



## Hematological Profile in Diagnosing Early Neonatal Sepsis

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### Abstract

**Introduction:** Systemic infection in first month of life have remained as major cause of mortality and morbidity despite the development of broad spectrum antimicrobial agents. In India incidence of sepsis is 38 per 1000 live births in tertiary care institutes and it contributes to 36% of deaths in hospitals. To prevent serious morbidity and mortality caused by untreated or late treated neonatal septicemia, it is important that the diagnosis is made early and the treatment is started as early as possible. The present study was aimed to evaluate the neonatal clinical manifestations and their hematological parameters, for rapid identification of early onset neonatal sepsis (EOS).

**Material and Methods:** This study was conducted at hematology section, Department of pathology, Government Medical College, Dungarpur. Blood samples of the neonates were collected at the time of admission and before initiation of antibiotic therapy. WBC < 5000 or >20,000 /mm<sup>3</sup> were considered abnormal. Absolute neutrophil count (ANC) was calculated, toxic granules, premature cells also noted.

**Observations and Results:** A total of 60 neonates were included in which 36% cases of culture positive and 11% cases of culture negative presented with low count while 10% cases of culture positive and 10% cases of culture negative presented with leucocytosis. Out of 35 proven cases 32% cases have ANC less than 1800/mm<sup>3</sup> and 26% cases had  $\geq$  to 1800/mm<sup>3</sup>. (35%) of proven cases have band cell count more than 20%. 52% proven cases showed I/T ratio  $\geq$  0.2 while 06% showed I/T ratio < 0.2. 19 (31%) of proven cases showed presence of cytoplasmic vacuoles in neutrophil. 41% cases showed presence of toxic granules.

**Conclusion:** It is simple, rapid, cheap and does not need special laboratory facilities which makes it useful in the early detection of neonatal sepsis.

**Keywords:** sepsis, neutrophils, ANC, toxic granules.

### Introduction

Systemic infection in first month of life have remained as major cause of mortality and morbidity despite the development of broad spectrum antimicrobial agents. Overall incidence

varies between 1-8 cases/1000 live births<sup>(1)</sup>. In India incidence of sepsis is 38 per 1000 live births in tertiary care institutes and it contributes to 36% of deaths in hospitals<sup>(2)</sup>. Although infection can be caused by virus, yeast, and parasite, yet it is

bacterial infection that play important role in neonatal sepsis<sup>(3)</sup>. Bacterial infection in the newborn still account for a considerable morbidity and mortality. This is because the newborn especially the premature are prone to serious infections by organisms and partly because the signs of these infections may be absent or minimal and hard to detect<sup>(4)</sup>. To prevent serious morbidity and mortality caused by untreated or late treated neonatal septicemia, it is important that the diagnosis is made early and the treatment is started as early as possible<sup>(5)</sup>. Early onset sepsis (EOS) is defined as sepsis occurring during the first 72 hours of life as a fetal response to an ascending infection (from the birth canal) or to the hematogenous dissemination of a maternal infection. (MLO). If the exposure occurs in utero or during delivery process, the sepsis is classified as early onset sepsis and if the exposure occurs after birth, it is classified as late onset sepsis<sup>(3)</sup>. "Suspected sepsis" is one of the most frequently encountered diagnosis in neonatology because: a) a large number of newborns are evaluated for early or late sepsis based on risk factors and for fear of missing a correct diagnosis and a prompt treatment; b) in neonates, the clinical signs of infection are not specific, late, and the differential diagnosis with neonatal respiratory distress syndrome, aspiration syndromes, or neonatal maladaptation to extra uterine life is difficult; c) blood culture - the golden standard in neonatal sepsis diagnosis - provides late information, has a poor accuracy, and is not universally available ; and d) we do not have yet an ideal diagnostic tool for neonatal Infection<sup>(6)</sup>. Although blood culture is the "Gold Standard" for the diagnosis of sepsis, reports are available after 48-72 hours and they may be affected by intrapartum antibiotic administration to the mother. Thus, the outcome of a neonate with sepsis largely depends upon its early identification. To meet this end, several rapid diagnostic tests have been described recently. Individual haematological parameters are least affected by antibiotic administration. However, they have low sensitivity and specificity, therefore

a combination of these tests was studied by many workers to formulate a reliable sepsis screen<sup>(7)</sup>. For the same reason, several rapid haematological tests are done as part of sepsis screen for early diagnosis of neonatal sepsis<sup>(2)</sup>. Sepsis screen = A battery of indirect markers of infection when collectively studied provide an extremely reliable index of neonatal sepsis much earlier and serve as a useful guide for initiating antibiotic therapy<sup>(5)</sup>. The present study was aimed to evaluate the neonatal clinical manifestations and their hematological parameters, for rapid identification of early onset neonatal sepsis (EOS)<sup>(2)</sup>. Peripheral blood smear examination provides information that cannot be obtained from automated cell counting<sup>(4)</sup>. Considering the limited supportive facilities in most area in country; a simple laboratory parameter is necessary which can predict sepsis thus helping the physician in diagnosis and treating neonatal sepsis<sup>(3)</sup>.

### **Aims and Objective**

Being cheap, simple, rapid and readily available, we aimed at re-evaluating the usefulness of hematological profile as there is still a need for a diagnostic test with high sensitivity, to help improve outcome of septic neonates by alleviating the delay in treatment, and high specificity, to decrease exposure of non-septic neonates to empirical antibiotic therapy.

### **Materials and Method**

This study was conducted at hematology section, Department of pathology, Government Medical College, Durgapur. Neonates in the department of pediatrics were evaluated thoroughly for their clinical course (i.e. gestational age, age of onset, birth weight) were noted. Based on blood culture positivity neonatal sepsis cases were classified as proven and probable sepsis. Blood samples of the neonates were collected at the time of admission and before initiation of antibiotic therapy. Blood sampling was done under all aseptic precautions in the NICU. Soon after admission two ml blood sample was

taken in EDTA vacutainer and processed for TLC on hematology autoanalyser. TLC < 5000 or >20,000 /mm<sup>3</sup> were considered abnormal. Blood smear were studied after giemsa stain for morphological features which were looked under 40X and oil immersion using cedar wood oil – Neutrophils = hyper segmented, band form, immature to total neutrophil ratio (I/T ratio), Absolute neutrophil count was calculated and toxic granules also noted. Noted data was entered in excel sheet and analyzed statistically.

**Study period:** Between August 2018 to January 2019

**Sample size:** 60 neonates admitted to NICU of pediatric department

**Inclusion criteria:** Neonates below the age of 28 days with suspected septicemia as per Signs and symptoms mentioned in proforma were included in this study.

**Exclusion criteria:** Newborn who were diagnosed with highly suspected sepsis and received antibiotic treatment before the samples were collected, had neonatal asphyxia, congenital anomalies, received resuscitation or any invasive procedures before sampling, born from mothers with co-morbid disease other than risk factor for sepsis, received antibiotic treatment for other indication, were not included in the study.

**Observations and Results**

**Table 1:** Gender distribution of cases

Gender	Clinical sepsis (Total 60)	
	Proven cases	Probable cases
Males	25 (41%)	17 (28%)
Females	10 (17%)	08 (14%)
Total cases	35	25

A total of 60 neonates were included in this study. Out of which 42 (69%) were males while 18 (31%) were females

**Table 2:** Incidence of Total leucocyte count in cases

Value of total count	Clinical sepsis (Total 60)	
	Proven cases	Probable cases
<5000/mm <sup>3</sup>	22 (36%)	07 (11%)
>20000/mm <sup>3</sup>	06 (10%)	06 (10%)
5000-20000/mm <sup>3</sup>	07 (11%)	12 (21%)
Total cases	35	25

Leucopenia was considered when count was <5000/mm<sup>3</sup> while leucocytosis was considered when count was >20000/mm<sup>3</sup>. In our study 22(36%) cases of culture positive and 07 (11%) cases of culture negative presented with low count. In our study 06 (10%) cases of culture positive and 06 (10%) cases of culture negative presented with leucocytosis. 07(11%) in culture positive and 12(21%) in culture positive showed WBCs within normal range.

**Table 3:** Incidence of Absolute Neutrophil Count (ANC) in cases

Value of ANC per cubic mm	Clinical sepsis (Total 60)	
	Proven cases	Probable cases
<1800/mm <sup>3</sup>	16 (26%)	10 (17%)
≥1800/mm <sup>3</sup>	19 (32%)	15 (25%)
Total cases	35	25

Out of 35 proven cases 19 (32%) cases have ANC less than 1800/mm<sup>3</sup> and 16 (26%) cases had more than or equal to 1800/mm<sup>3</sup>. Out of 25 probable cases 10 (17%) cases have ANC less than 1800/mm<sup>3</sup> and 15 (25%) cases had more than or equal to 1800/mm<sup>3</sup>.

**Table 4:** Incidence of Band Cell count in cases

Value of band cell count	Clinical sepsis (Total 60)	
	Proven cases	Probable cases
≥20%	22 (35%)	15 (26%)
<20%	13 (22%)	10 (17%)
Total cases	35	25

Out of 35 proven cases 22 (35%) cases have band cell count more than 20% and 12 (26%) cases had less than 20% 13 (22%). Out of 25 probable cases 15 (26%) cases have band cell count more than 20% and 10 (17%) cases had less than 20%.

**Table 5:** Cases showing incidence of positive I/T ratio

I/T ratio	Clinical sepsis (Total 60)	
	Proven cases	Probable cases
≥0.2	31 (52%)	19 (32%)
<0.2	04 (06%)	06 (10%)
Total cases	35	25

In the present study among the 35 cases of blood culture positive neonates 31(52%) showed I/T ratio ≥0.2 while 04 (06%) showed I/T ratio <0.2 .Out of 25 probable cases 19 (32%) showed I/T ratio ≥0.2 while 06 (10%) showed I/T ratio <0.2

**Table 6:** Cases showing incidence of toxic granulation of neutrophils

Toxic granulations in neutrophils	Clinical sepsis (Total 60)	
	Proven cases	Probable cases
Present	25 (41%)	13 (21%)
Absent	10 (17%)	12 (21%)
Total cases	35	25

Out of 35 proven cases 25 (41%) cases showed presence of toxic granules and 10 (17%) cases

**Discussions**

**Table 8:** Comparison of gender distribution among cases in different studies

Gender	Kalpana et al	Dalia et al	Champa et al	Vandana et al	Ravindra et al	Hijrah et al	Present study
Male	66%	57.2%	58.9%	56.4%	58%	57.5%	69%
Female	34%	42.8%	41.1%	43.6%	42%	42.5%	31%

In present study neonatal sepsis was more commonly seen in male in comparison to females which consistent with result of other studies.

**Table 9:** Comparison of total leucocyte count among cases in different studies

Study	Clinical sepsis	
	Proven cases	Probable cases
Champa et al	<5000/mm <sup>3</sup> - 15.2% 5000-20000/mm <sup>3</sup> - 54.3% >20000/mm <sup>3</sup> - 30.5%	<5000/mm <sup>3</sup> - 8.1% 5000-20000/mm <sup>3</sup> - 71.5% >20000/mm <sup>3</sup> - 20.4%
Ravindra et al	<5000/mm <sup>3</sup> or >20000/mm <sup>3</sup> - 49% 5000-20000/mm <sup>3</sup> - 51%	<5000/mm <sup>3</sup> or >20000/mm <sup>3</sup> - 35% 5000-20000/mm <sup>3</sup> - 65%
Dalia et al	<5000/mm <sup>3</sup> - 13.7.2% 5000-25000/mm <sup>3</sup> - 80% >25000/mm <sup>3</sup> - 06%	
Present study	<5000/mm <sup>3</sup> - 63% 5000-20000/mm <sup>3</sup> - 17 % >20000/mm <sup>3</sup> - 20%	<5000/mm <sup>3</sup> - 28% 5000-20000/mm <sup>3</sup> - 24 % >20000/mm <sup>3</sup> - 48 %

In present study leukopenia was found to be present in 63% of proven cases while leucocytosis was found to be in 20% of proven cases. In other studies maximum cases were found to be within

showed absence of toxic granules .Out of 25 probable cases 13 (21%) cases showed presence of toxic granules and 12 (21%) cases showed absence of toxic granules.

**Table 7:** Cases showing Incidence of positive cytoplasmic vacuoles in neutrophils

Cytoplasmic vacuolization of neutrophils	Clinical sepsis (Total 60)	
	Proven cases	Probable cases
Present	19 (31%)	12 (20%)
Absent	16 (26%)	13 (23%)
Total cases	35	25

Out of 35 proven cases 19 (31%) cases showed presence of cytoplasmic vacuoles in neutrophil and 16 (26%) cases showed absence of cytoplasmic vacuoles in neutrophil. Out of 25 probable cases 12 (20%) cases showed presence of cytoplasmic vacuoles in neutrophil and 13 (23%) cases showed absence of cytoplasmic vacuoles in neutrophil.

normal range suggesting no diagnostic significance in neonatal sepsis whereas in our study it is found to useful as marker of sepsis.

**Table 10:** Comparison of ANC among cases in different studies

Value of ANC per cubic mm	Ravindra et al		Hijrah et al		Present study	
	Proven sepsis	Probable sepsis	Proven sepsis	Probable sepsis	Proven sepsis	Probable sepsis
<1800	15.3%	06%	05%	45%	26%	17%
>1800	54.8%	24%	23%	27%	32%	25%

The results above show that higher ANC is associated with culture positive cases and also with probable cases except in study by Hijrah et al in which low ANC is associated with more number of probable cases. This result supports previous theories which implied that newborn from mother with infection risk factor and had high ANC are at high risk

of neonatal sepsis. In newborn infant, non specific immunity plays crucial role in eliminating pathogens, thus, if the newborn have high ANC it can be inferred that there's some severe infection going on. This prediction is more likely in newborn from mother with infection risk factors<sup>(3)</sup>.

**Table 11:** Comparison of I/T ratio in neonatal sepsis patients in different studies

Study	Clinical sepsis	
	Proven cases	Probable cases
Champa et al	I/T ratio >0.2 - 47.8%	I/T ratio >0.2 - 4.08%
Ravindra et al	I/T ratio ≥0.2 - 47% I/T ratio <0.2 - 23%	I/T ratio ≥0.2 - 06% I/T ratio <0.2 - 24%
Dalia et al	Normal I/T ratio <0.2 - 34.4% Moderate I/T ratio (0.2-0.29) -26% Severe I/T ratio (≥0.3) -39.6%	
Vandana et al	I/T ratio ≥0.2 - 50% I/T ratio <0.2 - 50%	I/T ratio ≥0.2 - 23.6% I/T ratio <0.2 - 66.4%
Present study	I/T ratio ≥0.2 - 52% I/T ratio <0.2 - 06%	I/T ratio ≥0.2 - 32% I/T ratio <0.2 - 10%

In the current study it was reported that neutrophil left shift (I/T ratio ≥ 0.2) was seen in maximum cases of both proven and probable cases which were consistent with most of the studies .During the bacterial infections increased number of neutrophils is released from bone marrow into the blood stream providing neutrophils to migrate at

the infected site. This increase in neutrophils appear essential for the host resistant to bacterial infection. As more neutrophils are released, more & more immature cell reaches the circulation, a process called as “shift to left”. This finding have been found valuable in early diagnosis of bacterial infection<sup>(5)</sup>.

**Table 12:** Comparison of presence of toxic granules in neutrophils in different studies

Study	Clinical sepsis	
	Proven cases	Probable cases
Champa et al	Present - 32.6%	Present - 00%
Ravindra et al	Present - 41 % Absent - 29%	Present - 07% Absent - 23%
Kalpana et al	Present - 70.5% Absent - 29.4%	Present - 36.3% Absent - 63.6%
Vandana et al	Present - 68.18% Absent - 31.82%	Present - 45.8% Absent - 54.6%
Present study	Present - 41% Absent - 17%	Present - 21% Absent - 21%

Toxic granules were present in majority of cases in proven cases which is in accordance with other

studies while in probable cases toxic granules were absent in most of the cases in present as well as

other studies. Xanthouin her study of neonatal infection, described toxic granulation as an important feature and that that toxic granulation was invariably present during sepsis change never seen in healthy new born babies<sup>(8)</sup>.

### Conclusions

Neonatal sepsis is a serious illness associated with high mortality so a high index of suspicion is important in the diagnosis and treatment of neonatal infection because it is hampered by vague and nonspecific clinical manifestations. It is simple, rapid, cheap and does not need special laboratory facilities which makes it useful in the early detection of neonatal sepsis as well as decreasing the exposure of non-septic neonates to antimicrobial therapy.

**Future Implications:** The accuracy of several biomarkers for diagnosing sepsis, as interleukins (IL6, IL8), CD64, tumour necrosis factor alpha in the early, procalcitonin in the middle and C reactive protein in the late phases of sepsis, have been studied and seemed promising if collaborated with current diagnostic hematological parameters like WBC count, I/T ratio, immature PMNs, thrombocytopenia, toxic granules, raised  $\mu$ -ESR and CRP.

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