OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com/sjpm

Original Research Article

Hematological Scoring System and its Significance in Early Diagnosis of Neonatal Sepsis

Dr. Manoj Maruti Sawadkar[1], Dr. Arpana Dharwadkar[2*], Dr Charusheela R. Gore[3], Dr Shirish Chandanwale[4], Dr. Archana Buch[4]

[1]Resident (PGY3), Department of Pathology, Dr. D.Y. Patil Medical University & Hospitals, Pimpri, Pune, Maharashtra, India
[2]Associate Professor, Department of Pathology, Dr. D.Y. Patil Medical University & hospitals, Pimpri, Pune, Maharashtra, India
[3]Professor & Head, Department of Pathology, Dr. D.Y. Patil Medical University & Hospitals, Pimpri, Pune, Maharashtra, India
[4]Professor, Department of Pathology, Dr. D.Y. Patil Medical University & Hospitals, Pimpri, Pune, Maharashtra, India

DOI: 10.36348/sjpm.2020.v05i02.012 | **Received:** 14.02.2020 | **Accepted:** 22.02.2020 | **Published:** 26.02.2020

***Corresponding author:** Dr. Arpana Dharwadkar

Abstract

Introduction: Neonatal septicaemia is one of the major factors contributing to the high perinatal and neonatal mortality and morbidity. The definite diagnosis of septicemia is made by a positive blood culture which requires a minimum period of 48-72 hours and yields a positive result in 30-70% of cases. Hence there is a critical need for laboratory tests that aid in the rapid diagnosis of neonatal sepsis. ***Objective:*** To evaluate the neonatal hematological parameters of clinically diagnosed cases of sepsis, as ones which can be used to formulate a scoring system in early diagnosis of neonatal sepsis. ***Design:*** A diagnostic study conducted at the Neonatal Intensive Care Unit of a tertiary care teaching hospital. ***Methods:*** This study consists of 100 neonates admitted at Neonatal Intensive Care Unit at the Dr. D.Y. Patil Medical Hospital, Pimpri, Pune, who were clinically suspected of sepsis. The neonatal hematological parameters included were total leukocyte count, total neutrophil count, lymphocytes, immature cells, immature to total leukocyte ratio, immature to mature cells ratio, nucleated red blood cells, platelet count, and degenerative cells (toxic granules & dhole bodies). These parameters were evaluated based on the standard reference values given by Rodwell et al and were graded as a) score >5- sepsis, score of 3 to 4 – probable sepsis and c) score of <3 as no sepsis. A blood culture was the standard indicator for proven sepsis. ***Results:*** There were 21 out of 100 neonates (21%) who had culture proven sepsis and they were predominantly males and less than one day old. Among the different parameters, the sensitivity of TLC was 100%, increased PMN count showed a sensitivity of 95%. The overall sensitivity of culture positive neonates with a score of more than 5 was 69%, specificity was 76% and disease prevalence was 29%. ***Conclusion:*** The sensitivities of the various screening parameters were found to be satisfactory in identifying early onset neonatal sepsis. Hematological scoring system is a simple and feasible diagnostic tool to guide towards the decision-making for a rationale treatment.

Keywords: Toxic Granules, Hematological scoring system, Leucocytosis, neonatal sepsis, early diagnosis, Antibiotic sensitivity.

INTRODUCTION

Neonatal septicemia is one of the commonest clinical problems encountered by the pediatricians. It accounts for major cases of neonatal mortality and morbidity and at the same time its diagnosis remains challenging.

The early signs of neonatal septicemia may be subtle and it is important not only to recognize the neonates with septicemia but also to identify the noninfected neonates. The illness progresses more rapidly in newborns than the adults.

The primary objective of the clinician caring for infants at risk for neonatal infections is to identify all potential cases of bacterial diseases quickly and begin antibiotic therapy promptly. It is important, however to determine which of these cases represent true infection and thus require a full course of antibiotics and which do not.

Definite diagnosis of neonatal sepsis requires positive blood culture, a process which takes around 48-72 hours.

Rodwell *et al*., [1] gave a haematological Scoring System (HSS) **(**Table-1**)** for early diagnosis of neonatal sepsis in high risk infants. This scoring system takes seven haematological parameters into account and assigns a score. The total score thus ranges from 0-8, and it has been suggested that if the total score is less than 2, sepsis is very unlikely and if the score is more than 5 the likelihood of sepsis is very high.

Measures of cytokines, acute phase proteins, cell surface antigens and bacterial genomes are used for the early diagnosis of sepsis either alone or in combination. Though these markers are sensitive and specific they are expensive and are not readily available in resource poor settings.

The haematological scoring system under such situation gives quick results and has good sensitivity, specificity so that unnecessary antibiotic is avoided and there would be a decrease in neonatal morbidity. Hence the current study was undertaken, to evaluate the haematological scoring system for early diagnosis of neonatal sepsis and to find out its significance.

MATERIALS AND METHODS

This diagnostic study was done for a duration of 2 years at Dr. D.Y. Patil Medical College & Hospitals, Pimpri, Pune, a total of 100 neonates admitted in NICU were included in the study. Neonates less than 28 days of age, with clinically suspected infection were included in the study, whereas those who already received antibiotics were excluded from the study.

A detailed history of all neonates was taken for detection of maternal risk factors of sepsis (ruptures of membranes >18hrs, maternal urinary tract infection, maternal intrapartum pyrexia >38 C) and infant risk factors like prematurity, low birth weight, asphyxia neonatorum, required resuscitation, invasive procedures, endotracheal intubation, ventilator, catheterisation infusion.

Blood samples were collected before starting antibiotics from neonates with suspected infection, 4 ml of peripheral venous blood was taken under sterile precautions. 1ml was used for preparation of peripheral smear and blood counts, 3 ml was used for blood culture which was collected in Brain heart infusion broth bottle and was sent for culture. The culture bottles were incubated and were monitored for 5 days, If the culture vials revealed no growth they are removed [2].

Complete blood count was done using automated hematological analyser. Accurate test results are obtained by carefully calibrating the instruments and ensuring correct operation by quality control practices [3].

Peripheral smear were prepared using fresh blood with EDTA. The smears were stained by Leishman stain.

The slides were studied under the microscope, first at scanner to rule out parasitic aetiology, later on the slides were viewed under 40X for RBC morphology. Oil immersion lens was used to study the morphology of neutrophils, toxic granules, degenerative changes and cytoplasmic vacuoles. The smears were also studied for Immature and mature cells of myeloid and erythroid series. Using these values Immature/Mature Polymorphonuclear cell, immature/total polymorphonuclear cell ratios were calculated. All these values were tabulated using the Haematological scoring system.

The diagnosis of sepsis was made when there were positive findings on blood culture. Infants were classified as having probable sepsis when the blood culture was negative, but there was a strong clinical history of infection. Infants were considered to be normal when blood culture was negative and there was no strong clinical evidence of infection. The Haematological scoring system as given by Dr. Rodwell in 1988 is as follows [1]. The HSS was assigned a score of one for each criteria found to be significantly associated with sepsis with one exception. An abnormal total count was assigned a score of 2 instead of 1, If no mature polymorphs were seen on the peripheral smear to compensate for the low immature/mature ratio. A total score of less than 2 showed that sepsis was very unlikely, score of 3 to 4 showed sepsis was possible and a score of more than 5 showed sepsis is very much likely.

STATISTICAL ANALYSIS

The data collected was statistically analysed and sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value) for each of the haematological parameter were evaluated.

OBSERVATION & RESULTS

Out of the 100 neonates enrolled in the study, there were 46 males and 54 females with a ratio of (M: F) 1:1.2, and among the neonates with sepsis the ratio was 1:1.3. The youngest neonate enrolled in the study was one day old, whereas the oldest was 25 days old. The mean age of the neonates was 13days. There were 71 neonates between the age 1day to 8 days, 31 of which were male and 40 were females. 21 neonates belonged to the age group 9 days to 16 days, 10 of which were males and 11 were females. A total of 8 neonates were between the ages 17 days to 25 days, out of which 5 were males and 3 females. Most of the neonates were between 1 to 8 days (40%) and were females.

Hematological Scoring System Classification (Table-2)

TLC, Total PMN count, Immature to Total PMN ratio, Immature to Mature PMN ratio, degenerative cells & platelets count of all the neonates were studied and hematological scores were calculated (Figure-1). The neonates were classified into groups, namely,

a. Sepsis (score of more than 5)
b. Probable sepsis (Score of 3 & 4)
c. No sepsis (score less than 3).

There were 37 neonates in the sepsis group, 33 and 30 neonates were in probable sepsis and no sepsis group respectively. Among the 37 neonates in the sepsis group, 68% of neonates were females and 32% were males.

Total Leucocyte Count. (Chart 1 & Figure 1):

A. **Neonates with TLC < 5000mm3**: Five neonates had a total leucocyte count of less than 5000 mm3, out of which 1 case was categorised in the group of sepsis and 4 in the group of probable sepsis and none in no sepsis group.
B. **Neonates with TLC of more than 25000 mm3 at birth:** There were 19 neonates who present with clinical signs of sepsis at birth had a TLC count of more than 25000 mm3, out of which 8 neonates were from sepsis category, 3 neonates from probable sepsis category and 8 neonates from no sepsis category.
C. **Neonates with TLC of more than 30000 mm3 12 to 48hrs after birth**: A total of 17 patient, between 12 to 48 hrs after birth had a TLC of more than 30000 mm3, wherein 6 neonates were from sepsis category, 7 neonates and 4 neonates were categorised as probable sepsis and no sepsis.
D. **Neonates with TLC of more than 21000 mm3 after 2 days of birth**: In 59 neonates who presented after 2 days of birth had a TLC of more than 21000 mm3, which constituted 22 neonates from sepsis category, 19 & 18 neonates from probable sepsis and no sepsis categories respectively.

Total Polymorph neutrophil (PMN) count (Figure 2 &Table 3)

There were 99 neonates who had an increase/decrease PMN, out of which 40 were grouped into sepsis group and 29 into probable sepsis group and a total of 30 neonates were grouped as having no sepsis. One neonate from sepsis group showed a normal PMN count.

Immature Polymorph neutrophils (Figure 3 & 4)

A total of 47 neonates showed an increase in immature polymorph neutrophil count. There were around 36 neonates in sepsis category, 10 neonates in probable sepsis category and 1 in no sepsis category. Immature cells like Band cells, myelocytes and metamyelocytes were noted. Promyelocytes were seen in few neonates. Out of the 47 neonates who showed increased in immature PMN count, 20 had a positive blood culture.

A ratio of immature to total PMN was also calculated, in which total 41 neonates had an increased ratio, out of which 35 neonates were in sepsis category and 6 neonates in probable sepsis category and none in no sepsis category.

31 neonates showed an increase in Immature to Mature PMN ratio out of which 30 neonates where from sepsis group and one from probable sepsis group and none from no sepsis group.

Of the 100 neonates in this study, total 19 neonates showed presence of degenerative cells (dhole bodies and toxic granules) (Figure 5 & 6), out of which 9 neonates were from the sepsis group and 10 from probable sepsis group.

Total of 30 neonates showed decrease in platelet count (<1,50,000), out of which 12 were from sepsis category and 18 from probable sepsis category.

Correlation of Culture positive neonates with HSS (Table-4)

After the hematological scoring classification was done of all the neonates, blood culture reports were followed up. Out of the 100 neonates enrolled, there were 29 culture positive patients and 71 culture negative neonates.

Out of the 37 neonates categorised in sepsis group, 20 (54%) were culture positive (female 51% & males 49%). A total of 33 neonates were grouped in probable sepsis group, from which 9 were culture positive (females 66% & males 34%) (Table-4).

Among the culture positive neonates 13 were 1 day old. Among these, 9 had score more than 5 and were categorised in sepsis group and 4 were classified in probable sepsis group.

Positive culture reports were also noted in neonates of 2 and 3 day old (1 each). The neonate of age 2 days was categorised as having probable sepsis and neonate with age 3 days was categorised as having sepsis as per the HSS. 14 neonates with age more than 4 days showed positive culture reports, 10 of them were in sepsis category, 4 from probable sepsis group and none from no sepsis Group (Table-4).

Sensitivity & Specificity of HSS variables (Table-5)

A sensitivity of 100%, specificity of 76%, PPV 54% and NPV of 100% was seen in neonates with increase in total leucocyte counts. Total polymorph neutrophil count showed a sensitivity of 95%, specificity of 76%, PPV of 53% and NPV of 98%. The

sensitivity and specificity of Immature PMN cells was 95 & 37% where as PPV and NPV were 53% and 91% overall. The ratio of Immature to Total PMN (>0.2) had a sensitivity of 51%, specificity of 19%, PPV of 51% & NPV of 19%. Immature to Mature PMN (>0.3) ratio had a sensitivity of 73%, specificity of 6%, PPV & NPV values of 46% & 17%. Platelets count showed the least sensitivity of 61%, specificity of 92%, ppv & NPV of 92% & 61%. Degenerative cells had a sensitivity of 86% & specificity of 75%, PPV & NPV of 67% and 90%.

Overall Statistics of HSS with culture positive neonates

The Culture positive neonates, with a haematological score of more than 5 were compared with those having a score less than 5. A sensitivity of 69% and specificity of 76% was seen, whereas the ppv and npv values were 54% and 85% with a disease prevalence of 29%.

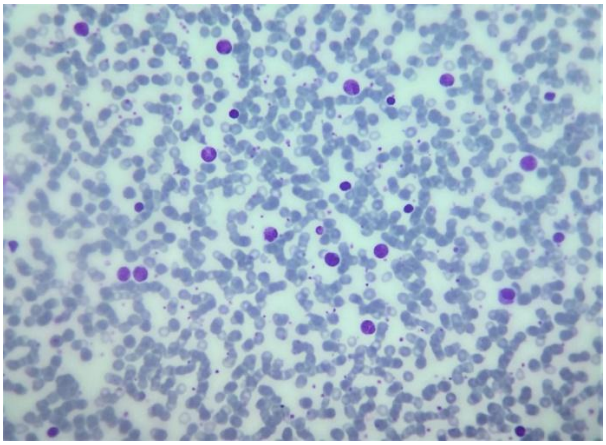

Fig-1: Smear showing Leukocytosis, (Leishman stain 400X)

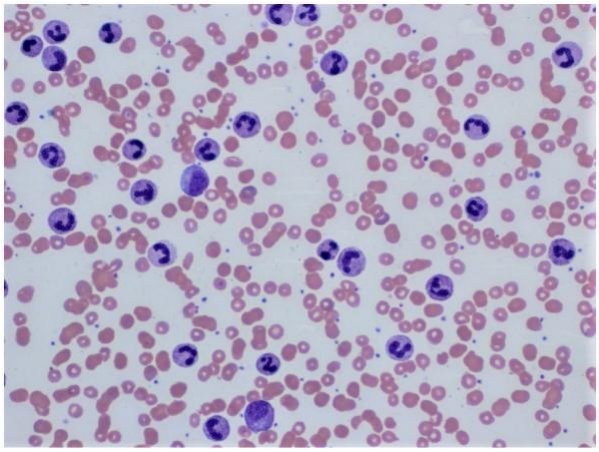

Fig-2: Smear showing Increase PMN Count (Leishman stain 400x)

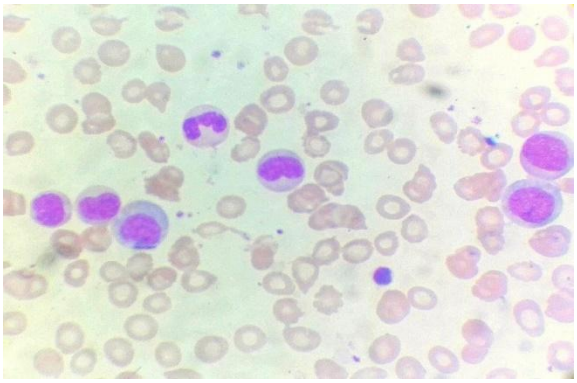

Fig-3: Smear Showing Immature forms (Leishman stain 1000x)

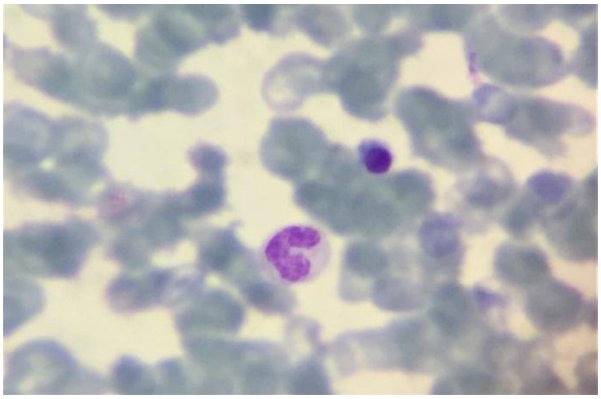

Fig-4: Smear Showing Band cells (Leishman stain, 1000X)

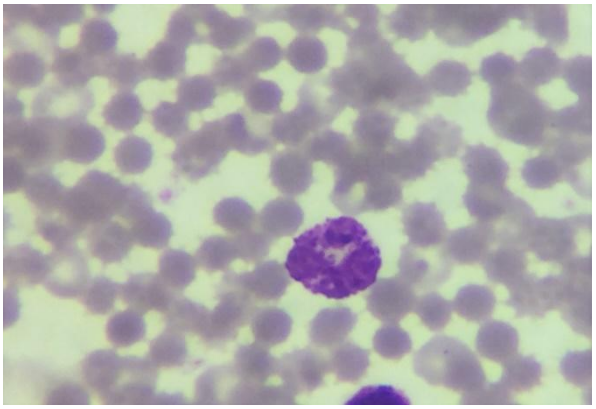

Fig-5: Smear Showing Toxic Granules in PMN (Leishman stain 1000x)

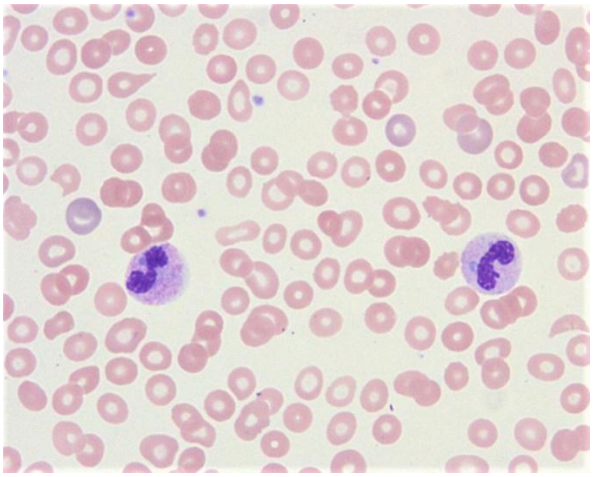

Fig-6: Dohle bodies (Leishman stain 1000x)

Table-1: Hematological scoring system

Criteria	Abnormality	Score
Total WBC count.	< = 5000/ microliter. >= 25000/ microliter at birth. >= 30000/microliter- 12 to 24 hours after birth. >= 21,000/microliter- day 2 onwards.	1
Total PMN count.	No immature PMN seen. Increased/Decreased	2
Immature PMN count.	Increased	1
I: T PMN ratio.	Increased.	1
I:M PMN ratio.	>=0.3	1
Degenerative changes in PMN	Toxic granulations/cytoplasmic Vacuoles	1
Platelet count.	<-1,50,000 microliter	1

Minimum score is 0 and maximum score is 8.

Table-2: Hematological scoring groups

Group	Number of cases (%)
Sepsis.	37
Probable sepsis.	33
No sepsis.	30

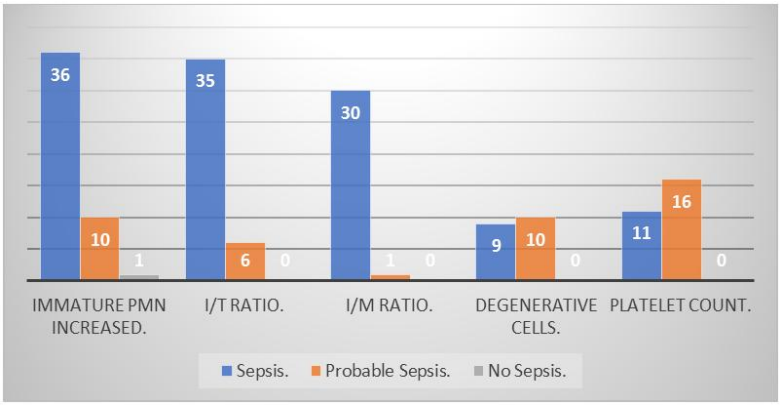

Chart-1: PBS count

Table-3: Total Polymorph neutrophil count

PMN	Increased/Decreased
Sepsis. (%)	40 (37%)
Probable sepsis (%).	29 (29%)
No sepsis.	30 (34%)
Total	99

Table-4: Differentiation of Culture positive neonates

Group.	Total	Culture Positive (%)	Culture positive sexwise
Sepsis.	37	20 (54%)	Male: 9 (49%)
			Female: 11. (51%)
Probable sepsis.	33	09 (28%)	Male: 3. (34%)
			Female: 6. (66%)
No Sepsis.	30	00	NA

Table-5: Sensitivity & Specificity of HSS variables

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
TLC Count.	100	76	54	100
Total PMNs.	95	76	53	98
Immature PMN.	95	37	53	91
I:T PMN ratio (>0.2)	51	19	51	19
I:M PMN ratio (>0.3)	73	6	46	17
Degenerative cells.	86	75	67	90
Platelets count.	61	92	92	61

DISCUSSION

Bacterial sepsis is one of the most common challenges in newborn medicine. Neonates are more susceptible to infection because of the immature development of the immune system [4]. The lack of specificity in clinical and laboratory examination for detecting neonatal sepsis is a difficult problem for clinicians as undiagnosed sepsis can lead to rapid deterioration use of antibiotics on the basis of non-specific findings and death. To overcome this problem there are various clinical and haematological parameters suggested to predict neonatal sepsis in advance [5]. In our study we have used the Hematological scoring system as described by Rodwell *et al.*, as a parameter for early detection of sepsis [1].

Our study showed female predominance, which concords with the study done by Majumdar A *et al*., [6], Debroy A *et al.*, [7], Makkar M *et al*., [8] and contradicts the study done by Suresh A Chaware *et al*., which showed male predominance [9]. Male predominance was noted in few studies, which could probably be due to location of factors regulating the production of a globulin, which are situated on the X chromosome, this makes the males less immunological protected than females. Neonates who were 1 day old were more susceptible to sepsis than the other age groups, 9 of them being in sepsis category and 4 in probable sepsis category. Preterm and Low birth weight babies were more susceptible to infection which can be attributed to low level IgG and low defense mechanism as described by Makkar M *et al*., [8] & Debroy A *et al*., [7].

Total leukocyte counts in this study were not consistent, all the neonates showed a varying level of TLC due to various preanalytical errors, but their overall sensitivity was 100% and specificity was 76%, which was also seen in the study done by Khair *et al*., [10], Mujumdar A *et al*., [6] showed no major significance of TLC in early diagnosis of neonatal sepsis.

In our study we had majority of patient with increase in neutrophil count, with a sensitivity of 96%, Specificity of 75%, ppv & npv values of 53% and 98%. Merina F *et al*., [11] mentions the significance of increase PMN count in diagnosing sepsis, whereas Majumdar A *et al*., [6] & Debroy A *et al.*, [7] showed no major significance of PMN count in diagnosing neonatal sepsis. In some studies Neutropenia was found to be more significant than neutrophilia because of increased adherence to altered endothelial cells and utilization at the site of infection in some other studies. Therefore, PMN values alone as per our study can be taken into consideration for diagnoses of Neonatal sepsis.

Immature PMN count has been documented to be increased in patient with bacterial infection. In this study nearly all of the enrolled patient showed an increase in Immature PMN count, out of which around 76% were from the sepsis category. A Sensitivity of 95% and specificity of 37% was noted, whereas the ppv & npv were calculated to 53% and 91%. This finding was similar to studies done by SG Derbala *et al*., [12] & Ghosh *et al*., [5].

Ratio of Immature PMN to Mature PMN & Immature to Total PMN showed increased in their levels with high sensitivity and ppv whereas low specificity and npv, which was similar to studies conducted by SG Derbala *et al.*, [12], Ghosh *et al.*, [5] & Arijit Majumdar *et al*., [6].

Our study has a moderate sensitivity & specificity for degenerative cells in diagnosing neonatal sepsis, which coincided with the study done by Derbala SG *et al*., [12], Ghosh *et al*., [5] and many other Antoniette W *et al*., [13] & Majumdar *et al*., [6], Polymorph neutrophil with degenerative changes was not found in all the neonates mostly due to underdevelopment of the granulocytic system in the neonates.

Thrombocytopenia is often seen in neonates with sepsis because of increased platelet destruction and sequestration. This can be secondary to infection and ineffective platelet production due to decrease megakaryocyte in the bone marrow and/or damaging effects of endotoxin in the blood vessel. A sensitivity of 61%, specificity of 92%, ppv and npv of 92% and 61% were noted, which was consistent with the study done by many authors [8, 14, 13, 1].

Kayode Adedeji *et al*., [15] mentions combination of neonates with high I/T & I/M ration with thrombocytopenia as significant in diagnosing

neonatal sepsis, which is similar to the findings in our study.

In our study, there were a total of 29 neonates who were culture positive. Out of which majority of the neonates were from age group 1 day (13 culture positive neonates) & age group more than 4 days (14 culture positive neonates) who were categorised into sepsis group and probable sepsis group. The risk factors of low birth weight, premature rupture of membrane and preterm which leads to decrease in immune response, could be the reason for these two age groups falling prey to sepsis. Among the neonates with culture positivity, hematological score of more than 5 showed sensitivity of 69% and specificity of 76%, this is consistent with the study done by Fathia Meirina *et al.*, [16]. The overall Negative predictive value of and Positive predictive of this study was calculated to be 54% and 85% with a disease prevalence of 29%.

CONCLUSION

- Hematological scoring system is a simple feasible, quick, cost effective tool which can be used as a screening test for early diagnosis of neonatal sepsis to decrease the death toll.
- TLC and total PMN counts are the most sensitive tests followed by immature PMN count in early diagnosis of sepsis.
- Combined analysis of Elevated levels of I/M PMN, I/T PMN & Immature PMN with degenerative cells and thrombocytopenia shows high specificity in early diagnosis of neonatal sepsis.
- Hematological scoring system with Hematological score of >5 can be of significance in increasing the sensitivity and would play a major role in early diagnosis of neonatal sepsis.
- Proper Antibiotics would help the clinician to decrease the antibiotic resistance in neonates and prevent unnecessary exposure of neonates.
- The usefulness of a scoring system based on the clinical manifestations of the neonate and mother supported by their hematological parameters can provide information in determining the probability of sepsis in neonates.

REFERENCES

1. Rodwell, R. L., Leslie, A. L., & Tudehope, D. I. (1988). Early diagnosis of neonatal sepsis using a hematologic scoring system. *The Journal of pediatrics*, *112*(5), 761-767.
2. Lee, A., Mirrett, S., Reller, L. B., & Weinstein, M. P. (2007). Detection of bloodstream infections in adults: how many blood cultures are needed?. *Journal of clinical microbiology*, *45*(11), 3546-3548.
3. Coulter, W. H. (1956). High speed automatic blood cell counter and cell size analyser. *Proceedings of National Electronics Conference*. 12: 1034–1040.
4. Pmc, J. E., & Api, G. R. (1997). Neonatal bacteraemia diagnosis and management (editorial), *British Medical Journal*, 2:1385-1386.
5. Ghosh, S., Mittal, M., & Jaganathan, G. (2001). Early diagnosis of neonatal sepsis using a hematological scoring system. *Indian journal of medical sciences*, *55*(9), 495-500.
6. Majumdar, J. D., & Manna, I. (2003). J Dutta Majumdar Articles written in Sadhana. *Laser*, *28*(3-4), 495-562.
7. Debroy, A., Joshi, D., & Sinha, T. (2016). Reappraisal of the Haematological Scoring System (HSS) for early diagnosis of neonatal sepsis in a remote geographical location of North East India. *Indian Journal of Pathology and Oncology*, *3*(3), 366-371.
8. Makkar, M., Gupta, C., Pathak, R., Garg, S., & Mahajan, N. C. (2013). Performance evaluation of hematologic scoring system in early diagnosis of neonatal sepsis. *Journal of clinical neonatology*, *2*(1), 25-29.
9. Chaware, S. M., Bagaria, V., & Kuthe, A. (2009). Application of the rapid prototyping technique to design a customized temporomandibular joint used to treat temporomandibular ankylosis. *Indian Journal of Plastic Surgery*, *42*(01), 085-093.
10. Khair, M., Lopez, S., Ng, R., Ghaem, S., & Olson, W. L. (2005). *U.S. Patent No. 6,897,788*. Washington, DC: U.S. Patent and Trademark Office.
11. Merina, F., & Trihadiningrum, Y. (2011). Produksi bioetanol dari eceng gondok (Eichhornia crassipes) dengan Zymomonas mobilis dan Saccharomyces cerevisiae. In *Prosiding Seminar Nasional Manajemen Teknologi XIII* (Vol. 5).
12. Derbala, S. G., Handoka, N. M., Hasan, B. E., & El-Sayed, H. F. Performance of the Hematological Scoring System for Early Diagnosis of Neonatal Sepsis in a Neonatal Intensive Care Unit of a Developing Country.
13. Mayuga, W. A. B., & Isleta, P. F. D. (2005). Clinical correlation of neonatal and maternal hematological parameters as predictors of neonatal sepsis. *PIDSP J*, *9*(2), 36-42.
14. Speer, A. (1905). Albert Speer. *Architektur. Arbeiten 1933–1942*.
15. Kayode-Adedeji, T., Ige, O., & Ekanem, T. (2019). Women Entrepreneurs in Nigeria: Where Is the Mass Media?. In *Gender Economics: Breakthroughs in Research and Practice* (pp. 56-73). IGI Global.
16. Meirina, F. (2013). *Hematological scoring system (HSS) sebagai alat uji diagnostik dini sepsis pada neonatus* (Master's thesis).