



## EARLY DIAGNOSIS OF NEONATAL SEPSIS – A LABORATORY CHALLENGE

**Dr. Sourabh Mandwariya**

**Dr. Ashok Panchnia\***

\*Corresponding Author

**Dr. C. V. Kulkarni**

**ABSTRACT** Sepsis in newborn is a devastating problem leading to morbidity and mortality. Inability to adequately exclude the diagnosis of neonatal sepsis can result in unnecessary and prolonged exposure of the newborn to antibiotics. Objectives of this study are to evaluate peripheral smears to establish an appropriate hematological parameter for early diagnosis of neonatal sepsis even before the blood culture.

We found that abnormal Total Neutrophil count is highly sensitive and increase Band cell count : Mature Neutrophil count ratio is highly specific for early diagnosis of neonatal sepsis. The positive predictive value is highest for increase Band cell count : Mature Neutrophil count ratio (B : M) and negative predictive value is highest for abnormal Total Leukocyte count. Leukery test is a good predictor for early diagnosis of neonatal sepsis. Group of Haematological parameters are more reliable indicator for early diagnosis of neonatal sepsis than any single parameter.

**KEYWORDS :** leukery test, neonatal sepsis, degenerative changes in neutrophils

### Introduction:

Neonatal sepsis, sepsis neonatorum and neonatal septicemia are the terms that are used to describe the systemic response to infection in the newborn infant. Sepsis in newborn can be a devastating problem leading to major morbidity and mortality. Neonates with one or more predisposing factors (e.g., low birth weight, premature rupture of membranes, septic or traumatic delivery, fetal hypoxia, maternal peripartum infection) are at increased risk for sepsis. The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births.

Diagnosis of neonatal sepsis may be difficult as the early signs of sepsis may be subtle and different at different gestational ages. The definitive diagnosis of septicemia is made by a positive blood culture, which requires a minimum of 48-72 hrs and yields a positive result in only 50-60% of cases.

Inability to adequately exclude the diagnosis of neonatal sepsis can result in unnecessary and prolonged exposure of the newborn to antibiotics. Thus laboratory tests that assist the clinician in diagnosis of infection in neonates have considerable relevance.

### Material and Method:

The present study is hospital based prospective study includes 500 neonates admitted in Neonatal Intensive Care Unit (NICU) with different symptoms during the period of July 2011 to April 2012. Peripheral blood smears are made from freshly collected specimen in anticoagulant containing vial and stained with standard hematoxylin and eosin staining method. The following hematological parameters are evaluated:

**Leukery Test :** On each peripheral blood smear slide 300 white blood cells of all types were counted and the percentage of leukery (agglomerated cells) was calculated. Agglomeration was considered to be positive at least three leukocytes were in close proximity, the distance between their nuclei being less than the diameter of one cell<sup>2</sup>. The count was made twice and the final leukery result was the average of the two readings.

### Grading of Leukery Test:

Grade – 0 - Leukery in less than 10% of leukocytes.

Grade – 1 – Leukery in 11-19% of leukocytes.

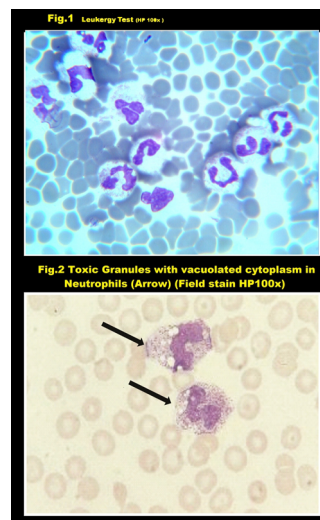
Grade – 2 – Leukery in 20-34% of leukocytes.

Grade – 3 – Leukery in >34% of leukocytes.

### Other hematological parameters<sup>3</sup>

1. Hemoglobin estimation was done on automated cell counter (Sysmex-kx21) by cyanide-free method<sup>4</sup>.

2. Presence of normoblast
3. Total Leukocyte count : Decrease or Increase (Decrease less than 5000/mm<sup>3</sup> or Increase more than 25,000; 30,000; and 21,000/mm<sup>3</sup> respectively at birth, 12-24 hrs; and day 2 onward)
4. Total Neutrophil count : Decreased or Increased (Normal Value - 7,800 to 14,500/mm<sup>3</sup> {less than 72 hrs}; 1,750 to 4500/mm<sup>3</sup> {more than 72 hrs})



5. Band cell count : Increased {Normal Value - 500 to 1450/mm<sup>3</sup> (<72 hours), <500/mm<sup>3</sup> (Upto 28 days)}
6. Band cell count : Total Neutrophil count ratio : Increased (More than 0.2)
7. Band cell count : Mature Neutrophil count ratio : Increased (more than 0.3)
8. Presence of degenerative changes in Neutrophils (Toxic granules, Vacuolated cytoplasm, Döhle bodies)
9. Platelet count : Decreased (Less than 1,00,000/mm<sup>3</sup>)<sup>5</sup>

### Blood Culture and Sensitivity

The blood is collected using aseptic technique and sent to Department of Microbiology. Sample was inoculated on Blood agar and MacConkey agar; and reported by microbiologist after 48 hrs of incubation<sup>6</sup>. Positive results are further conformed by gram staining and other biochemical test<sup>7</sup>.

### Observations

The results and observations of various haematological changes are presented in Table I and Table II.

**Table I : AGE AND SEX DISTRIBUTION OF STUDY CASES**

Age group	Number of males	Number of females	Total
0-24 hr (D1)	12 (04.78%)	12 (04.82%)	24 (04.80%)
25-48 hr (D2)	36 (14.34%)	24 (09.64%)	60 (12.00%)
49-72 hr (D3)	83 (33.07%)	78 (31.33%)	161 (32.20%)
73-96 hr (D4)	36 (14.34%)	39 (15.66%)	75 (15.00%)
97-120 hr (D5)	39 (15.54%)	42 (16.87%)	81 (16.20%)
>120 hr	45 (17.93%)	54 (21.68%)	99 (19.80%)
Total	251 (100%)	249 (100%)	500 (100%)

Out of 500 study cases, males (50.20%, n=500) are more in number than females (49.80%, n=500). Maximum numbers of cases (32.20%, n=500) are belonging to age group 49-72 hr (D3).

**Table II : PERFORMANCE OF INDIVIDUAL PARAMETER**

Parameter	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Leukergy test	59.12	60.27	70.25	52.74
Total Leukocyte count	50.45	80.67	72.00	59.02
Total Neutrophil count	77.87	15.31	58.07	39.02
Band cell count	71.15	55.57	68.65	53.40
Band cell count: Total Neutrophil count ratio	33.17	86.48	79.05	46.57
Band cell count: Mature Neutrophil count ratio	29.54	99.27	81.29	50.36
Degenerative changes in Neutrophils	14.35	92.14	77.14	51.56
Platelet count	41.45	92.56	77.48	49.51
Haemoglobin level	59.22	37.14	59.49	42.54
Normoblast	46.21	81.41	81.20	56.52

Abnormal Total Neutrophil count (77.87%) is highly sensitive followed by increase Band cell count (71.15%) in early diagnosis of neonates with sepsis.

Increase Band cell count : Mature Neutrophil count ratio (99.27%) followed by decreased Platelet count (92.56%) is highly specific test helpful in early diagnosis of neonates with sepsis.

The positive predictive value is highest for increase Band cell count : Mature Neutrophil count ratio (81.29%) followed by presence of Normoblast ( 81.20%) which is helpful in identifying neonates who really have sepsis.

The negative predictive value is highest for abnormal Total Leukocyte count (59.02%) followed by presence of Normoblast (56.52%) which indicate that neonates do not have any evidence of sepsis<sup>8</sup>.

## Discussion

Leukergy test and Degenerative changes in Neutrophils (Toxic granules, Döhle bodies and Vacuolisation of cytoplasm) are good predictors for early diagnosis of neonatal sepsis. Total Leukocyte count and Total Neutrophil count will be abnormal in neonatal sepsis; it may increase or decrease. Band cell count, Band cell count : Total Neutrophil count ratio, Band cell count : Mature Neutrophil count ratio and Normoblast count are increased in neonates with sepsis. Platelet count and Haemoglobin level will be lower in the cases of neonatal sepsis as compared to neonates without sepsis.

We found that Total Neutrophil count will show maximum abnormality (77.87% sensitive) if a neonate is having septicaemia. Band cell count : Mature Neutrophil count ratio will be normal if a neonate is not having sepsis (99.27% specific). Increased Band cell count : Mature Neutrophil count ratio is having maximum positive predictive value (81.29%). The negative predictive value is highest for Abnormal Total leukocyte count (59.02%) which indicate that

neonates do not have any evidence of sepsis. Group of tests are more reliable in early diagnosis of neonatal sepsis than any single laboratory parameter<sup>7</sup>.

Blood culture may be sterile if antibiotics are administered before withdrawing the blood sample for laboratory investigations<sup>10</sup>. In these cases haematological changes are much more useful for diagnosis and treatment.

Our endeavour here is to evaluate haematological changes in neonatal sepsis to help the clinicians in early diagnosis of neonatal sepsis, for deciding the treatment and improve the outcome of neonates.

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