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Original Article

Nucleated Red Blood Cell Count as Earliest Prognostic Marker for Adverse Neonatal Outcome in Neonatal Sepsis

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Abstract

Introduction: The most common cause of neonatal mortality is neonatal sepsis, the diagnosis of which depends on blood culture, which has low sensitivity and takes time. We hypothesize that demonstration of elevated NRBC levels in neonatal sepsis might help in predicting an adverse neonatal outcome.

Aim of the Study: To analyse elevated NRBC it can serve as a prognostic marker for neonatal sepsis and an increased risk for adverse neonatal outcome.

Materials and Methods: This is a hospital based prospective study done in neonates who are admitted in NICU of Madurai Medical College with risk factors or clinical features of sepsis. After getting informed consent, the maternal details and examination findings were recorded and blood sample taken for sepsis screen, blood culture and peripheral smear for NRBC.

Results: The sensitivity of NRBC in identifying sepsis was 81.5%, its specificity was 61.76%, positive predictive value was 70.4% and negative predictive value was 75%. In the neonates who expired, serial NRBC counts (mean – 22.4) were significantly increased from baseline value (mean 17.3).

Conclusion: NRBC is significantly elevated in the neonatal sepsis and is a predictor of adverse neonatal outcome.

Key words: Neonatal sepsis, NRBC, Outcome

INTRODUCTION

The most common cause of neonatal mortality in developing countries is neonatal sepsis.[1] The definitive diagnosis of sepsis rests on the isolation of pathogenic bacteria in blood cultures, which has low sensitivity and takes time to influence the initiation of antibiotic therapy. A higher incidence of false-negative results due to antenatal antibiotic use and inadequate blood sampling further limits the test.

Thus, early, accurate, and rapid diagnosis of neonatal sepsis remains a major diagnostic challenge in neonatology, revealing the need for reliable and timely diagnostic biomarkers to enable clinicians to efficiently diagnose sepsis risk during the early phases of sepsis, provide effective antibiotic management tailored to causative organisms, and provide a useful guide for therapy during recovery.

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Neonates with sepsis are showing excess nucleated red blood cell (NRBC) in peripheral blood and are correlating with the adverse outcome. We hypothesize that the demonstration of elevated NRBC levels in neonatal sepsis might help in predicting an adverse neonatal outcome, and hence, we can improve the care by prioritizing them. If we come to know that the NRBCs are increased at an early stage, we can resort to higher antibiotics or other intensive management to prevent poor outcome. There are limited studies evaluating the role of NRBCs in neonatal sepsis, and hence, this study has been undertaken.

Many studies have shown that a value of up to 10 NRBC/100 white blood cell (WBCs) or an absolute

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NRBC count of 500/mm^3 is normal in a healthy term neonate.[2,3]

MATERIALS AND METHODS

The study was a prospective, hospital-based study conducted on neonates admitted in the NICU of Madurai Medical College for 6 months from March 2017 to August 2017.

Inclusion Criteria

- All term live neonates admitted in Level II NICU with risk factors of sepsis or clinical features of sepsis will be included in the study.

Exclusion Criteria

The following criteria were excluded from the study:

- Maternal pre-eclampsia or eclampsia.
- Gestational diabetes mellitus.
- Intrauterine growth retardation.
- Birth asphyxia.
- Pre-term and post-term babies.
- Hemolytic anemia (ABO and Rh incompatibility).
- Maternal smoking.

All the patients selected for the study were valuated in detail, comprising detailed history including maternal details and risk factors for sepsis, clinical examination, and relevant investigations.

Following investigations were done in all patients:
C-reactive protein (CRP): CRP was taken only after 6–12 h in early onset sepsis (EOS). CRP is estimated using semi-quantitative test using latex agglutination test. A value of 1.2 mg/dL is taken as cutoff.

Sepsis Screen

The components of the sepsis screen include total leukocyte count, absolute neutrophil count (ANC), immature/total neutrophil ratio (I/T ratio), micro-ESR, and CRP. Due to non-availability of the test, micro-ESR was not done in any patients. The cutoff values for total leukocyte count is <5000/mm^3 and immature-to-total neutrophil ratio is >0.2. The age-specific ANC values in the immediate neonatal period and the normal reference ranges for term babies are available from Manroe's charts.[2] For very low birth weight infants, the values are available from Mouzinho's chart.[3]

The presence of two or more abnormal parameter was considered as a positive screen and the neonate was started on antibiotics. If the screen was negative but clinical suspicion persisted, then it is repeated within 12 h. If the screen was still negative, the diagnosis of clinical sepsis was excluded.

Peripheral Smear

It is prepared using Leishman stain and is then examined under microscope for the presence of NRBC. NRBC count is expressed relative to 100 WBCs. A value of 10 NRBCs/100 WBCs or more is considered elevated. A repeat peripheral smear was taken on day 3 of admission, and the value was compared with the previous value. These neonates were followed up until discharge and repeat smear examination was done if the clinical condition of the neonate deteriorated.

Blood culture

The sample was collected by the resident doctor under strict aseptic precautions. Other investigations such as lumbar puncture, chest X-ray, abdominal X-ray, and other radiological studies were done in indicated cases.

The study group was divided into the following three groups based on the clinical findings and investigations.

1. Proven sepsis (Group I) – Neonates with positive blood culture.
2. Probable/clinical sepsis (Group II) – Neonates with strong clinical features, a positive sepsis screen but a negative blood culture.
3. No sepsis (Group III) – Neonates with negative blood culture and a sepsis screen. They presented with features of suspected sepsis or with associated risk factors. On further evaluation, they were found to be suffering from other disorders. Both Groups I and II were included in the sepsis group.
4. Data were entered into MS Excel and analyzed using SPSS v20. Qualitative data were summarized as frequencies and percentages. Quantitative data were checked for normality. Normally distributed data were summarized using mean and standard deviation.

RESULTS

A total of 72 neonates were included in the study, of which 48 neonates (66.6%) were <72 h old and were suspected to have EOS depending on the risk factors. The rest 24 neonates (33.34%) were >72 h old who presented with clinical features of sepsis or associated risk factors and suspected to have late-onset sepsis (LOS).

Of the 72 cases, 41 (56.9%) were male and 31 were female. All the cases included in the study were term babies who were appropriate for gestational age. Majority of the cases fit into the birth weight of 2.5–3.5 kg. Of the 72 cases, 42 were born by labor natural, of which two were assisted delivery (vacuum delivery). The rest 30 cases were lower segment cesarean section. The difference in the two groups was not found to be statistically significant ($P = 0.57$).

The most common risk factor for sepsis was foul smelling or meconium stained liquor (18 cases each) followed by maternal fever (13 cases). 13 neonates presented with clinical features of sepsis. Three cases had a history of unclean vaginal examination and delivery.

Of the 72 cases, 20 cases were culture-positive sepsis, 18 cases were clinical sepsis or culture-negative sepsis, and the rest 34 cases were negative for sepsis (no sepsis).

The most common symptomatology was respiratory distress seen in 30 cases, of which 15 cases had desaturation. 13 cases presented with shock, 11 cases were hypothermic during admission, and four cases had fever. Four cases presented with abdominal symptoms and two cases had convulsion.

The most common organism isolated from culture is *Klebsiella pneumonia* (35%) followed by coagulase-negative Staphylococcus (30%).

Of the total cases, NRBC was negative in 41 cases and positive in 31 cases. NRBCs were positive in 16 culture-positive cases, 15 culture-negative cases, and 13 cases with no sepsis.

The mean NRBC value in each group is given in Table 1.

The sensitivity of NRBC in identifying sepsis was 81.5%, its specificity was 61.76%, positive predictive value was 70.4%, and negative predictive value was 75%.

Of the 72 cases, 63 survived and 9 expired with a mortality of 13%. Of the 9 expired cases, 6 were culture positive. Mean NRBC in the mortality group was 17.3 on day 1, while a repeat count on day 3 showed an increase in the number of circulating NRBC in the mortality group and the mean value was 22.4. In the group that survived, NRBCs decreased on day 3 and were undetectable in most of the cases with a mean value of 3.47. NRBC is a better predictor of mortality and adverse neonatal outcome.

Table 1: The mean NRBC value

Final diagnosis	Mean
Culture-positive sepsis	13.75
Clinical sepsis	12.44
No sepsis	4.97

Table 2: Comparison of baseline investigations in sepsis and no sepsis group

Measure	Group	Mean±SD	*P* value
Hemoglobin	Sepsis	10.34±2.41	0.002
	No sepsis	13.05±1.24	
Total count	Sepsis	8526±5778	0.1096
	No sepsis	6867±1593	
Platelet	Sepsis	1,83,000±87,872	0.0001
	No sepsis	3,34,000±93,111	
NRBC	Sepsis	13.13±5.48	0.0003
	No sepsis	4.97±4.6	

SD: Standard deviation

DISCUSSION

In our study, of the 38 sepsis cases, 25 cases (65.7%) were EOS, of which 13 were culture positive, and 13 cases (34.2%) were LOS, of which 7 were culture positive. This is comparable to a study by Pramila *et al.* which shows 55.1% of EOS and 44.8% LOS.[4] In another study from Egypt, 44.2% were classified as EOS (≤72 h) and 55.8% as LOS (>72 h). The association between the culture positivity and the onset of sepsis was not found to be statistically significant ($P = 0.924$).

Among the sepsis screen, CRP positivity, thrombocytopenia, and I/T ratio were found to be statistically significant ($P = –0.0001$).

Comparison of Baseline Investigations in the Sepsis and no Sepsis Group given in Table 2

Mean Hb in the sepsis group was 10.34 and in the no sepsis group the mean was 13.05 and the difference was statistically significant.

Total count was normal in 48 cases.19 cases had leucopenia of which 13 are in sepsis group and 6 in no sepsis group.5 cases had leucocytosis; all of them were in sepsis group. But the difference between the two groups were not statistically significant ($P = 0.109$).

Immature to total cells (I/T) ratio was positive in 41 cases of which 37 turned out to be positive for sepsis and 4 were negative for sepsis and the difference is found to be stastically significant ($P <0.0001$).

Mean platelet count was 1.16 lakh in the sepsis group and 3.34 lakh in the no

sepsis group and the difference was statistically significant ($P = <.0001$)

CRP was positive in 35 cases of which 31 cases were in sepsis group and 4 cases were in no sepsis group and the difference was found to be statistically significant. ($P = <0.0001$)

The baseline NRBC was 13.75 in culture-positive cases, 12.44 in clinical sepsis, and 4.97 in no sepsis group, and the difference was found to be statistically significant. In a study done by Rathi *et al.*,[5] 47 cases of 56 neonates

with proven sepsis had a NRBC score of >10/100 WBCs accounting to 83.9%.

The sensitivity of NRBC in identifying sepsis was 81.5%, its specificity was 61.76%, positive predictive value was 70.4%, and negative predictive value was 75%. In a study done by Abhishek and Sanjay,[6] the sensitivity of the test in detecting proven sepsis was 35%, specificity 53.4%, positive predictive value 23.07%, and negative predictive value 67.64%.[6] In another study done by Rathi *et al*., the sensitivity of NRBCs was found to be 86.15%, specificity of 51.06%, positive predictive value 54.9%, and negative predictive value of 84.21%.[5]

Of the total 72 cases, 9 cases expired with a mortality of 13%. Mean NRBC in the mortality group was 17.3 on day 1, and on day 3, the mean value was 22.4. In our study, the mortality was high in cases with increased NRBC counts. NRBC is a better predictor of mortality and adverse neonatal outcome. In a study by Mădălina *et al*., it has been found that the daily screening for the presence of NRBCs seems to be a useful tool to estimate the mortality risk.[7] Mean NRBC in the mortality group was 17.3 on day 1, while a repeat count on day 3 showed an increase in the number of circulating NRBC and the mean value was 22.4. In the group that survived, NRBCs decreased on day 3 and were undetectable in most of the cases with a mean value of 3.47. The difference was found to be statistically significant.

Leiken *et al*.[8] found an increase in NRBCs when histological chorioamnionitis was present without signs of clinical chorioamnionitis. Salafia *et al*.[9] postulated that the increase in NRBCs could be a fetal response to an inflamed environment and not due to fetal hypoxia.

In a study done by Dulay *et al*. to determine if fetal inflammation is associated with an elevation of neonatal NRBC count in the setting of inflammation-associated preterm birth, it has been found that neonates with EOS had higher absolute NRBC count ($P = 0.011$). NRBC counts were directly correlated with cord blood IL-6 levels ($P < 0.001$) but not with erythropoietin, cortisol, or parameters of acid-base status levels.[10]

Acute chorioamnionitis has been associated with increased levels of erythropoietin and increased NRBCs. Maier *et al*.[11] found significantly elevated erythropoietin levels in neonates whose placentas showed signs of chorioamnionitis. Increased NRBCs have been reported in preterm infants' born after pregnancies complicated by chorioamnionitis without cord acidosis or hypoxemia.[9]

The main limitations of the study were a small sample size, and NRBC count was done by peripheral smear examination rather than by automated analyzer, which can lead to interobserver variation. Micro-ESR was not done as a part of sepsis screening due to non-availability of the test.

CONCLUSION

Estimation of NRBC in suspected neonatal sepsis can predict sepsis earlier. Serial NRBC measurement between survivors and non-survivors was found to be significant with $P < 0.05$. NRBC count is helpful in assessing the prognosis of sepsis in response to therapy. It is a better predictor of mortality in neonatal sepsis.

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