Original research article

A Study of Nucleated Red Blood Cell count as a Prognostic Marker for Adverse outcome in Neonatal Sepsis

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Abstract

Background : Neonatal Sepsis is the major cause of neonatal mortality among developing countries, the diagnosis of which depends on blood culture, which has low sensitivity and takes time.

Aim & Objective : To know whether Nucleated red blood cell count can be the first prognostic marker for adverse outcome in neonatal sepsis.

Materials and method: This was a hospital based prospective study conducted among neonates with clinical feature of sepsis in department of paediatrics, Govt Medical College, Siddipet, Telangana State, for the duration of one year. 50 patients were selected for the study after getting their informed consent and following inclusion and exclusion criteria, the maternal details and examination findings were recorded and blood sample taken for sepsis screen, blood culture and peripheral smear for NRBC.

Results: Sensitivity of NRBC in identifying sepsis was 86.9%, its specificity was 69.3%, positive predictive value was 74.2%, and negative predictive value was 77.56%, with cut off value of 16.4. Also study observed 6/26(23%) mortality among neonates with sepsis. And its was 12% among all study samples.

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

Keywords: NRBC, Peripheral Smear, Blood Culture etc.

Introduction

Neonatal sepsis refers to an infection involving the bloodstream in newborn infants less than 28 days old. It remains a leading cause of morbidity and mortality among neonates, especially in middle and lower-income countries[1]. Bacterial infections are the most common cause of sepsis in newborns. Bacteria such as *E. coli*, *Listeria* and Group B *streptococcus* (GBS) are common bacteria that can cause infections that lead to sepsis.

Neonatal sepsis is divided into two groups based on the time of presentation after birth: early-onset sepsis (EOS) and late-onset sepsis (LOS). EOS refers to sepsis in neonates at or before 72 hours of life (some experts use seven days), and LOS is defined as sepsis occurring at or after 72 hours of life[2]. The immature immune system is the major contributing factor for increased neonatal susceptibility to sepsis. Signs and symptoms of neonatal sepsis can range

from nonspecific or vague symptoms to hemodynamic collapse. Early symptoms may include irritability, lethargy, or poor feeding.

Others may quickly develop respiratory distress, fever, hypothermia or hypotension with poor perfusion and shock. Sometimes the diagnosis may only be suspected on the basis of laboratory findings, which may reveal hyperglycemia or hypoglycemia, acidosis, or hyperbilirubinemia.

Gold standard diagnostic modality for neonatal sepsis is blood culture which is time consuming, unavailable and has low positivity.[3] As a result there has been a constant search for a simple, rapid, and cost effective test. Thus, early, accurate, and rapid diagnosis of neonatal sepsis remains a major diagnostic challenge in neonatology, 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.

Thus we have undertaken this study to know whether Nucleated red blood cell count can be the first prognostic marker for adverse outcome in neonatal sepsis.

Materials and Method
This was a hospital based prospective study was undertaken in the department of Paediatrics, Govt Medical College, Siddipet, Telangana State, for the duration of one year. 50 patients were selected for the study after getting their informed consent and following inclusion and exclusion criteria and approved by Institutional review board.

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 :
- Maternal pre-eclampsia or eclampsia.
- Gestational diabetes mellitus.
- Intrauterine growth retardation.
- Birth asphyxia.
- Pre-term and post-term babies
- Maternal smoking.

Method
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.

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/mm3 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. For very low birth weight infants, the values are available from Mouzinho's chart.

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.

On the basis of clinical findings and investigation study group was divided into the following three groups.

1. Proven sepsis (Group I) – Neonates with positive blood culture.
2. (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.

Statistical Analysis :

Collected data were entered into MS Excel 2016 for further analysis, Qualitative data were expressed as frequencies and percentages. Quantitative data were checked for normality by using Shapiro wilk test. Normally distributed data were summarized using mean and standard deviation. Statistical analysis were done with the help of software SPSS version 25. Difference between the means were done by using t-test and difference in the proportion or association were assessed by using chi-square test. P-value<0.05 was considered statistically significant at 5% level of Significance.

Observation and Results

This study was a hospital based prospective study to evaluate whether a significant increase in NRBC is seen in neonatal sepsis. A total of 50 neonates were included in the study of which, 28 neonates (56%) were male and 22 neonate were female.

Table 1: Demographic profile of study population

Parameter	Group I (N= 26)	Group II(N= 24)	Total	Chi-square	P-value
Gender					
Male	14(285%)	14(28%)	28(56%)	0.102	0.74
Female	12(24%)	10(20%)	22(44%)		
Birth Weight					
<2500gms	14(28%)	5(10%)	19(38%)	2.39	0.122
≥ 2500 gms	16(32%)	15(30%)	31(62%)		
Maturity					
Preterm	19(38%)	16(32%)	35(70%)	0.0038	0.95
Term	8(16%)	7(14%)	15(30%)		

There was no significant difference was observed in the group I and group II for gender, birth weight and maturity of neonates shown in above demographic table.
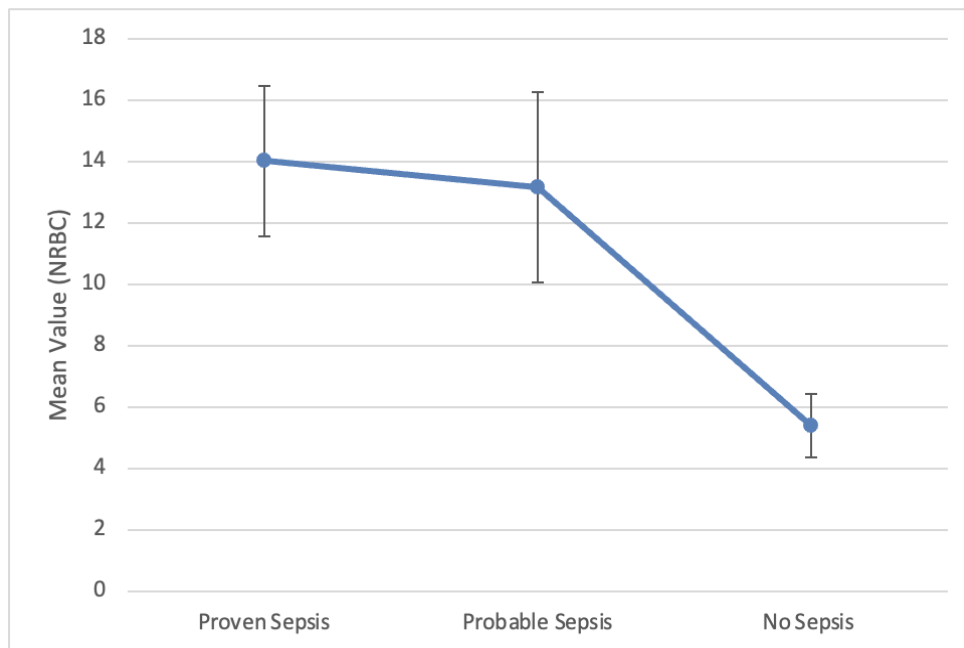

Figure 1: Mean distribution of NRBC among different sepsis

Table 2 : Mean distribution of investigation between the groups

Investigations	Group I (N= 26)	Group II(N= 24)	t-test	P-value
NRBC	13.58±4.45	5.4±1.02	15.17**	<0.001
Total Count	9106±6241	5421±1142	2.84**	0.0064
Haemoglobin	11.02±2.45	13.42±1.04	4.44**	<0.001
Platelets	1.45±0.83	3.82±0.91	9.71**	<0.001

**P-value<0.05, statistically highly significant at 5% level of significance

Study observed that NRBC, total count, haemoglobin level and platelets count were statistically highly significant between the groups.
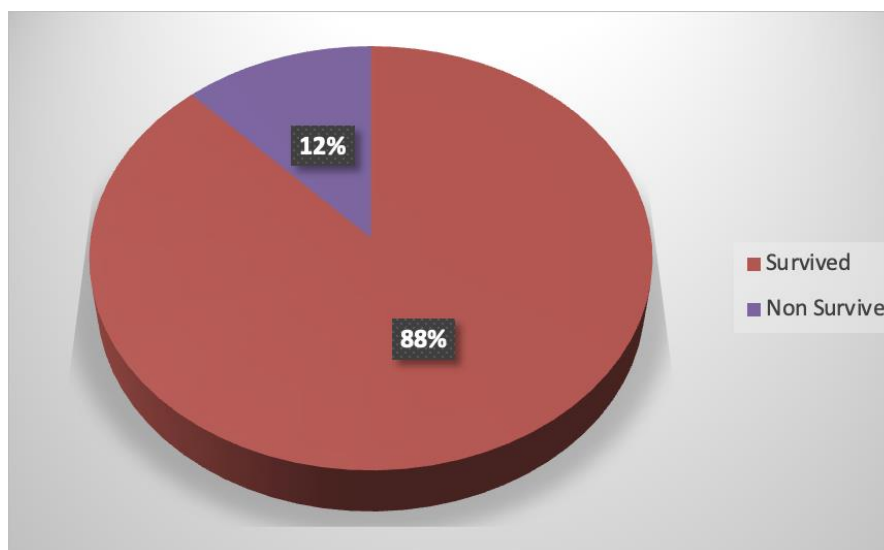
Figure 2 : Distribution of adverse outcome among study population.

Among all , study observed mortality in 6(12%) neonates, and all the non-survived neonates were from group I(Proven and probable Sepsis) which was counted 23%

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 [4]. The definite diagnosis of septicaemia is made by a positive blood culture which requires a minimum period of 48-72hrs and yields positive result in 30-40% of cases [5]. 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 increases risk of preventable mortality, thus in order to diagnose early septicaemia and to prevent adverse outcome we have undertaken this study, considering NRBC is a prognostic marker for adverse outcome in neonatal sepsis. We have included 50 neonates among which we have observed 26 were with sepsis and 24 were with no sepsis. Among neonates with sepsis 14 were males and 12 were females and gender difference between the group was not statistically significant.

In the present study we have observed that, sensitivity of NRBC in identifying sepsis was 86.9%, its specificity was 69.3%, positive predictive value was 74.2%, and negative predictive value was 77.56%, with cut off value of 16.4. Study conducted by Rathi et al[6] observed that, sensitivity of NRBC,s was found 86.15%, specificity 51.06%, PPV 54.9% and NPV was 84.21% another study by Dulay *et al.* 2008 [7] where NRBC,s showed increase number of NRBC,s in neonatal sepsis, Tripathi *et al.* 2010 [8] observed that activated macrophages releases cytokines which play important role in stimulating NRBC,s in absence of hypoxia. She also revealed that Nrbc were significantly increased in early and late neonatal sepsis.

In our study we have observed 6/26(23%) mortality among neonates with sepsis. And its was 12% among all study samples. Study conducted by Mădălina et al [9] observed that the daily screening for the presence of NRBCs seems to be a useful tool to estimate the mortality risk. Another study conducted 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 ($P = 0.011$).

Some studies observed that haematological parameters were poor predictors of neonatal sepsis[10] but in contrast many studies have established that the correlation between NRBC,s and neonatal sepsis. Though we have established relation between NRBC,s and adverse outcome, but also we had some limitation of study was sample size, also we have not done Micro-ESR which was part of sepsis screening.

Conclusion:

From overall observation and after discussing with another studies we can conclude that NRBC,s can be the early prognostic marker in neonatal sepsis in combination with other laboratory tests, this test can be tool for timely diagnosis and prompt interventions especially in rural areas, even before blood culture positivity. Also It will guide clinicians in instituting early treatment and adopting aggressive treatment whenever applicable, thus reducing the neonatal morbidity and mortality.

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