e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(8); 204-208

Original Research Article

Eosinopenia and Nucleated Red Blood Cells as Diagnostic Markers in Neonatal Sepsis and in Predicting Morbidity and Mortality in Neonatal Intensive Care Unit

Satish Kumar[1], Rakesh Kumar[2]

[1]Senior Resident, Department of Pediatrics, GMCH, Purnea
[2]Senior Resident, Department of Pediatrics, GMCH, Purnea

Received: 10-06-2023 / Revised: 04-07-2023 / Accepted: 30-07-2023
Corresponding author: Rakesh Kumar
Conflict of interest: Nil

Abstract:

Background and Objectives: Neonatal sepsis is a clinical syndrome consisting of bacteremia with systemic signs and symptoms of infection accompanied by evidence of the bacterial growth in blood cultures, urine or cerebrospinal fluid, in the first four weeks o/f life. Neonatal sepsis is frequent and important cause of morbidity and mortality particularly in the developing countries like India and accounts for early-half of all neonatal death in ourcountry. To evaluate the diagnostic efficacy of eosinopenia in neonatal sepsis and to determine the value of eosinopenia and nucleated red blood cells in predicting morbidity and mortality in NICU.

Methodology: A prospective comparative study done in Neonatal intensive care unit of GMCH, Purnea. 254 neonates admitted to neonatal intensive care unit with a clinical suspicion of sepsis were included in the study. All neonates fulfilling the inclusion criteria were included as study subjects after obtaining detailedinformed consent from the parents.

Conclusion: As an inexpensive, easily available test to diagnose sepsis on ICU admission, eosinopenia and nucleated red blood cells offer a moderate degree of certainty in diagnosis of neonatal sepsis and can be used as an adjuvant test along with the other currently available septic markers.

Keywords: NRBC, Eosinopenia, Neonatal sepsis, morbidity, mortality, NICU.

Introduction

Neonatal sepsis is a clinical syndrome consisting of bacteremia with systemic signs and symptoms of infection accompanied by evidence of the bacterial growth in blood cultures [1], urine or cerebrospinal fluid, in the first four weeks of life. Neonatal sepsis is frequent and important cause of morbidity and mortality particularly in the developing countries like India and accounts for early-half of all neonatal death in our country [2]. According to the Lancet review 2018,[3] the lowest incidence rates were found in the USA, with 450 cases of neonatal sepsis per 100 000 live-births, and the highest incidence was reported in India, with 17 000 clinical sepsis cases per 100 000 live-births. Mortality ranged from 11% in the USA4 to 19% in India.[5] Some population-based studies have reported clinical sepsis rates ranging from 49 to 170/1000 live births in rural India.6 Incidence is not changed much over the past decade, and the fatality due to sepsis is between 30% and 65%.[7] The risk factors include lack of antenatal care, unsupervised or poorly supervised home deliveries, unhygienic and unsafe delivery practices and cord care, prematurity, low birth weight, lack of exclusive breast-feeding, and delays in recognition of danger signs in both mother and baby.[8,9] Gold standard for the diagnosis of neonatal sepsis is by demonstration of the organisms in blood culture, but it takes a long time of 2-8 days for positive culture, the cost is relatively expensive and has low positive rate [10] and hence various other investigations are necessary for early diagnosis or to rule out sepsis. In addition, it is also important to limit inappropriate antibiotic exposure leading to antibiotic resistance and lowering the cost of therapy. Therefore, there is a need for a test that is cheap, accurate, easily performed with quick availability of reports so that prompt and appropriate treatment is ensured. Rapid diagnostic test, which can distinguish infected infants from uninfected infants will have a significant impact on newborn care. There are no much studies done in India regarding the role of eosinopenia as diagnostic marker of neonatal sepsis. The available literature is mainly from the paediatric group and adult population. Also, there is relative lack of information about the role of nucleated red blood cells in neonatal sepsis. Much has been studied about the role of this component in asphyxia, but little data is available with respect to neonatal

infection. Hence this study was undertaken to provide information about the diagnostic value of eosinopenia, nucleated red blood cells and other subcomponentsof WBC in detection of bacterial infection in neonates and also in predicting the morbidity and mortality in neonatal intensive care unit.

Objectives

- To evaluate the diagnostic efficacy of eosinopenia in neonatal sepsis.
- To determine the value of eosinopenia and nucleated red blood cells inpredicting morbidity and mortality in NICU.
- To evaluate the diagnostic efficacy of the following parameters in the earlydiagnosis of neonatal sepsis and in predicting morbidity and mortality in neonatal intensive care unit

Material and Methods

A prospective comparative study, All neonates admitted to neonatal intensive care unit of Government Medical College Hospital Purnea, Bihar. during the study period of 2 years. As per the previous admission statistics minimum sample size was calculated to be 100. We took total of 245 neonates in the period of 2 years

Inclusion Criteria

All neonates admitted in neonatal intensive care unit of GMCH, during the study period with clinical signs of sepsis

Exclusion Criteria

Outborn neonates who have already been started on antibiotics before admission to NICU. Neonates with congenital anomalies. Neonates admitted in NICU for pure surgical corrections. Neonates on steroids for any cause. Hematological disorders (Hemolytic diseases, Rh incompatibility etc.

All neonates fulfilling the inclusion criteria were included as study subjects after obtaining detailed informed consent from the parents. In all cases, blood was collected prior to starting antibiotic treatment. In case ofsuspected early onset sepsis, blood was obtained after 6 hours of life through either a central venous access or a peripheral venous accessunder strict aseptic precautions as per our neonatal intensive care unit protocol. 1ml of blood was collected for blood culture and was processed through BACTEC method. Blood sample of 2cc each was collected in plain vacutainer as well as in EDTA vacutainer. Plain vacutainer for CRP estimation and EDTA vacutainer for leucocyte count, preparation of peripheral smears for estimation of Immature: Total neutrophil (I: T) ratio, immature granulocytes, to see the morphology of neutrophils and tosee for nucleated red blood cells. Nucleated red blood cells were counted under microscopy in high power field and was reported as number ofcells per 100 white blood cells.

The eosinophil count, C-reactive protein, peripheral smear for immature granulocytes, I:T ratio(immature neutrophils to totalneutrophils), nucleated red blood cells and micro ESR was done and the results were analysed among all the subgroups. The study population was further evaluated and followed up till the time of discharge, for theduration of hospital stay, need for mechanical ventilation, oxygen requirement, morbidity parameters (Necrotising enterocolitis, respiratory distress syndrome, intraventricular haemorrhage, retinopathy of prematurity, ventilator associated pneumonia, hearing abnormalities, etc.)and mortality.

The results were compared and analysed according to the sub groups. The test results were analysed as per the values. Three or more of the parameters positive was considered as septicscreen positive and was included in group 2 i.e., Probable sepsis

Septic screen parameters

Sl. No.	Laboratory Parameter	Positive for sepsis
1.	Total leucocyte count	<5000/mm^3, >30,000/mm^3
2.	Immature/total neutrophil ratio	>0.2
3.	Micro-ESR	>15 mm in first hour
4.	C reactive protein (CRP)	>6 mg/dl
5.	Absolute neutrophil count	As per Manroe and Mouzinho's chart[8]
6.	Platelet count	< 1.5 * 10^5 cells/mm^3

Results

Total of 306 cases with clinical suspicion of sepsis. Out of which 24 were outborn neonates, 8 were already started on antibiotics before including in the study, 7 newborns had haematological disorders (haemolytic disease of newborn, Rh incompatibility etc) and 13 cases didn't have all the investigation reports and hence were excluded. 254 newborns met the inclusion criteria and were included in the analysis.

Table 1: Mean birth weight in different groups and standard deviation

	Mean	Std. Deviation	Minimum	Maximum
Group 1	2.07846	0.839061	0.000	3.600
Group 2	1.94625	0.682777	0.800	3.900
Group 3	2.32325	0.650674	1.050	3.700
Total	2.15504	0.728916	0.000	3.900

there was statistical difference in the distribution of cases among the three groups based on gestational age, though more number of term neonates were seen in group 3. The mean gestational age in group 1 and group 2 were34.635 weeks and 34.440 weeks respectively. More term neonates were seen in Group3 with a mean gestation age of 36.19+/- 4.0372 weeks. The minimum and maximum gestation age.

Table 2: Mean gestational age in different groups

	Mean gestation in weeks	Std. Deviation	Minimum gestational age.	Maximum gestational age
Group 1	34.635	2.6873	28.0	40.0
Group 2	34.440	3.2937	28.0	42.0
Group 3	36.197	4.0372	32.0	40.0

Klebsiella sepsis was the maximum in our neonatal intensive care unit during the study period accounting for 22.7% followed by Staphylococcus epidermidis and Citrobacter koseri. Only in one case, Streptococcus agalactiae was isolated. 59.4% (n=151) cases were early onset sepsis and 40.6% (n=103) cases were late onset sepsis. The division of early onset and late onset sepsis based on the timing of presentation.

Table 3: Mean, SD, minimum and maximum value of NRBC in different groups

	Groups	Mean	SD	Minimum	Maximum
NUCLEATED	Group 1	38.841	41.671	0.000	140.000
RBCS	Group 2	26.455	62.116	0.000	400.000
	Group 3	5.060	7.270	0.000	34.000

NRBC in each limb was analysed further with a cut-off value of 40cells/100 WBCs. Group 1 had 21 cases and group 2 has 7 cases above the cut off value. Group 3 didnot have any cases above the cut-off value. We further analysed the NRBCs with different cutoff values. NRBCs showed a sensitivity of 33.3%, specificity of 95.6%, PPV of 75% and a NPV of 78.5% in diagnosing neonatal sepsis, The duration of stay in NICU in group 1 was 20.03 days as opposed to 5.069 days in group 3 which was statistically significant. . Table 4 shows the mean duration of stay in NICU, which was more in group 1 and 2 as compared to group 3.

Table 4: Duration of stay in NICU among the three groups

		Mean (in days)	Std. Deviation
Days In NICU	Group 1	20.030	12.485
	Group 2	14.653	9.972
	Group 3	5.069	3.466

The Comparison of specificity, sensitivity, PPV and NPV of different parameters in diagnosing neonatal sepsis. Eosinopenia exhibited an excellent sensitivity of 76.9% which was better than that of the established markers, i.e., IT and CRP. NRBC had relatively lower sensitivity (33.3%). But eosinopenia had a lower specificity of 25.1% as opposed to all other established markers. NRBC had an excellent specificity of 95.6% which was even better than that of IT. Along with these, MPV exhibited excellent sensitivity of 92.4% and a relatively moderate specificity of 64.4%.

Table 5: Area Under the Curve of differentseptic markers in diagnosing neonatal sepsis

Test Result Variable(s)	Area
MPV	0.770
IT^2	0.743
IT	0.735
NRBC	0.624
CRP	0.593
Eosinopenia	0.518

The ROC curve of various other markers of sepsis used in the study. Area under the curve for various parameters has been shown in table 20. Area under the curve was maximum for MPV. We found a similar value for IT^2 as that of IT. The area under the curve for CRP was 0.593 which was comparable

with that of eosinopenia. Area under the curve for NRBC was better than that of the CRP which isa known marker of sepsis.

Discussion

In the study done by Hornik et al [11], 166,092 neonates with sepsis were included. The mean GA and birth weight of all neonates was 34.6 weeks (5th, 95th percentile: 27, 40) and 2420 g (860, 3960), respectively. In the study done by Newmann et al [12], the mean gestational age of sepsis group was 38.8 and no sepsis group was 38.7 weeks. The mean gestational age in our study was 34.635 weeks, 34.440 weeks and 36.197 weeks in group 1, group 2 and group 3 respectively. This was statistically significant and hence stressed that lower gestational age was associated with higher incidence of proven and probable sepsis. The mean birth weight was 2.07 kgs, 1.94 kgs and 2.32 kgs in group 1, group 2 and group 3 respectively which was not having any significant difference statistically. In our study early onset sepsis was more common than late onset sepsis. 59.4% (n=151) cases were early onset sepsis and 40.6% (n=103) cases were late onset sepsis. These results were similar to the study conducted by Prutha Desai et al, Eko Bagus et al [13] mentioned that sensitivity of eosinopenia at the cut off of 50cells/µL was 28.6% but it can be increased to 52.4% if cutoff is taken as 129cells/µL but at the cost of decrease in specificity to 64.3% as opposed to 100% with present cutoff. But contrary to this study, we got a sensitivity of 76.9% and a very poor specificity of 25.1% with a cut off value of 50 cells/µL. The variation of result can be attributed to lesser sample size and low blood culture positivity rates in the study done by Eko Bagus. Amal Sabry et al [14] investigated the impact of eosinophil percentage on admission to ICU on mortality of critically ill adult patients admitted to ICU. Their results showed no significance in relation to total ICU mortality, age, gender or mechanical ventilation. Also, eosinopenia showed significance with the APACHE II score. However, their results showed significant relation of eosinopenia to the length of ICU stay (P<0.001). Hence it was concluded that, with longer duration of ICU stay, patients tend to be more eosinopenic and mortality risk tend to increase significantly with eosinopenic patients. In contrast, Abidi et al [15] proved that eosinopenia could be considered a reliable marker of infection with a specificity of 80% and sensitivity of 80%. In our study, eosinopenia was associated with longer duration of ICU stay and had higher morbidity in sepsis. Also, eosinopenia was significantly present in proven and probable sepsis groups as opposed to clinical sepsis group. Though Eosinopenia was directly proportional to the mortality in sepsis neonates, the number was very lessto have a statistical significance. Eosinopenia showed a sensitivity of 15.9% and specificity of 79.2% in detecting mortality in the sepsis patients. Prutha Desai et al [16] found the highest sensitivity of CRP in diagnosing neonatal sepsis, which was 86.66%. But in our study the sensitivity of CRP was only 69%. Desai et al also concluded that I:T Ratio also has reasonably good sensitivity (83.33%) and it is more specific (95%) than CRP which can more accurately be used for ruling out proven sepsis. Similar to their results, we had a very good specificity of IT accounting for 87.8%, but a low sensitivity of 65.2%. Micro ESR has lower sensitivity and specificity, so can't be used alone to confirm or rule out sepsis. We also calculated another parameter, i.e., the IT2 which showed similar results as that of IT in its diagnostic value. As its established role in the study done by Newmann et al, we also found a sensitivity and specificity of 62.1% and 91.4% respectively. Thus, stressing the point that calculating the I/T2 enhances the prediction of early onset sepsis.

In our study we also analysed another new parameter for diagnosing neonatal sepsis i.e., the mean platelet volume (MPV). In the study done by Ferhat et al [17] A MPV value of 10.35 fL was identified as the cut off value in patients probably resulting in sepsis with a sensitivity of 97.8% and specificity of 78.7%. In our study also, we found an excellent sensitivity of 92.4% but a moderate specificity of 64.4% of this parameter in the diagnosis of neonatal sepsis. This enhances the role of MPV in diagnosis sepsis and also in follow-up of sepsis and the response of antimicrobial treatment.

Conclusion

Eosinopenia has a better sensitivity than CRP but showed poor specificity in diagnosing sepsis. Nucleated red blood cells has better specificity which is comparable with that of the IT ratio, an established marker in diagnosis of neonatal sepsis. Though these results cannot be used as gold standard, it definitely can be used as an adjuvant along with the other septic screen parameters in the diagnosis of neonatal sepsis. Both eosinopenia and nucleated red blood cells shows good specificity but poor sensitivity in detecting the morbidity and mortality in neonates admitted with sepsis in NICU.

References

1. Ghai OP, Gupta P, Paul VK. Essential paediatrics, 5th ed., 2003; 141-142.
2. Nelson Waldo: Textbook of Paediatrics, volume -1 Chapter 109, Page 915. 18th ed. 23-639.
3. Carolin Fleischm2007; 6ann-Struzek, David M Goldfarb, Peter Schlattmann, Luregn JSchlapbach, Konrad Reinhart, Niranjan Kissoon: The global burden of paediatric and neonatal sepsis: a systematic review, The Lancet, Respiratory medicine, March 2018;6(3): 223-230
4. RS Watson, JA Carcillo, WT Linde-Zwirble, G Clermont, J Lidicker, DC Angus, The

epidemiology of severe sepsis in children in the United States. Am J Respir Crit Care Med, 2003;167: 695-701
5. K Swarnkar, M SwarnkarA study of early onset neonatal sepsis with specialreference to sepsis screening parameters in a tertiary care centre of rural India. The Internet Journal of Infectious Diseases, 2012;10: 1-8.
6. Thaver D, Zaidi AK. Burden of neonatal infections in developing countries: A review of evidence from community-based studies. Pediatr Infect Dis J. 2009;28: S3-9.
7. Mathur NB. Neonatal sepsis. Indian Pediatr1996;33:663-74.
8. Lawn JE, Cousens S, Zupan J, Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? Lancet. 2005; 365:891-900.
9. Stoll BJ. The global impact of neonatal infection. Clin Perinatol. 1997;24:1-21.
10. Saleh MT, Sherif LS, Elwakkad AS, Assal WAM. Leptin: does it have a role in neonatal sepsis? Journal of Applied Science Research. 2008; 4: 353-359.
11. Hornik CP, Benjamin DK, Becker KC, Benjamin DK Jr., Li J, Clark RH, Cohen-Wolkowiez M, Smith PB. Use of the complete blood cell count in early-onset neonatal sepsis. Pediatr Infect Dis J. 2012; 31:799-802.
12. Newman TB, Draper D, Puopolo KM, Wi S, Escobar GJ. Combining immature and total neutrophil counts to predict early onset sepsis in term and late preterm newborns: use of the I/T2. The Pediatric infectious disease journal. 2014 Aug;33(8):798.
13. Eko Bagus, Hartono Kahar, Puspa Wardhani, Diagnostic values of immature granulocytes, eosinopenia and I/T ratio in detection of early onset neonatal sepsis in neonates with bacterial infection risk Folia Medica Indonesiana January - March 2014;50(1): 43-47
14. Amal Sabry, Amr Abd Allah, Lamiaa Salama, Alexandria University, anesthesia and surgical intensive care department, Alexandria, Egypt Nucleated red blood cells and eosinopenia as a high-risk mortality marker in patients of the intensive care units; Journal of American Science, 2012; 8.
15. Abidi K, Khoudri I, Belayachi J, Madani N, Zekraoui A, Zeggwagh AA, Abouqal R. Eosinopenia is a reliable marker of sepsis on admission to medical intensive care units. Critical Care. 2008 Apr;12(2):R59.
16. Prutha Desai, Amrish N. Shah, Tejas Pandya et al: C- Reactive protein, Immature to total Neutrophil Ratio and Micro ESR in early diagnosis ofNeonatal Sepsis, International Journal of Biomedical and Advance Research ISSN: 2229-3809
17. Ferhat Catal, Cuney Tayman, Alparslan Tonbul et al, Mean Platelet Volume (MPV) may simply Predict the Severity of Sepsis in Preterm Infants: Clinical laboratory. August 2014.