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Original Research Article

A Potent Marker of Neonatal Sepsis- Morphological Changes in Leukocytes

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Abstract: Hematologic scoring system (HSS) can improve the efficiency of the complete blood count as a screening test for sepsis and permits an objective assessment of hematological changes. In present study 150 cases of neonatal sepsis were studied in a period of one and a half year from March 2015 to July 2016. Out of 150 cases, 56 cases had proven sepsis of which 49 cases showed morphological changes in their leukocytes which is the most sensitive marker of neonatal sepsis. The best parameter amongst all appears to be the study of morphological changes with both high sensitivity and specificity. Overall impression goes in the favour of the battery of investigation as the whole combination is good to assess and compare the septic and aseptic cases early.
Keywords: Neonatal sepsis, hematological scoring system

INTRODUCTION

The leading cause of morbidity and mortality amongst neonates is the neonatal sepsis [1]. Though, there has been significant reduction in complications and improvements in survival in preterm infants, sepsis in very low birth weight (VLBW<1500 g) infants in Neonatal Intensive Care Units (NICUs) [2, 3] contributes significantly to the mortality and morbidity. Early screening methods for the detection of neonatal sepsis are a must because its diagnosis is difficult. There are no specific signs and symptoms of neonatal sepsis [4]. Symptoms like fever, cyanosis, feeding difficulties, apnea, lethargy, irritability, hypotonia, abdominal distention, seizures, bulging fontanel, poor perfusion, respiratory distress bleeding problems, hepatomegaly, positive culture of stools, jaundice of unknown cause [5, 6]. There may be gasping in uterus along with hypoxia and acidosis causing pneumonia and meconium aspiration [7]. The incidence is not negligible in asymptomatic neonates as well [4].

MATERIALS AND METHODS:

Blood sample are collected in EDTA tubes. The samples are labelled with subjects' age and identification number. All the samples were taken from patients attended OPD and IPD of index medical college, Indore in duration of 16 months from March 2015 to June 2016 and IEC approval was done on 21/02/2015.

Using automated haematology analyser values are noted. Peripheral smears are prepared from the samples. Smears are stained with leishman stain and observed under microscope. Detailed analysis of the peripheral smear is done and reported accordingly. A Hematological scoring system as in a study by Rodwell *et al.;* in 1988 [8], is framed using different parameters to be studied by the peripheral smear as shown in table 1. The scoring system includes the following parameters, TLC, Absolute Neutrophil Count, mature and immature neutrophil count, immature to mature ratio, immature to total ratio, morphological changes and platelet count and n RBC count. Maximum score of 11 is given. According to the scores, neonates are further categorized in to 4 categories, no sepsis with score <4, low risk with score of 4-6, high risk with score 7-8 and sepsis with score of 9-11, as shown in table 02. The category is informed to the pediatrician so that the treatment is planned accordingly.

INCLUSION CRITERIA:

Neonates suspected on clinical background. Neonates with confirmed clinical diagnosis of sepsis and not on treatment.

EXCLUSION CRITERIA:

Neonates on treatment. Parents not willing to give the consent. Neonates severely jaundiced due to blood group incompatibility.

RESULTS

All the hematological parameters were combined and scores were prepared based on the variation from the normal reference values as shown in table 1 , for early and simple approach towards8 management of the new born as in a study by Rodwell et al[8] and thereby, further preventing morbidity and mortality.

Table 1: The hematological scoring system

Parameters	Findings	Score
Total leukocyte Count	≤ 5,000 /mm^3	02
	≥ 25,000 /mm^3	01
Absolute Neutrophil Count	No mature Neutrophils seen	02
Absolute Neutrophil Count	< 1,500 /mm^3	02
[Mature Neutrophils]	> 8,000 /mm^3	01
I/T Ratio	> 0.2	01
I/M Ratio	> 0.3	01
Neutrophil Morphology	> 30%	01
nRBCs	> 10 /100WBCs	01
Platelet Count	< 1,50,000 /cu mm	01
	Total	11

According to pediatric reference values Total leukocyte Count is considered to be 5000 as lower limit and 25000 as upper limit at the first day which gradually fall to 20000 within 24 hrs. Scoring is done accordingly. According to WHO normal platelet count are 1, 50,000 - 4, 00,000/cu mm. anything less than the normal count is allotted score 1 in this scoring system. The categorized groups (no risk, low risk, high risk and sepsis group) as shown in table 2, were based on the results obtained from the screening test. The feedbacks of the results obtained were given to the respective pediatrician.

Table 2: Categorized groups from the screening test

	Score
No sepsis	<4
Low risk	4-6
High risk	7-8
Sepsis	9-11

Neonates at risk of developing sepsis by HSS:-

Table 3: Neonates at risk of developing sepsis

	DEATH	DEVELOPED SEPSIS
No sepsis (n-70)	00	00
Low risk (n-23)	01(05%)	05
High risk (n-27)	04(13.5%)	21
Sepsis (n-30)	08(22.22%)	30

Out of 150 neonates with features of sepsis admitted to the pediatric department of Index Medical College, Indore, were classified on the basis of HSS in to 4 categories as shown in table 2. On the above basis as depicted in table 3, most of the children fell in to no sepsis group and had excellent prognosis with none of the children developing sepsis, as later confirmed by the culture report. There was no mortality in the children falling in to this category.

- 23 neonates with score (4-6) falling in to low risk group had 5/23 neonates developing sepsis. The mortality was 1 in 23.

- 27 neonates on the basis of HSS fell in to high risk group of developing sepsis. 21 out of these 27 neonates were later on confirmed of having sepsis by culture. This group had higher mortality ratio i.e 4/27 (14.81%) as mentioned in table 3.
- 30 neonates with score of 9-11 were confirmed of having sepsis by peripheral blood smear examination and the findings were confirmed by culture positivity. This group had significant mortality of 8/30 (26.6%).
- Various parameters of hematological scoring system were studied and scoring was done accordingly.

MORPHOLOGICAL CHANGES IN NEUTROPHIL IN RELATION TO NEONATAL SEPSIS:

As discussed in table 4, Morphological changes included the changes in neutrophil morphology, toxic granulation, and cytoplasmic vacuolation and Dohle bodies. Neonates with morphological changes in >30% of leukocytes were given a score of 01 in our hematological scoring system.

Table 4: Morphological changes in neutrophils

Morphological changes	With sepsis	Without sepsis	Total
Toxic granules	20	08	28
Cytoplasmic vaculations	09	03	12
Toxic granules + cytoplasmic vacuolation + Dohle bodies	20	01	21
Total	49	12	61

- Out of 150 neonates studied, 94 cases had no sepsis of which only 12 showed morphological changes.
- Of 56 cases of proven sepsis 49 cases showed morphological changes, when compared with neonates without sepsis (12/94).
- Toxic granules was the most common change seen in the present study i.e 20 cases had toxic granules alone, while 20 had toxic granules in combination with other morphological changes like Dohle bodies, cytoplasmic vaculations, (9/96) i.e 9.37% without sepsis.
- Cytoplasmic vacuolation was found in only 29/56 cases of sepsis and 4/96 cases i.e 4.16% without sepsis.

MORPHOLOGICAL CHANGES IN NEONATES WITHOUT SEPSIS

Morphological changes were infrequent in neonates without sepsis. Only 12/96 cases showed morphological changes, of which toxic granules were the most common, i.e. 8/96; equally distributed amongst the low and high risk cases.

MORPHOLOGICAL CHANGES IN NEONATES WITH SEPSIS

Out of 56 sure shot cases of sepsis, 49 cases showed some morphological changes which included toxic granules, cytoplasmic vacuolation, Dohle bodies. Toxic granules alone were most commonly found in neonates of probable risk category. The combination of all 3, i.e, toxic granules, cytoplasmic vacuolation and Dohle bodies were seen in high risk (09-12) score group i.e, 17/30. Cytoplasmic vacuolation was also seen mostly in high risk cases.

ABSOLUTE NEUTROPHIL COUNT:

From the total leukocyte count and the mature and immature neutrophil count, absolute neutrophil count is calculated. Absolute neutrophils with no mature neutrophils are given a score of 02 in our hematological scoring system. Absolute neutrophil count of <1500/mm3 is given a score of 02 in HSS and those with count of >8000/mm3 is given a score of 01.

- Our study showed 02 neonates out of 94 cases of no sepsis with a count of <1500 and 34 cases with >8000/mm3.
- Out of 56 cases of proven sepsis, 35 cases has a count of <1500 and 21 cases with >8000/mm3.

TOTAL LEUKOCYTE COUNT:

Another important parameter is the TLC. Out of 150 neonates studied, those with total leukocyte count <5000/mm3 were given a score of 02, whereas those with the total leukocyte count >25000/mm3 were given the score of 01. A score of 0 was given to those lying with the total leukocyte count ranging from 5000-25000/mm^3. Our study shows 94 cases of no sepsis of which only 01 cases had a total count <5000/mm3 and 12 cases had a count of >25000/mm^3. Of 56 cases with

proven sepsis, 18 cases had a total count of <5000/mm3 and 36 cases had a count of >25000/mm$^{3.}$

IMMATURE: MATURE RATIO

Ratio of immature leukocyte count which included the left shift, to mature leukocyte count which included the matured neutrophils was calculated, and a score of 01 was given to the neonates with the ratio >0.3.

Of the 94 neonates with no sepsis, the ratio of >0.3 was found in 22 neonates.

Of the 56 neonates of proven sepsis, ratio of >0.3 was found in 51 neonates.

IMMATURE: TOTAL LEUKOCYTE COUNT:

Ratio of immature to total leukocyte count was calculated and the ratio of >0.2 was assigned a score of 01 in our hematological scoring system. Out of 150 neonates studied, ratio of >0.2 was found in 31 neonates out of 94 cases who had no sepsis. Ratio of >0.2 was found in 50 neonates out of 56 neonates who had sepsis to whom a score of 01 was given in our scoring system.

NUCLEATED RED BLOOD CELL COUNT:

Out of 150 neonates studied a score of 01 is given in our hematological scoring system when >10/100 WBCs are seen. Our study showed 94 cases with no sepsis of which 46 neonates had n RBC count >10/100 WBCS and 47 cases out of 56 neonates with proven sepsis had a score of >10/100 WBCs scoring 01 in our hematological scoring system.

PLATELET COUNT:

- Platelet count of <1, 50,000/mm^3 was considered and a score 01 was given in our hematological scoring system.
- Out of 94 cases with no sepsis, 31 cases had a platelet count of <1,50,000/mm^3 and out of 56 cases with proven sepsis, 30 cases had a platelet count <1,50,000 per mm^3

Table 5: SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE AND NEGATIVE PREDICTIVE VALUE OF INDIVIDUAL PARAMETERS OF THE HEMATOLOGICAL SCORING SYSTEM:

Parameter	No sepsis/ sepsis	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Morphological changes	12/94 49/56	88.88%	87.02%	82.3%	92.13%
Immature: mature Ratio	22/94 51/56	91.80%	76.59%	71.79%	93.50%
Immature: total count	31/94 50/56	90.03%	67.00%	64.36%	91.30%
Absolute neutrophil count	65/94 54/56	96.55%	30.85%	46.28%	93.54%
Total leukocyte count	32/94 50/56	90.32%	65.95%	63.63%	91.17%
Platelet count	31/94 30/56	68.29%	67.02%	64.36%	70.78%
pRBCs	46/94 47/56	86.15%	51.06%	54.90%	84.21%

We studied the peripheral blood smears of 150 neonates suffering from clinical signs and symptoms of sepsis and framed a hematological scoring system based on the parameters as total leukocyte count, absolute neutrophil count, I/T ratio, I/M ratio, morphological changes in leukocytes, nucleated RBC count and platelet count. On the basis of scoring, neonates were categorized in to no sepsis to high risk category and treatment was planned accordingly. A score <_3 suggested that sepsis was unlikely, score ranging from 4-6 fall in to low risk, score from 7-8 fall in to the category of probable risk whereas those with score ranging from 9-11 fall in to the high risk group.

Immature to mature neutrophil ratio (92%) was highly sensitive followed by Immature: Total PMN count (91%) in identifying infants with sepsis. Morphological changes (77%) followed by immature to mature ratio (76%) were highly specific tests helpful in diagnosing sepsis. Of the morphological changes, toxic granulation was the commonest change observed in probable risk category whereas combination of morphological changes i.e toxic granules, cytoplasmic vacuolation and Dohle bodies were frequent in cases with sepsis. The best parameter amongst all was the study of morphological changes

with both high sensitivity and specificity. The most accurate parameter for true sepsis was absolute neutrophil count with sensitivity of 96.55%, but it lacks when finding out the true negative cases (i.e specificity of 30.85%). Of all the tests platelet count was the least sensitive 68.29% yet having an almost similar specificity 67.02%, thus it can also be well utilized for assessment. Overall impression goes in the favour of the above battery of investigations as the whole combination is good to assess and compare the septic and aseptic cases so as to plan the treatment and avoid unnecessary use of antibiotics on neonates.

DISCUSSION

Sepsis neonatorum and neonatal septicemia are terms that are used to describe the systemic response to infection in the newborn infant according to a study by Narasimha A *et al.; in 2011* [9]. Kumar GD *et al.;* in 2002 [10] studied that delayed treatment until clinical recognition of signs and symptoms of sepsis entails risk of preventable mortality. In the present study, the incidence of EONS among full term newborn was 72% (108/150) which can suggest the burden of the disease. In the present study, 51/57 i.e 91.07% high risk group and 5/23 i.e 21.7% low risk group developed sepsis but lacked clinical evidence. According to a study by Chandna A *et al.;* 1988 [11] the definite diagnosis of septicemia is made by a positive blood culture which requires a minimum period of 48-72hrs and yields positive result in 30-40% of cases. In our study considering all four parameters i.e.: sensitivity, specificity, positive predictive value and negative predictive value, I: M ratio and I: T ratio were the most reliable tests for diagnosing sepsis.

Study by Shankar MJ *et al.;* 2008 [12] observed that sepsis is the commonest cause of neonatal mortality. Our study showed that out of 150 neonates presenting with features of sepsis 56 neonates i.e 37.33% of neonates developed sepsis as later confirmed by culture. Out of these 56 neonates suffering from sepsis 13 neonates i.e 23.21 % died of sepsis related causes. The HSS is simpler, quick, cost effective and readily available tool in the early diagnosis of neonatal sepsis and could provide a guideline to decision regarding antibiotic therapy as studied by Rodwell *et al*, Ghosh S *et al*[8].The higher the score, the greater the certainty that sepsis is present. Therefore it simplifies the interpretation of hematological profiles. In the present study, along with existing HSS parameters like total leucocyte count, I/T ratio, I/M ratio, ANC we included new HSS parameters like nRBCs, morphological characteristics of neutrophils and platelet counts.

The combination of all these parameters gives better results rather than individual. The abnormal variations in parameters like nRBCs, ANC, I/T ratio, I/M ratio and Neutrophil morphology showed > 65% risk in developing sepsis among newborns, whereas other parameters like Total leucocyte count, platelet count and showed >50% risk in developing sepsis. Dulay *et al.;* in 2008 [13] has studied the NRBC count of neonates with early onset neonatal sepsis were higher in sepsis and significantly seen in 83.9% of early onset neonatal sepsis cases in proven sepsis and 48.9% in probable sepsis. Immature to mature ratio (92%) followed by immature to total ratio (91%) were found to be the most sensitive indicator of sepsis.

CONCLUSION:

Hematological scoring system is an easy and cheap method. Results are obtained early when compared to culture which is the gold standard. The results of the hematological scoring system categorize the neonates in to no sepsis to high risk groups. This battery of investigations used in our hematological scoring system, as a whole combination is good to assess and compare the septic and aseptic cases, which if informed early to the pediatricians, appropriate treatment can be started thereby avoiding unnecessary antibiotic usage in neonates. The best parameter of all was the study of morphological changes with high sensitivity and high specificity. Hematological scoring system can be used as an early predictive screening method for all the newborns to identify early onset neonatal sepsis. Apart from morphological changes additional investigations like blood culture and ESR can be considered.

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