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Original Research Article

Study to correlate sepsis markers and blood culture in neonatal sepsis

Harshitha M. Swamy[1], Lakshmi[1]*, Mallesh K.[1], Asima Banu[2]

[1]Department of Paediatrics, [2]Department of Microbiology, Bangalore Medical College and Research Institute, Bangalore, Karnataka, India

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***Correspondence:**
Dr. Lakshmi,
E-mail: lakshmikeshava39@gmail.com

ABSTRACT

Background: Neonatal sepsis forms the second most common cause of neonatal mortality resulting in more than one million neonatal deaths per year. Neonatal sepsis, pneumonia and meningitis together result in one- fourth of all newborn deaths. Objectives of the study was to correlate sepsis markers with blood culture in neonatal sepsis.
Methods: A cross sectional study was carried out in the NICU unit under department of Pediatrics, between November 2017 and May 2019. Sample size was 50. Babies admitted to NICU with clinical suspicion of sepsis were included in the study. Blood samples from these babies were collected under aseptic precautions and subjected to rapid diagnostic tests- sepsis markers and blood culture.
Results: Male were predominant (64%). Important risk factors were preterm and low birth weight. Blood culture positivity was 20% (*E. coli* being most commonly isolated organism). CRP had a high sensitivity of 90% and low specificity of 47%. Procalcitonin had highest sensitivity of 100% and low specificity of 47.5%.
Conclusions: CRP and PCT were found to be statistically significant (p=0.036 and 0.01), can be used as a diagnostic tool in neonatal sepsis.

Keywords: Blood culture, Diagnosis of sepsis, Neonatal sepsis, Sepsis markers

INTRODUCTION

Neonatal sepsis is one of the leading causes of neonatal mortality contributing to one- fourth of all neonatal deaths along with pneumonia and neonatal meningitis.[1]

Neonatal sepsis refers to the clinical syndrome characterized by signs and symptoms of infection with or without bacteraemia in the first 28 days of life.[2] The clinical signs of sepsis are non- specific and can be mimicked by other disorders.

The organisms causing neonatal sepsis are different in different countries. Most commonly, gram negative organisms are found to be associated as per Indian studies.[3] In the presence of high risk factors and clinical suspicion, early treatment with the support of indirect marker will help us decrease the neonatal morbidity and mortality.[4] Help of indirect markers such as neutropenia, leukopenia, thrombocytopenia, CRP and procalcitonin can be taken for early treatment with antibiotics.

The gold standard for the diagnosis of neonatal septicaemia is a positive blood culture. Definitive culture results take at least 48-72 h, resulting in treatment delays. Hence, certain rapid diagnostic tests such as C reactive protein (CRP), Procalcitonin, total leucocyte count (TLC), absolute neutrophil count (ANC), and platelet count (PC) collectively can be used. These indirect markers will help detect neonatal sepsis earlier which will

enable the clinician to treat the infection timely and thus it will help to reduce neonatal morbidity and mortality.[5]

Septic markers that will be considered in this study will be: CRP considered positive if >1mg/dl., Total leukocyte count (TLC) (leukopenia): <5000 cells/cumm., ANC (neutropenia): <1800 cells/cumm., PC (thrombocytopenia): <1.5 lakhs/cumm.

METHODS

The neonates who were admitted in the NICU unit under Department of Pediatrics, Tertiary care hospital; Bangalore Medical College and Research Institute, between the period of November 2017 to May 2019 were considered for the study.

Statistical analysis

The collected data was analysed by software- Stata version 12, the following statistical methods were used to test the hypothetical results

- Chi-square test
- Fischer's exact test

Inclusion criteria

Neonates admitted to NICU with signs and symptoms of clinical sepsis. (as per NNF criteria)

Neonates satisfying any of the following will be termed as clinical sepsis. The criteria include: Poor feeding, Irritability/ excessive cry, lethargy, poor cry and reflexes, fever, hypothermia, jaundice, vomiting, abdominal distension, tachypnoea and grunting, convulsions, diarrhoea, pustules, sclerema, cyanosis, bulged fontanelle, DIC/ bleeding, poor perfusion/ shock, apnoea.3

Exclusion criteria

- Congenital anomalies of gastro-intestinal system, e.g. tracheoesophageal fistula, malrotation of the gut.
- Congenital anomalies of respiratory system, e.g. lobar agenesis
- Congenital anomalies of the cardiovascular system, e.g. TGA, complex heart diseases.
- Inborn errors of metabolism
- Congenital anomalies of central nervous system, e.g. microcephaly, anencephaly, other neural tube defects etc., 4
- Neonates who have received antibiotics before admission.[5]

After obtaining institutional ethics committee clearance and written informed consent (Annexure I), the outpatients in the department of medicine fulfilling the inclusion and exclusion criteria will be enrolled in the study.

The blood samples will be collected from the 50 neonates with clinical suspicion of sepsis and will be sent for CBC, CRP, procalcitonin and microscopy and Blood culture via BACTEC. Paired 2-3-ml blood samples will be drawn at the same time and by the same route (peripheral puncture, central line, or percutaneously inserted central catheter). The first sample will be used for the reference standard method, TBC, and will be inoculated into paediatric blood culture BACTEC PEDS PLUS/F bottles, following the manufacturer's recommendations.

RESULTS

In the present study, of the total neonates, 26 (52%) were males and 24 (48%) were females and 38(76%) were preterm (<37 weeks of gestation) and 12(24%) were term neonates (>37 weeks of gestation), 41(82%) were ≤2.5 kg and 9(18%) were of >2.5 kg. Among the 50 neonates with clinical sepsis, most common maternal risk factor was found to be maternal fever and maternal UTI seen in 6 cases each (12%), followed by >3 per- vaginal examination and chorioamnionitis as risk factors. Seen in 4 cases each (8%). The least common maternal risk factor was found to be premature rupture of membranes 3(6%).

Data wise 20(40%) neonates had non- specific signs, followed by cardiovascular and respiratory system signs in 16(32%) babies each, then by 14(28%) neonates with gastrointestinal tract symptoms, then central nervous system signs in 11(22%) neonates and lastly 7(14%) babies with haematological signs (Table 1).

Table 1: Sign of sepsis in different systems among neonates with clinical sepsis.

Sign of sepsis in	Frequency	Percentage*
Cardiovascular system	16	32.0
Respiratory system	16	32.0
Gastrointestinal tract	14	28.0
Central nervous system	11	22.0
Haematology	7	14.0
Non-specific	20	40.0

* Percentage calculated against 50 and does not add to 100%

Blood culture in 40(80%) neonates showed no growth. 10(20%) had blood culture positive with 4(8%) showing Escherichia coli (E. coli) growth, 3(6%) each showing Gram negative non- fermentous bacteria (GNNF) and klebsiella growth (Table 2).

On analysing the Table 3, CRP has Sensitivity= 90%; Specificity= 47.5%; Positive predictive value= 30%; Negative predictive value= 95%; Cohen's Kappa= 0.2143 (no strong agreement) and p Value= 0.0366 (significant association).

Table 2: Blood culture results among neonates with clinical sepsis.

Blood culture	Frequency	Percentage
Escherichia Coli	4	8.0
Gram negative non-fermentative (GNNF)	3	6.0
Klebsiella	3	6.0
Sterile	40	80.0
Total	50	100

Table 3: Diagnostic validity of C-reactive protein in diagnosis of bacterial infection among neonates with clinical sepsis.

C-reactive protein	Blood culture		Total	p value*
	Bacteria detected, n (%)	Sterile, n (%)		
>1 mg/dl	9 (90.0)	21 (52.5)	30 (60.0)	0.0366
≤1 mg/dl	1 (10.0)	19 (47.5)	20 (40.0)	
Total	10 (100.0)	40 (100.0)	50 (100.0)	

* Fishers Exact test

Leucopenia has Sensitivity= 20.0%; Specificity= 90.0%; Positive predictive value= 33.3%; Negative predictive value= 81.8%; Cohen's Kappa= 0.118 and p value= 0.586. Thus, there was no strong agreement (Cohen's kappa= 0.118) and no significant association between TLC< 5000cells/ cumm and blood culture (p value= 0.586) (Table 4).

Table 4: Diagnostic validity of leucopenia in diagnosis of bacterial infection among neonates with clinical sepsis.

Total leukocyte count	Blood culture		Total	p value*
	Bacteria detected, n (%)	Sterile, n (%)		
<5000 cells/cumm	2(20.0)	4(10.0)	6(12.0)	0.586
≥5000 cells/cumm	8(80.0)	36 (90.0)	44(88.0)	
Total	10 (100.0)	40 (100.0)	50 (100.0)	

* Fishers Exact test

Table 5 shows, ANC has Sensitivity= 20.0%; Specificity= 97.5%; Positive predictive value= 66.7%; Negative predictive value= 83.0%; Cohen's Kappa= 0.237 and p value= 0.098. Thus, there was no strong agreement (Cohen's kappa= 0.237) and no significant association between ANC< 1800 cells/cumm and blood culture (p value= 0.098).

Table 5: Diagnostic validity of neutropenia in diagnosis of bacterial infection among neonates with clinical sepsis.

Neutrophil count	Blood culture		Total	p value*
	Bacteria detected, n (%)	Sterile, n (%)		
<1800 cells/cumm	2(20.0)	1(2.5)	3(6.0)	0.098
≥1800 cells/cumm	8(80.0)	39(97.5)	47 (94.0)	
Total	10 (100.0)	40 (100.0)	50 (100.0)	

* Fishers Exact test

In the present study, thrombocytopenia has Sensitivity= 60.0%; Specificity= 45.0%; Positive predictive value= 21.4%; Negative predictive value= 81.8%; Cohen's Kappa= 0.030 and p value= 0.776. Thus, there was no strong agreement (Cohen's kappa= 0.030) and no significant association between Platelet count <1.5 lakh/cumm and blood culture (p value= 0.776) (Table 6).

Table 6: Diagnostic validity of thrombocytopenia in diagnosis of bacterial infection among neonates with clinical sepsis.

Platelet count	Blood culture		Total	p value*
	Bacteria detected, n (%)	Sterile, n (%)		
<1.5 lakh/cumm	6(60.0)	22(55.0)	28(56.0)	0.776
≥1.5 lakh/cumm	4(40.0)	18(45.0)	22(44.0)	
Total	10 (100.0)	40 (100.0)	50 (100.0)	

* Chi-square test

Table 7: Diagnostic validity of elevated procalcitonin in diagnosis of bacterial infection among neonates with clinical sepsis.

Procalcitonin	Blood culture		Total	p value*
	Bacteria detected, n (%)	Sterile, n (%)		
>0.15 ng/ml	10 (100.0)	21 (52.5)	31 (62.0)	0.005
≤0.15 ng/ml	0 (0.0)	19 (47.5)	19 (38.0)	
Total	10 (100.0)	40 (100.0)	50 (100.0)	

* Fishers exact test

As per table 7, procalcitonin has Sensitivity= 100.0%; Specificity= 47.5%; Positive predictive value= 32.3%; Negative predictive value= 100.0%; Cohen's Kappa=

0.267 and p value= 0.005. Thus, there was no strong agreement (Cohen's kappa= 0.267) whereas, significant association between Procalcitonin >0.15ng/ml and blood culture (p value= 0.005).

DISCUSSION

In the present study, of the total neonates, 26 (52%) were males and 24 (48%) were females. It is noted that the male to female ratio from our study is comparable with the study by Khinchi et al, with a total sample size of 411, conducted in the year 2010.[6] This male preponderance that has been noted in the present study may be due to the x-linked immunoregulatory gene leading to host susceptibility for septicaemia.

Study by Hasan et al, carried out with a total of 50 babies in the year 2011, has similar distribution of gestational age among the neonates as compared to this study.[7]

El- din et al, studied neonatal sepsis in 304 neonates in the year 2015 and found that low birth weight babies predominated which was similar to the present study.[8]

PROM as a risk factor was seen 6% of the cases in our study had PROM which was close to the study by Bhat et al, Maternal fever was associated in 33.3% cases in the study by Verma et al., whereas it was associated in 12% of cases. Chorioamnionitis was seen in 8% of cases which was comparable with the study by Escobar et al, Maternal UTI was seen in 12% of the neonates in our study, which was comparable to the study by Bhat et al. The differences in the maternal risk factors in different studies could be due to the differences of rate of occurrence of the risk factors.[9-11]

Non-specific symptoms were most commonly observed, supported by the study by Verma et al, and Kabwe et al, this is followed by CVS and RS symptoms supported by Verma et al.[10, 12]

In the present study, author had 20% blood culture positive cases. Of that, 6% showed positive for *Klebsiella* species, which was comparable to the study by Madavi et al, and Jiang et al, study showed positive for *E. coli* in 8% cases, which was comparable to the study by Jiang et al, and Lancet Global- DeNIS collaboration.[13-15]

The present study showed sensitivity of CRP- 90%, which was comparable to the study by Dhanlakshmi et al, Negative predictive value was 95%, which was comparable to the study by Galhotra et al.[5,16]

Present study showed total leucocyte count had low sensitivity of 20%, which was comparable with the study by Rimon et al, and Hasan et al.[7,17]

Present study showed Absolute neutrophil count had a low sensitivity (20%) and a specificity of 97.5% which was comparable to Hasan et al.[7]

Study showed Thrombocytopenia had a sensitivity of 60% and specificity of 45%, comparable with Hasan et al.[7]

Sensitivity and negative predictive value in our study was 100% which was comparable to the study by Kocabas et al, carried out in the year 2007.[18]

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ANNEXURE I

Proforma for written informed consent

Date:

Place:

I, Mr/Ms/Mrs_____ son/ daughter/ wife of _____, aged _____ years have been explained in detail in my own understandable language regarding the study being conducted namely "A STUDY TO CORRELATE SEPSIS MARKERS AND BUFFY COAT CULTURE WITH BLOOD CULTURE IN NEONATAL SEPSIS". At Department of Paediatrics, Bangalore Medical College and Research Institute, Bengaluru.

I have also been briefed about the need for the blood investigations.

I have no issues about sharing my details in case records and would co-operate for the study. I have been informed that I will not be sharing any incentives. Personal identity will not be revealed but data can be used for publication purpose.

During the discussion with the paediatrician at any time, there has been no compulsion to undergo the treatment and hence I will fully give consent to participate in the study for the specified duration and adhere to the study protocol as explained.

I shall not hold the doctor or the hospital authorities responsible for any untoward incident occurring during the study or as a consequence of the treatment.

Signature of the patient Signature of the doctor

Signature of the attendant/witness