Original article

"Diagnostic utility of nucleated red blood cells and modified hematological score in diagnosis of early onset neonatal sepsis"

Dr. Disha B S[1], Dr. Ramyashree G[1], Dr Mamatha K[2], Dr S S Kalyanshettar[3]

[1]Assistant professor,Department of Pathology, SS Institute of medical sciences and research centre, Davangere
[2]Associate professor,Department of Pathology, Shri B M Patil Medical college, Vijayapura
[3]Professor,Department of Pediatrics, Shri B M Patil Medical College, Vijayapura

Corresponding author:Dr.Mamatha K

AssociateProfessor,Department ofPathology,Shri B M Patil Medical college, Vijayapura

Abstract

Background:
Neonatal sepsis is one of the principal causes of neonatal morbidity and mortality in the developing countries. Early onset neonatal sepsis presents with vague and non specific signs and symptoms. The blood culture which is considered to bethe standard test, will be available after 48-72 hours. The nucleated RBCs are commonly found in neonatal blood. Increased counts are often the result of prematurity, increased erythropoiesis, acute stress mediated release from marrow stores, and post natal hypoxia.

Objective:
To study the utility of nucleated red blood cells in the diagnosis of early onset neonatal sepsis

Materials and methods:
It was a hospital based, cross sectional study.Blood samples were collected in plain and EDTA anticoagulated vacutainers from umbilical cord blood or arterial or venous blood for culture and analysis of hematologic parameters respectively.On peripheral smear nRBCs were counted per 100 WBCs and immature granulocytes, toxic granules in neutrophils, platelet counts and B:N ratio were also noted. Modified hematological score was calculated and analyzed.

Results:
The nRBC counts were increased in 18 out of 19 culture proven cases of sepsis. The sensitivity, specificity, positive predictive value and negative predictive value were 95%, 46%, 23% and

98% respectively. The sensitivity and specificity of Modified hematological scoring system was 94.7% and 83.9% respectively.

Conclusion:

The nucleated red blood cells are an immediate reliable marker in the diagnosis of early onset neonatal sepsis. It is an easy, rapid, and cost effective test. Modified hematological scoring system uses six hematological parameters including nRBC counts which has better sensitivity and specificity.

Keywords: Nucleatedred blood cells, early onset sepsis, modified hematological score

Introduction

"Neonatal sepsis, a systemic response by host to the bacterial infection, is one of the prime causes of neonatal morbidity and mortality in India".[1]According to the data obtained from National Neonatal Perinatal Database (NNPD) 2002-2003, the incidence of neonatal sepsis in India was 30 per 1000 live births.[2,] All the neonates with clinical suspicion of sepsis should get a septic screen done for the validation of diagnosis. Blood culture is regarded as the gold standard diagnostic test for diagnosis of neonatal sepsis but a minimum of 48-72 hours have to be waited for the culture and sensitivity report.[2,3]The significance of various factors like C reactive protein, hematological parameters like total neutrophilic count, total leucocyte count, immature to total mature cells ratio, platelet count and toxic granules in the neutrophils have been studied in diagnosing neonatal sepsis.[1-4]The nRBCs are found normally in circulation in neonates. The counts can increase as result of premature birth, post natal hypoxia, acute stress mediated release from the bone marrow stores and increased erythropoiesis in chronic conditions [4,5]

There is a persistent search for a test which is rapid, fundamental and cost effective that can be done in the early newborn period, which can significantly impact the neonatal health care.This study was undertaken to understand the role of nucleated red blood cells in the diagnosis of early onset neonatal sepsis.

Methodology

Source of data :A cross sectional hospital based study was carried out on neonates fulfilling the inclusion and exclusion criteria attending inpatient Department of Paediatrics, referred to the Department of Pathology in BLDE Deemed to be University Shri B.M.Patil Medical College, Hospital and Research centre, Vijayapura.

Study period: 1st October, 2016 to 30th June, 2018

Methods of collection of data.

Data of neonates with clinical suspicion of sepsis admitted to the NICU were collected. Clinical, perinatal history and demographic data like age, sex, gestational age -preterm/full term, weight and manner of delivery were noted. Neonates with suspected inborn errors of metabolism, congenital anomalies, and neonates with hemolytic disease of newborn were excluded from the study.

Based on clinical and laboratory parameters, neonates were grouped into

1. Proven sepsis
2. Clinical sepsis
3. Suspected sepsis

Neonates with positive culture reports were considered as proven cases of sepsis. Neonates with clinical features of sepsis along with positive septic screen were considered as clinical sepsis. Neonates with clinical features of sepsis were considered as suspected sepsis.Blood samples were collected in plain and EDTA anticoagulated vacutainers from umbilical cord blood or arterial or venous blood for blood culture and analysis of hematologic parameters respectively. Blood sample collected in plain vacutainer was inoculated into glucose broth and incubated for 48 hours and streaked to Mcconkeyagar.Blood samples collected in EDTA anticoagulated vacutainers were run in quantitative haematology analyzer Sysmex XN-1000 to obtain various hematologic parameters. Peripheral smears were prepared, stained using Leishman's stain.Modified hematological septic score was done using various parameters and scores were assigned accordingly.**Table 1**

Criteria	Abnormality	Score

Total WBC count	<5,000/µl >25,000/µl at birth >30,000/µl 12–24 h >21,000/µl Day 2 onwards	2 1
Total PMN count	No mature PMN seen Increased/decreased	2 1
I:T PMN ratio	Increased	1
Degenerative changes in PMN	Toxic granules/cytoplasmic vacuoles	1
Platelet count	<1,50,000/µl	1
nRBC count	>10% <10%	1 0

Table No.1:Modifiedhematological scoring system

Results

Among 131 neonates, 109 (83.2%) were 1 day old, 10 (7.6%) were 2 days old and 12 (9.2%) cases were three days old. 78 cases were males (59.5%) and 53 (40.5%) were females. 38 cases were positive for septic screen and 19 were positive for blood culture. An analysis of sex wise distribution was also done, with male neonates having more number of proven cases of sepsis in comparison to females.

Out of 19 culture proven cases of sepsis, Escherichia coli(5.3%) was the most common isolated organism followed by Klebsiella pneumonia (3.1%). Other organisms were Citrobacter freundi (2.3%), Staphylococcus (2.3%) and Coagulase negative Staphylococcus (1.5%). Considering the gestational age, 83.2% of the neonates were term and the remaining 16.8% were preterm. Among 19 cases of proven sepsis, 18 were term neonates and a single case belonged to the preterm category. Even in the 38 cases of clinical sepsis, 26 were term neonates and 12 were preterm neonates. The same trend continued in the 74 cases of suspected sepsis as well with 65 term neonates and 9 preterm neonates.

Amongst the 131 cases, elevated C reactive protein and Absolute neutrophil count were noted in 125 and 98 cases respectively, followed by elevated nucleated Red blood cells seen in 79. Considering these parameters along with the nature of the sepsis diagnosis, elevated CRP levels were found in 100% of the cases of proven sepsis, whereas, elevated nRBCs were noted in 94.7% of the cases followed by toxic granules and B:N ratio with 78.9% each. However, elevated nRBCs, toxic granules in neutrophils and B:N ratio were having a statistically significant difference with a p-value of <0.005.

Elevated nRBC s was seen in 18 (94.7%) cases out of 19 cases of proven sepsis, 21 (55.3%) cases out of 38 cases of clinical sepsis and 40(54.1%) cases out of 74 cases of suspected sepsis. Elevated ESR was seen in 3 (15.8%) cases out of 19 cases of proven sepsis, 2 (5.3%) cases out of 38 cases of clinical sepsis and 2 (2.7%) cases of suspected sepsis. B:N ratio was elevated in 15 (78.9%) cases out of 19 cases of proven sepsis, 37 (97.4%) cases out of 38 cases of clinical sepsis, 8 (10.8%) cases out of 74 cases of suspected sepsis. 15 (78.9%) cases of proven sepsis showed toxic granules in neutrophils. C reactive protein was elevated all 19 culture proven cases of sepsis and in all 38 cases of clinical sepsis. Absolute neutrophil count was increased in 14 (73.7%) cases out of 19 cases of proven sepsis, 37(97.3%) cases out of 38 cases of clinical sepsis and in 47 (63.5%) cases out of 74 of suspected sepsis.**Table 2**

Table no 2: distribution of parameters according to sepsis groups

PARAMETERS	PROVEN SEPSIS (N=19)		CLINICAL SEPSIS (N=38)		SUSPECTED SEPSIS (N=74)		p value
	N	%	N	%	N	%	
Elevated nRBC s	18	94.7	21	55.3	40	54.1	0.004*
Elevated Total WBC count	6	31.6	6	15.8	2	2.7	0.001*
Elevated ESR	3	15.8	2	5.3	2	2.7	0.073
Elevated B:N ratio	15	78.9	37	97.4	8	10.8	<0.001*
Toxic granules in neutrophils	15	78.9	8	21.1	16	21.6	<0.001*
Elevated CRP(mg/dl)	19	100.0	38	100.0	68	91.9	0.089
Elevated ANC	14	73.7	37	97.4	47	63.5	<0.001*
Total	19	100.0	38	100.0	74	100.0	

Note: * significant at 5% level of significance (p<0.05)

With respect to the number of nRBCs per 100 WBC analysis, >30 nRBCs were seen in 63.2% of proven sepsis cases and the number of nRBCs were more than 10 per 100WBCs in all the remaining 7 cases. In clinical sepsis and suspected sepsis, 31.6% and 17.6% of the cases were having more than 30 nRBCs. These results are found to be statistically significant with a p-value of 0.020.**Table 3**

Tableno 3: Distribution ofnrbc/100wbcs

NRbc/100wbc	PROVEN SEPSIS (N=19)		CLINICAL SEPSIS (N=38)		SUSPECTED SEPSIS (N=74)		p value
	N	%	N	%	N	%	

0-9	0	0.0	15	39.5	30	40.5	0.020*
10-19	4	21.1	7	18.4	23	31.1	
20-29	3	15.8	4	10.5	8	10.8	
≥30	12	63.2	12	31.6	13	17.6	
Total	19	100.0	38	100.0	74	100.0	

According to modified hematological scoring system, among proven cases of sepsis, 13 cases showed a score of ≥5 indicating sepsis was very likely. Among clinical sepsis 22 cases showed a score of 3-4 indicating possibility of sepsis. Among suspected cases of sepsis 51 cases showed a score of ≤2 indicating sepsis was unlikely and 21 cases showed score of 3-4 indicating possibility of sepsis. **Table 4**

Table no 4: Distribution of modified HSSaccording to sepsis groups

HSS	**PROVEN SEPSIS (N=19)**		**CLINICAL SEPSIS (N=38)**		**SUSPECTED SEPSIS (N=74)**		**p value**
	N	**%**	**N**	**%**	**N**	**%**	
UNLIKELY (≤2)	0	0.0	11	28.9	51	68.9	<0.001*
POSSIBLE(3-4)	6	31.6	22	57.9	21	28.4	
VERY LIKELY (≥5)	13	68.4	5	13.2	2	2.7	
Total	19	100.0	38	100.0	74	100.0	

Note: * significant at 5% level of significance ($p<0.05$)

At a cut off value of ≥21 for nRBCs, the sensitivity and specificity was 73.7% and 69.6% respectively and at cut off score of ≥3.5 the sensitivity and specificity of HSS was 94.7% and 83.9% respectively.

Discussion

The current study was undertaken to evaluate the efficacy of nucleated red blood cells in the diagnosis of early onset neonatal sepsis along with other hematological parameters.

Of the 131 neonates in the study, 78 were males and 53 were females. Among the 19 proven cases of sepsis, 13 were males. Several other workers such as Abhishek M G *et al*[1], Chandna *et al*[6], and Krishnamurthy V *et al*[7] have reported similar findings.Septicemia is more common in male neonates than female neonates. The probable reason to this is the fact that, the factors that regulate the synthesis of Y globulins are situated on X chromosome. Males have less immunological protection than females since they have a single X chromosome. [6]
In present study, 109 cases were term neonates and 22 cases were preterm neonates. These findings were similar to the findings Abhishek MG *et al*[1] and Krishnamurthy V *et al*[7].

Among 131 cases of suspected sepsis, 79 cases showed elevated nRBCs, and 18 cases out of 19 culture proven sepsis showed increased nRBCs. The result of this study was almost similar with the result of the study done by Abhishek MG et al [1](17 out of 60 cases showed increase in nRBCs and 6 cases out of 14 culture positive cases). [Table No 6.3]

Table No 5: Comparison of ESR, Total WBC count, Toxic granules in neutrophils, nRBCs among positive blood culture cases

Parameters	Present study	Abhishek MG et al

	(Out of 19 cases)	(Out of 14 cases)
Increased nRBCs	94% (18)	42.8% (6)
Elevated ESR	15.7% (3)	14.2% (2)
Elevated Total WBC count	31% (6)	28.5% (4)
Toxic granules in neutrophils	78% (15)	28.5% (4)

Out of 19 cases of proven sepsis, 12 cases showed a nRBC count of >30/ 100WBCs.

In a study conducted by Boskabadi H *et al*[8] , there was a significant association between the nRBC counts of the case group and mortality (p value < 0.0001). The diagnostic cut-off point for nRBC count to predict infant mortality was more than 7 per 100 WBCs. At this cut-off point, sensitivity was 65% and specificity 85%, while positive and negative predictive values were 44% and 93%, respectively.

In present study, the sensitivity of nRBC parameter was 95%, and its specificity was 46%, positive predictive value was 23%, and negative predictive value was 98%.The present study showed a better sensitivity and positive predictive value of nRBC parameter for identifying neonatal sepsis. The test showed a higher sensitivity because, 18 out of 19 culture proven cases of sepsis showed increased nRBCs. Increase in the nRBC count can be attributed to the factor that most of the neonates in this study were one day old, where nRBC counts will be on higher side. It is most commonly associated with early onset sepsis. They have several virulence factors which might result in increased IL 6 production, which causes increased production of nRBC.[9,10]

Dulay *et al*[5] found that the elevated nRBC counts directly correlated with Interleukin 6 present in the cord blood. They demonstrated that, in the absence of stress or hypoxia, the production and release of nRBCs into circulation is an adaptive response to an inflammation in the intrauterine period. The nRBCs and other hematological indices are good predictors of short term neonatal outcome.

According to the study conducted by Boskabadi H *et al*[8], the mean nRBC count was ten times higher in the infection group than in the control group. Sepsis is an inflammatory response of the body to pathogens. This causes the release of many inflammatory mediators including cytokines which cause production of nRBCs.

According to ROC analysis of cut off values for detecting proven cases of sepsis, nRBC at a cut off value of >21 showed a sensitivity and specificity of 73.7% and 69.6%.

Hematological scoring system is a score devised using various hematological parameters like total WBC count, total PMN count, immature PMN count, I:M PMN ratio, degenerative changes in PMN, and platelet count. In present study, a modified hematological scoring system was used including nRBC parameter.

In present study, among 19 culture positive cases, 13 cases showed a score ≥5 and 6 cases showed a score between 3 -4. Score of more than ≥5 shows that sepsis is very likely, a score of 3-4 indicates possibility of sepsis and a score of 0-2 indicates sepsis is unlikely. In a study conducted by Narasimha *et al*[11] on hematological scoring system, 26 cases showed score of 3-4 and 12 cases showed score of ≤2. All the 12 proven cases of sepsis showed a score of >5. In a study done by Makkar M *et al*[13], out of 42 cases with culture proven sepsis, 35 (83.33%) infants had score ≥5.

Table No 6: Comparison of score distribution among culture proven cases of sepsis

	Score 0-2	Score 3-4	Score ≥5
Narasimha *et al*[11]	-	-	100% (12 cases)
Ahirrao et al[12]	-	-	100%(77 cases)
Makkar M *et al*[13]	-	16.6%(7 cases)	83.3%(35 cases)
Present study	-	31.6%(6 cases)	68.4% (13 cases)

In present study, at a cut off score of ≥3.5,the sensitivity and specificity of Modified hematological scoring system were 94.7% and 83.9% respectively. The positive predictive value and negative predictive value were 28% and 100% respectively. It was concordant with Krishnamurthy *et al*[7] with sensitivity and specificity were 84% and 82% respectively.

The sensitivity, specificity and negative predictive value of modified hematological scoring system was better than hematological scoring system. In modified HSS

immature neutrophil count and I:M neutrophil ratio were excluded since they were repetitive and falsely increasing the scores. The nRBC count was included considering its role in neonatal sepsis.[7] Considering high sensitivity, specificity and negative predictive value, it implies that a score of >4 was more reliable for diagnosing sepsis than individual hematological parameter.

Conclusion

nRBC counts is an easy, rapid and cost effective test, could be used along with other hematological parameters in diagnosing neonatal sepsis.
Combining the values of biochemical markers along with modified HSS could improve the diagnostic efficacy in neonatal sepsis.

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