitivity and specificity. Serum procalcitonin (PCT) and C Reactive Protein (CRP) have been proposed as an early marker of infections in neonates.

Aims & Objectives: - To study the sensitivity and specificity of CRP and PCT in diagnosis of Neonatal sepsis and to study the role of serial quantitative CRP and PCT in guiding course and duration of antibiotic therapy in Neonatal sepsis.

Method:- Neonates undergoing sepsis evaluation at the Neonatal Intensive Care Unit, Hindu Rao Hospital between September 2013 to September 2015 were included. Blood samples were obtained for white cell count, blood cultures, serum CRP and PCT analysis. Neonates were categorised into Proven Sepsis, Suspected Sepsis and Clinical Sepsis groups on the basis of laboratory findings and risk factors. A control group with no clinical and biological data of infection was also included. Predictive values and area under the receiver operating characteristic curve (AUC) of PCT were evaluated.

Results: Out of the various individual tests for rapid diagnosis of neonatal septicemia, in combined definite & clinical sepsis group, CRP was positive in (73.33%) with sensitivity of (73.33%), specificity of (86.67%), PPV (92.27%), NPV (58.21%) with p value of (<0.001) which is statistically highly significant in combined sepsis. For early and rapid detection of neonatal sepsis in definite & clinical sepsis group, PCT was positive in (91.43%) with sensitivity of (91.43%), specificity of (91.11%), PPV (96.00%), NPV (82.00%) with p value of (<0.001) which is statistically highly significant in definite & clinical sepsis. In our study it is evident that PCT is early and better marker than CRP in detecting septicemia, PCT has better sensitivity, specificity, PPV & NPV than CRP in all weight groups. It is also observed that sensitivity, specificity, PPV and NPV of the PCT & CRP were higher in >2500 grams babies than other two groups. From our study it was evident that the recommended mean duration of therapy was much lesser than what was used routinely. In definite sepsis mean duration of therapy was (9.43+-3.93 days), in clinical sepsis (7.11+-2.84 days), in sepsis ruled out group (2.42+-1.1days) by serial measurement of PCT.

Conclusion: - These findings support the usefulness of the PCT in diagnosis and duration of treatment of Neonatal sepsis.

KEYWORDS

Neonatal Sepsis, Procalcitonin, Receiver Operating Characteristic Curve.

INTRODUCTION

Neonatal sepsis is one of the important causes of neonatal morbidity and mortality particularly in the developing countries1. An early diagnosis of neonatal septicaemia helps the clinician in instituting antibiotic therapy at the earliest, thereby reducing the mortality rates in the neonates. Early recognition and diagnosis of neonatal sepsis are difficult because of its variable and nonspecific clinical presentation. Isolation of the causative microorganisms by using blood culture has been the gold standard method for its diagnosis. However, as pathogens in blood cultures are only detected in approximately 25% of patients, the sensitivity of blood culture is suspected to be low2. Besides, it is impractical to ob tain blood sample for serial blood culture from infants3. serum procalcitonin (PCT) is one of the most promising4 test. Firstly demonstrated to increase at the onset of bacterial infection and sepsis by Assicot et al in 19935, this acute phase reactant has the characters of acute phase proteins, hormones and cytokines. This study aims at evaluating procalcitonin as an early or first line marker in the diagnosis of neonatal septicemic infection.

AIMS & OBJECTIVES: - To study the sensitivity and specificity of CRP and PCT in diagnosis of Neonatal sepsis. To study the role of serial quantitative CRP and PCT in guiding course and duration of antibiotic therapy in Neonatal sepsis.

METHODS: -This study was conducted in Neonatal intensive care unit, department of pediatrics, Hindu Rao Hospital, New Delhi from September 2013 to September 2015. Study design- Hospital based Prospective and Observational study. Inclusion Criteria- 1. Newborns (Upto age 28 days)admitted in NICU for suspected sepsis (Newborn with high risk factors, and /or sepsis screening is indicated on the basis of Perinatal Risk Scoring System given). 2. Patient population: All newborns (Preterm or Term or Intra Uterine Growth Retardation (IUGR), different gestational age admitted in NICU with Early Onset Sepsis (EOS) and Late onset sepsis (LOS).Exclusion Criteria- 1. Newborns having major congenital malformations. 2. Birth weight <1000 grams. 3. Newborn infants who received antibiotics prior to septic work-up excluded. 4. Second episode (2nd episode) and/or Reinfection. Enrollment of subject: Minimum 150 eligible neonates with above inclusion criteria were studied. 1. Very low birth weight/ <1500grams= minimum 50 cases. 2. Low birth weight/ 1500-2500grams= minimum 50 cases. 3. Normal / >2500grams= minimum 50 cases. Outcome- Primary- Duration of antibiotic treatment days for first episode of infection. Secondary- 1. Clinical Cure. 2. Death due to Sepsis. Stratified group according sepsis: 1. Definite sepsis (culture proven sepsis). 2. Clinical sepsis / probable sepsis. 3. Sepsis ruled out / No sepsis. The standard unit protocol for management of infants with suspected sepsis was to obtain the blood for complete blood count (for Differential white blood cell count, ANC, I/T ratio, m-ESR, peripheral smear for toxic granules and platelets), CRP, PCT and a blood culture at presentation. The sterile BAC-TEC system was used for blood cultures. Repeat blood cultures were done only in those patients with suspected nosocomial sepsis. Lumbar punctures were performed only if there was a suggestion of central nervous system involvement. All those babies eligible for inclusion criteria in the study were investigated for suspected sepsis within the first 6 h of the disease onset and or admission in the NICU and complete hemogram, blood culture and serial PCT & CRP was done on 3rd, 5th, 7th, 10th, 14th, 18th& 21st day if needed in persistent sepsis cases. CRP: analysis was done using Immunoturbidometry Method, This is a quantitative analysis where levels greater than 10mg/ liter is considered as positive. Procalcitonin: PCT level analysis was done using IMMUNO NEPHLOMETRY ASSAY FOR PROCALCITONIN BY VIDAS BRAHMS PCT KIT manufactured by BIOMERIUX INDIA (P) LTD. This is a quantitative assay where levels less than 0.5ng/ml is normal and greater than 0.5ng/ml is high risk for neonatal septicemia. The study group included those neonates with a negative PCT (<0.5ng/ml) or CRP (<10 mg/L) both at presentation and after 24 to 48 h, Antibiotic therapy was stopped after 48 h in these infants. For the study purpose

the babies were not discharged at this point but were kept under observation until the final blood culture results at 72 h were available. If the culture was positive or the baby's condition deteriorated, antibiotic therapy could be recommenced. If the babies were clinically well and the cultures were negative, the babies were eligible for discharge. This was in line with the standard unit practice. The attending physician could override the study protocol and continue antibiotic therapy, despite the negative PCT & CRP results, if it was thought that the baby's clinical condition warranted this. If the CRP & PCT levels abnormal then neonates were divided into definite sepsis (culture proven sepsis) v/s clinical sepsis (signs & symptoms of sepsis without culture positivity), then antibiotic therapy monitored by serial CRP & PCT levels, and antibiotic were stopped if the consecutive two PCT and or CRP levels are negative. Guidelines for starting antibiotics: Clinical sepsis or sepsis screen positive i. e 2 of the 5 criteria, blood culture (Bactac method) sample was taken before starting antibiotics. 1. Positive sepsis screen (any of two): A. TLC <5000 cells/cm. B. ANC <1500cells/cm. C. I/T Neutrophil ratio > 0.2. D. CRP >10 mg/l. E. Micro-ESR >15mm in one hour. 2. C- Reactive Protein (CRP) Group: >10mg/l taken as abnormal. 3. Procalcitonin (PCT) Group: Procalcitonin level >0.5ng/ml taken as abnormal. Serial measurement of C - reactive protein (CRP) and Procalcitonin (PCT) every 48 or 72 hours to monitor, guiding and stoppage of antibiotic therapy. Guidelines for Stopping Antibiotics- 1. C-Reactive Protein: When the serial two consecutive CRP values 24hours apart (CRP) <10mg/l in all sepsis groups and or 2. Procalcitonin: When the serial two consecutive PCT values 24hours apart <0.5ng/ml, and child clinical condition improved was under observation for 48 hours after stoppage of antibiotics. Criteria used: Early onset sepsis: Age at onset- Birth to 7days, usually <72 hours. Late onset sepsis: age at onset- 7 to 28 days. Proven or definite sepsis: Any newborn presents with clinical picture of sepsis along with isolation of organism from blood. Probable or clinical sepsis: any newborn having clinical picture suggestive of sepsis with any of following criteria:- 1. Existence of high risk factors >3 (risk factors are additive and present of more than 2 factors increases the risk of sepsis many fold). Sepsis ruled out: sepsis was ruled out in all symptomatic infants with a negative blood culture and normal values of PCT and CRP on two occasions 24-48 hours apart with improved clinical condition, as well as in all asymptomatic infants with a negative blood culture and 2 normal values of PCT and CRP. The statistical programme for social sciences (SPSS) computer software was used to analyse the data. - 1. Chi Square test to calculate P values where the data is in groups. 2. Student's T-Test to calculate P values where the data is in actual values. The P values are interpreted as:- A. Significant- if p<0.05. B. Highly significant- if p<0.01 C. Very highly significant- if p<0.001

RESULTS: - of the 150 neonates 58 were definite sepsis, 47 were clinical sepsis and sepsis ruled out in 45 neonates. It is observed that in our study 86(57.33%) male babies were affected by septicemia and 64(42.66%) Female babies were affected by septicemia.

In our study, the mean gestational age of the neonates who had definite sepsis was 35.44+ 3.03 (SD) weeks and for the neonates who had clinical sepsis and sepsis ruled out was 35.19 +3.40 (SD) weeks, 36.77+2.31 (SD) weeks respectively and this difference was statistically significant (p=0.05) in the both definite sepsis v/s sepsis ruled out group. CRP has higher sensitivity (77.42%), specificity (89.47%), PPV (92.31%) and NPV (70.83%) which is better in detecting >2500gms neonatal sepsis group than other 2 groups which has slightly lower sensitivity, specificity, PPV and NNP. CRP was statistically significant in detecting neonatal sepsis in all 3 groups of sepsis (p<0.001).(Table-1)

Table-1: Significance of CRP in different weight groups according to sepsis and gestational weight

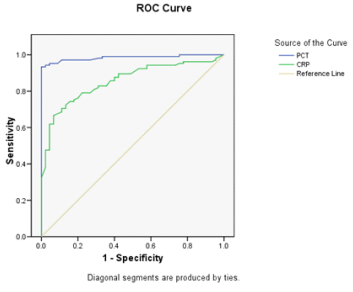
Sepsis →	Definite& Probable		No sepsis		p-value	Odds Ratio	95% CI for Odds Ratio		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Birth weight (gms) ↓	n	%	n	%			Lower	Upper				
1000-1500	29	72.50	3	30.00	0.006	6.152	1.484	25.496	72.50	70.00	90.63	38.89
1500-2500	24	70.59	2	12.50	<0.001	16.800	3.973	71.043	70.59	87.50	92.31	58.33
>2500	24	77.42	2	10.53	<0.001	29.143	6.917	122.790	77.42	89.47	92.31	70.83

PCT has higher sensitivity (93.55%), specificity (89.47%), PPV (93.55%) and NPV (89.47%) which is better in detecting >2500gms neonatal sepsis group than other 2 groups which have slightly lower sensitivity, specificity, PPV and NNP. PCT has statistically significant in detecting neonatal sepsis in all 3 groups of sepsis (p<0.001). (Table-2)

Table-2: Significance of PCT in different weight groups according to sepsis and gestational weight

Sepsis →	Definite Probable sepsis		No sepsis		p-value	Odds Ratio	95% CI for Odds Ratio		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Birth weight (gms) ↓	n	%	n	%			Lower	Upper				
1000-1500	35	87.50	3	30.00	<0.001	16.333	3.879	68.778	87.50	70.00	92.11	58.33
1500-2500	32	94.12	2	12.50	<0.001	112.000	22.557	556.113	94.12	87.50	94.12	87.50
>2500	29	93.55	2	10.53	<0.001	123.250	24.702	614.948	93.55	89.47	93.55	89.47

DISCUSSION: - Neonatal sepsis still remains a diagnostic and treatment challenge for the neonatal health care providers. This challenge leads to the over treatment of large number of neonates who present with clinical suspicion of sepsis6. In recent years measurement of procalcitonin and other inflammatory mediators have been reported as sensitive parameters for the early diagnosis of neonatal sepsis and evaluating its outcome7. The aim of this study was to evaluate PCT as diagnostic marker for neonatal sepsis. The incidence of culture-proven sepsis was low (38.8%). This was higher than the reports of incidence of 20% and 25.7% by Adeleke and Belonwu, 20068, and Naher and Khamel, 20139 respectively. In our analysis of this study evaluating PCT in neonates with and without sepsis, the area under the ROC curve for PCT was 0.984 with 95% CI (0.967-1.001), and the sensitivity and specificity were 95.24% and 91.11%, respectively with p-value (p<0.001) and for CRP area under the ROC curve for was 0.855 with 95% CI (0.795-0.915), and the sensitivity and specificity were 80.95% and 71.11%, respectively with p



value (P<0.001). (Fig-1)

Fig-1. ROC Curve of PCT and CRP for the diagnosis of the neonatal sepsis

PCT levels were high in the neonates with proven and suspected sepsis cases. This finding was similar with reports of some studies10, 11. The postnatal increase of PCT observed in the healthy neonate with peak values at 24 h of age most likely represents endogenous synthesis12. In this study, at a cut-off point of 0.5ng/ml, the sensitivity and specificity, PPV and NPV of PCT in neonatal sepsis was found to be 96.43% and 91.11%, 96%(PPV) and 82%(NPV) respectively for proven infection. This high sensitivity and NPV of PCT is consistent with the reports of Ballot et al., 200413in South Africa and Sucilathangam11, et al., in 2012 in India. White, et al., in 200714in South Africa recorded similar report of NPV of 80%, but a lower sensitivity of 48%. NPV and Sensitivity increased to 100% and specificity 56% as cut off values increased to 10 while PPV dropped to 21%.In the study of White, et al., in 200714, increased cut-off value (10.1 ng/ml) had no effect on the NPV, worsened the sensitivity (98% v. 22%, respectively), but

improved the PPV (78% v. 79%, respectively), and the specificity (74%v.98%). ROC analysis for PCT had an area under the curve (AUC) of 0.984 which is higher than reports of White, et al., 200714, where ROC analysis had an area of 0.631. Boraey, et al., 201215 reported an AUC value of 0.92 for PCT at a cut off value of 1.3ng/ml. AUC values were 0.662 and 0.658 for Preterm and Term neonates respectively without any significant statistical difference. This is in agreement with the reports of White et al., 200714.Also, no significant statistical difference was found between the AUC values of PCT in EONS and LONS cases. This suggests that the PCT seems to be equally accurate for the diagnosis of neonatal sepsis in preterm and term neonates; as well as in EONS and LONS cases. Overall mortality was (11.3%), mortality in definite sepsis was (9.33%), in clinical sepsis was (1.3%) and sepsis ruled out was (0.66%).

The result from this study suggests PCT as a good predictor of mortality as almost all neonates who died (96.3%) had elevated PCT. This is in agreement with Adib, et al., 201216. In our study it is evident that PCT is early and better marker than CRP in detecting septicemia, PCT has better sensitivity, specificity, PPV & NPV than CRP in all weight groups. It is also observed that sensitivity, specificity. PPV and NPV of the PCT & CRP were higher in >2500 grams babies than other two groups. From our study it is evident that the recommended mean duration of therapy is much lesser than what was used routinely. In definite sepsis mean duration of therapy was (9.43+-3.93 days), in clinical sepsis (7.11+-2.84 days), in sepsis ruled out group (2.42+-1.1days) by serial measurement of PCT. (Table-3)

Table- 3: PCT guided duration of antibiotic therapy (in days)

Diagnosis	n	Days of Antibiotic		p-value
		Mean	±SD	
Definite Sepsis	58	9.43	3.93	< 0.001
Clinical Sepsis	47	7.11	2.84	
No Sepsis	45	2.42	1.10	

CONCLUSION:- Our study concludes that PCT is the earliest marker to rise in neonatal sepsis and better marker in diagnosing neonatal sepsis, it helps in starting the antibiotics as early as possible in neonatal sepsis. Benefit of the study is a possible limitation of unnecessary use of antibiotics. On a population level, unnecessary long-term use of broad-spectrum antibiotics is a serious concern because it can promote the development of resistant bacteria, which will result in untreatable infections over time.

Limitations: - The sample size in our study was small. Therefore, larger sample size needs to be considered in future endeavour of study.
Source of Support: - Nil

Conflict of Interest: - None declared

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