Original Research Article

Nucleated red blood cells and hematological scoring system – Future trends in early onset neonatal sepsis

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Abstract

Background: Neonatal sepsis, sepsis neonatorum and neonatal septicemia are terms that are used to describe the systemic response to infection in the newborn infant. Infection is more common in the neonatal period than at any other time in life. This is partly attributable to exposure to a large number of organisms, but is also due to a relative failure of the neonatal host defenses to clear microorganisms from blood and tissues. **Material and Methods:** The present study is a prospective analysis of hematological parameters of 110 neonates with suspected sepsis, and correlation with blood culture and C-reactive proteins had been interpreted. **Result:** Out of 110 infants, 23(20.9%) cases with proven sepsis, 30(27.2%) cases with probable sepsis and 57(48.18%) cases were on safer side. 74.5% infants had early onset neonatal sepsis and 25.45% infants had late onset. 73.9% of male infants were of proven sepsis and 60.8 preterm infants were more prone. Nucleated RBC (nRBC) values were higher in sepsis. Significant nRBCs were seen in 78.2% of EONS cases in proven sepsis and 50% of EONS in probable sepsis. nRBCs were not significant in late onset(4.3%). **Conclusion:** The advantage of studying the hematological parameters of neonates suspicious of having sepsis is that these can be done rapidly even in small hospitals, allowing prompt treatment to neonates with sepsis and minimizing therapy to those without infection.

Key Words: Rodwell's Hematological Scoring System, Neonatal sepsis, Nucleated RBCs, Early onset neonatal sepsis

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Received Date: 08/08/2019 Revised Date: 12/09/2019 Accepted Date: 03/10/2019
DOI: https://doi.org/10.26611/10512112

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 23 October 2019

INTRODUCTION

Neonatal sepsis is defined as a clinical syndrome of bactermia with systemic signs and symptoms of infection in the first 4 weeks of life [1]. Systemic infection in the newborn is the commonest cause of neonatal mortality. Data from National Neonatal Perinatal Dataase 2000 suggest that Klebsiella pneumonia and Staphylococcus aureus are the commonest causes of neonatal sepsis in India.[1] Clinical features of sepsis are nonspecific in neonates and a high index of suspicion is required for its identification. Although blood culture is the "**Gold Standard**" for the diagnosis of sepsis, reports are available after 48-72 hrs and they may be affected by intrapartum antibiotic administration to the mother.[2] In order to diagnose septicemia early, several rapid diagnostic tests have been described recently. These can be performed rapidly in an hour or two and antibiotics can then be used judiciously, thereby reducing the incidence of drug resistance and improving the survival rate of septicemia. These tests are C-reactive protein (CRP) test, band cell/total neutrophil ratio, buffy coat smear examination, gastric aspirate cytology for

How to cite this article: Shivendra Vikram Singh, Megalamane Supreetha, Satyavathi R Alva. Nucleated red blood cells and hematological scoring system – Future trends in early onset neonatal sepsis. *MedPulse International Journal of Pathology.* October 2019; 12(1): 53-58. https://www.medpulse.in/Pathology/

polymorphs, micro ESR, haptoglobin levels, serum orosomucoid, serum fibrinogen levels etc [2]. Neonatal septicemia is one of the major factors contributing to the high perinatal, neonatal mortality and morbidity. Several authors have reported that the morality due to neonatal sepsis ranges between 40-65% [2].In the present study we evaluate the utility of the peripheral smear, a hematological scoring system along with certain tests like C-reactive proteins as aids in early diagnosis of neonatal sepsis. Hence we can see that there is a critical need for laboratory tests that aid in the rapid diagnosis of neonatal sepsis and at the same time are cost effective and simple. Hence we aimed to study various hematological parameters on peripheral smear and importance of Rodwell's Hematologic Scoring System.

MATERIAL AND METHODS

We analyzed 110 neonates admitted to the NICU at KVG medical college, Sullia. The blood samples were collected by peripheral venipuncture using aseptic precautions and sent to the pathology laboratory in EDTA vacutainers. Neonates were divided into 3 groups mainly,

Group 1 (Proven sepsis): neonates with sepsis (with positive blood culture)

Group 2 (Probable sepsis): neonates with probable infection (with strong
clinical history and negative blood culture)

Group 3 (No sepsis): normal neonates (with minimal h/o or signs of sepsis).

The routine hematological investigations included hemoglobin, hematocrit, red blood cell indices (MCV, MCH and MCHC), total WBC count, differential count and platelet count. These investigations were performed on multichannel automated cell counter—MINDRAY model BC1000 with standard calibration. For every sample a peripheral smear was prepared and the blood film was stained with Leishman's stain. The WBC count was corrected for nucleated red cells and a differential count was performed manually. Immature neutrophils include promyelocyte, myelocyte, metamyelocyte and band forms. A band cell was defined as a neutrophil in which, the nucleus was indented by more than half, but in which the isthmus between the lobes was wide enough to reveal two distant margins with nuclear material between. The polymorphonuclear leucocytes were also examined for degenerative morphological changes such as toxic granulation, toxic vacuolization and Dohle bodies. The hematological findings were analyzed according to the hematological scoring system of Rodwell *et al*.[9]

Table 1: Rodwell's Hematological Scoring System

Criteria	Abnormality	Score
Total WBC count	≤5,000/µl	1
	≥25,000 at birth ≥30,000—12-24hrs ≥21,000—day2 onwards	1
Total PMN count	No mature PMN seen	2
	Increased/ decreased	1
Immature PMN count	Increased	1
I:T PMN ratio	Increased	1
I:M PMN ratio	≥0.3	1
Degenerative changes in PMN	Toxic granules/cytoplasmic vacuoles	1
Platelet count	≤150,000/µl	1
The normal values are according to Manore *et al* [17]: Total PMN count- 1800-5400 Immature PMN count – 600 Immature : Total PMN ratio- 0.12 Immature : Mature PMN ratio- ≥0.3		

C-reactive protein levels were also recorded. This test was done in the immunology laboratory by immune turbidimetric method using MISPA-i analyser. For culture 2ml blood in 20 ml glucose broth were taken before administering any antibiotic and sent to Microbiology department immediately. Subcultures were observed after 24-48 and 120 hours. If growth was observed, material was further analyzed for isolation of organism and antibiotic sensitivity. If no growth was observed after 5 days, culture was reported negative. The data collected was statistically analyzed, to find out the performance of the test individually and as a scoring system under the following parameters. Sensitivity, Specificity, Positive predictive value, Negative predictive value, Chi-square test, Contingency coefficient and P value < 0.05 was considered as significant.

OBSERVATION AND RESULTS

The present study was conducted on 110 neonates who clinically presented with symptoms of sepsis. Based on

clinical findings and laboratory data infants were classified into three categories.

Proven sepsis (23/110cases): The diagnosis of sepsis was made when there were positive findings on blood culture.

Probable sepsis (30/110*):* Infants were classified as having probable infection when blood cultures were negative but there was a strong clinical history indicating infection. Certain high risk factors such as prolonged rupture of membranes, meconium aspiration, prolong labour and maternal fevers were noted in these neonates. The infants presented with clinical features such as respiratory distress and grunting, apnea, lethargy, poor feeding/sucking, abdominal distension and shock.

No sepsis (57/110): This group consisted of neonates with negative blood culture, who finally presented with feature of suspected sepsis or with associated risk factors. On further investigation they were found to be suffering from other disorders such as hyaline membrane disease, transient tachypnea of the new born and hypoglycemia. The study had males 72(65.5%) as well as females 38(34.5%). We had 71 (64.5%) term infants and 39 (35.4%) pre-term infants with the age ranging from 24 h to 7 days.

Neonatal sepsis was divided into 2 groups according to the onset of symptoms.

(i) **Early onset sepsis**: usually presents within the first 72hrs of life.

(ii) **Late onset sepsis**: usually presents after 72hrs.

Many of the neonates presented with more than one symptom. The commonest form of presentation was respiratory distress (82%) and lethargy/poor feeding (59%).

Immature: Mature PMN ratio (94%) was highly sensitive followed by Immature: Total PMN count (91%) in identifying infants with sepsis. Total leucocyte count (TLC) (94%) followed by platelet counts (75%) were highly specific tests helpful in diagnosing sepsis. The positive predictive value was high for Immature: Mature PMN ratio (94%) followed by Total WBC counts (82%) which were helpful in identifying infants who really had sepsis. Negative predictive value was high in Immature: Mature PMN ratio (97%) along with degenerative changes (82%) which indicated that the infants did not have any evidence of sepsis.

Table 6: Performance of individual hematological findings and CRP levels in 23 infants with sepsis from 110 evaluations in the first month of life

Hematological findings	Sensitivity (%)	Specificity (%)	Positive prediction value (%)	Negative prediction value (%)
↑ or ↓ WBC	45	94	82	66
↑ or ↓ Total PMNs	78	87	80	67
↑Immature PMNs	65	87	49	90
↑ I:T ratio	91	79	78	51
↑ I:M ratio	94	97	94	97
Degenerative changes	53	89	68	82
Platelet <150 x 10^3 /µL	48	93	54	68
CRP levels	82	70	54	91

Table 8: Performance of HSS – present study

Hematological score	Sensitivity (%)	Specificity (%)	Positive prediction value (%)	Negative prediction value (%)
≥1	100	44	31	100
≥2	100	65	35	100
≥3	96	75	38	98
≥4	87	93	80	96
≥5	43	97	88	85
≥6	39	100	91	72

Nucleated Red blood cells (nRBC) values were higher in sepsis and significantly seen in 78.2% of early onset neonatal sepsis cases in proven sepsis and 50% in probable sepsis. nRBC were not significant in late onset (4.3%).

Table 9: Nucleated RBC in early and late onset neonatal sepsis case studied

Infection status	EONS (n=82)		LONS (n=28)	
	<10 nRBCs	>10 nRBCs	<10 nRBCs	>10 nRBCs
Probable sepsis(n=30)	5	15	4	6
No sepsis(n=57)	34	8	13	2
Proven sepsis(n=23)	2	18	2	1

p-value - 0.001 contingency coefficient- 0.629

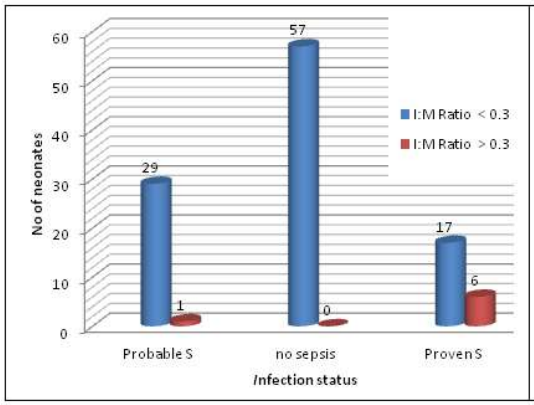
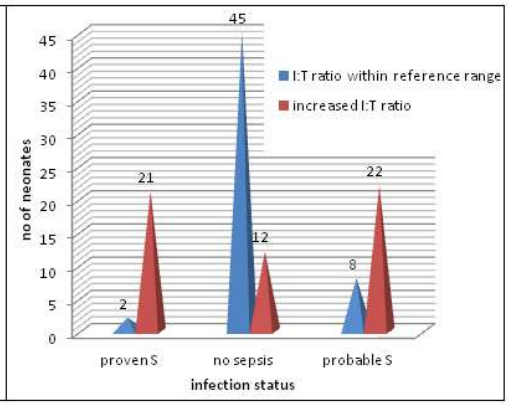


Figure 1



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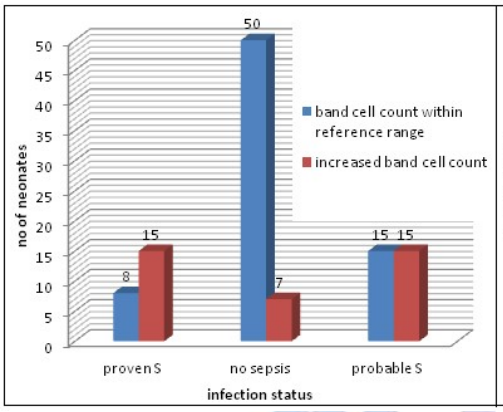

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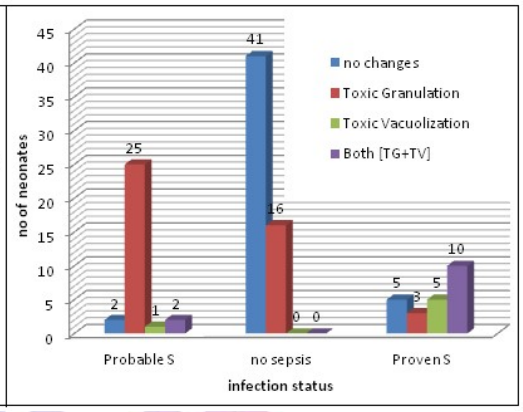

Figure 4

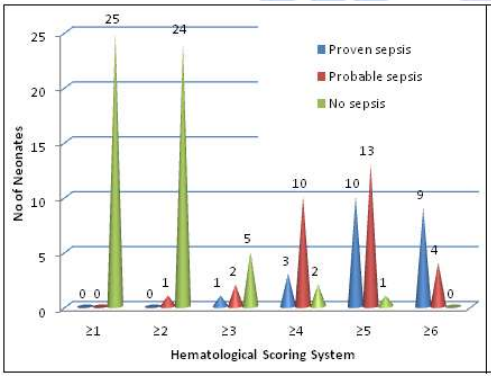

Figure 5

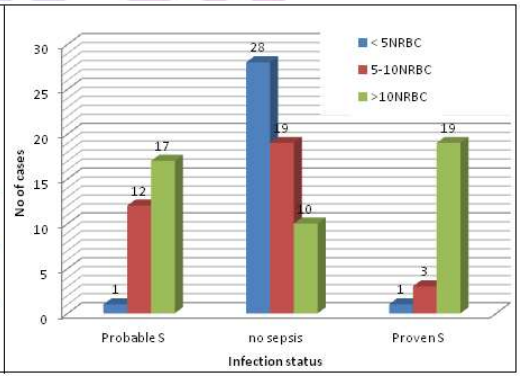

Figure 6

Figure 1: I: M Ratio (Immature to Mature neutrophil ratio) in neonates studied; **Figure 2:** I: T Ratio (Immature to Total Neutrophil ratio) in neonates studied; **Figure 3:** Immature polymorphonuclear leucocyte (Band cell) in neonates studied; **Figure 4:** Degenerative changes in neutrophils in neonatal sepsis; **Figure 5:** Hematological scores of neonates of present study; **Figure 6:** Nucleated RBC in neonatal sepsis

DISCUSSION

Sepsis is the commonest cause of neonatal mortality. It is responsible for about 30-50% of the total neonatal deaths in developing countries. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes [3].Infants are deficient in their inherent protective mechanisms, humoral and cellular immunity [4]. In the preterm VLBW infant, though all molecular and cellular elements necessary for adequate host defense are present, their number/capacity or function is reduced (newborn's immune naivety) accounting for decreased magnitude of immune response. This immune naivety is made worse by sepsis. Unless this is adequately addressed in the management package along with killing of the pathogen/s it is unlikely that mortality rates from sepsis will come down [5]. The definite diagnosis of septicemia is made by a positive blood culture which requires a minimum period of 48-72hrs and

yields positive result in 30-40% of cases [1].An early and accurate etiological diagnosis is not always easy, especially since the disease may start with minimal or non-specific symptoms. Delayed treatment until clinical recognition of signs and symptoms of sepsis entails risk of preventable mortality, notwithstanding the fact that presumptive antibiotic therapy may result in overtreatment [6]. The unnecessary use of stronger antibiotics for minor infections and for prophylaxis should be discouraged [7, 8]. In order to diagnose septicemia early, several rapid diagnostic tests have been described, which are easily performed and have the benefit of quick availability of reports [1].In our study considering all four parameters i.e.: sensitivity, specificity, positive predictive value and negative predictive value, I:M ratio and I:T ratio were the most reliable tests for diagnosing sepsis. Degenerative changes in neutrophils were not found to be a very sensitive indicator of sepsis. Thrombocytopenia was consistently associated with poor prognosis. These findings were in comparison with other studies.[9,10,11] The higher the score, the greater was the likelihood of sepsis. A score ≤2 suggests that sepsis was unlikely. Hematologic scoring system (HSS) can improve the efficiency of the complete blood count as a screening test for sepsis and permits an objective assessment of hematological change [9]. The HSS is simpler, quick, cost effective and readily available tool in the early diagnosis of neonatal sepsis and could provide a guideline to decision regarding antibiotic therapy [9, 12]. The higher the score, the greater the certainty that sepsis is present. Therefore it simplifies the interpretation of hematological profiles. 78.2% of infants with culture positive early onset neonatal sepsis showed significant number of nucleated RBCs in the peripheral blood on the day one only. 3.5% of infants with late onset neonatal sepsis showed no significant nucleated RBCs. Tripathi *et al* stated that cytokines released in sepsis have an important role in stimulating nucleated RBC production independent of hypoxia. She has concluded in her study that there is significantly elevated nRBCs demonstrated in early onset sepsis and increased nRBC count immediately after birth could be an interesting marker of early onset neonatal sepsis in absence of hypoxia [13]. Hermansen has mentioned that increase in nRBC count in EONS infants without asphyxia [14]. Dhananjay *et al* concluded in his study that along with CRP, platelet count, total leucocyte count, differential count, nucleated RBCs are also effective predictor markers of sepsis.[15] Dulay *et al* have studied the nRBC count of neonates with early onset neonatal sepsis was significantly higher independent of gestational age at birth, erythropoietin (EPO) or hypoxia [16]. Though there are several methods for rapid detection of microorganisms in blood cultures of newborn infants using automated blood culture system, DNA probe and fluorometric detection systems[11], still HSS can be employed as a useful test to distinguish the infected from the non infected infants. It has high sensitivity and specificity, the certainty of sepsis being present with higher scores.

CONCLUSION

The advantage of HSS lies in the fact that it is applicable to all infants, including those who have received antibiotic therapy prior to sending for blood culture. The successful recovery of microorganism from blood culture on the other hand depends on many complex factors. The advantage of studying the hematological profile of neonates having suspicious of sepsis is that tests can be done rapidly even in small hospitals, allowing prompt treatment to neonates with sepsis and minimizing therapy to those without infection. The importance of correlating both clinical and laboratory data needs to be stressed. The HSS is a useful test to distinguish the infected from the non-infected infants. It has high sensitivity and specificity, the certainty of sepsis being present increasing with higher scores. In the setting of inflammation associated preterm birth and in absence of hypoxia, elevations in nucleated RBCs in early neonatal period may a direct response of exposure to inflammatory mediators in utero. The HSS should improve the efficiency of the CBC as a screening test for sepsis and permits an objective assessment of hematological changes. Hematological indices with HSS and nRBCs are good predictors of short term neonatal outcome, independent of gestational ages or birth weight. It carries diagnostic and prognostic value and also provides insight into the pathophysiology of fetal adaptation to a microbial inflammatory attack.

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Source of Support: None Declared
Conflict of Interest: None Declared