Role of Hematologic Scoring System in Early Diagnosis of Neonatal Septicemia

Khalada Binte Khair[1], Mohammad Asadur Rahman[2], Tuhin Sultana[3], Chandan Kumar Roy[4], Md. Quddusur Rahman[5], Mohammod Shahidullah[6], A.N. Nashimuddin Ahmed[7]

[1]Assistant Professor, Department of Pathology, East West Medical College Hospital, Dhaka, [2]Department of Gastroenterology, BSMMU, [3]Associate Professor, Department of Clinical Pathology, BSMMU.[4]Assistant Professor, Department of Clinical Pathology, BSMMU, [5]Associate Professor, Department of Clinical Pathology , BSMMU. [6]Professor and Chairman, Department of Neonatology. BSMMU, [7]Professor and Chairman, Department of Clinical Pathology, BSMMU, Dhaka

Abstract:

Background: Neonatal septicemia is one of the major health problems throughout the world. Infections are a frequent and important cause of morbidity and mortality in neonatal period. **Objective**: The objective of this study was the role of hematologic scoring system (HSS) in the early diagnosis of neonatal septicemia. **Methods**: This is a prospective study consisted of 100 neonates admitted at neonatal ICU, BSMMU, who were clinically suspected cases of septicemia. The neonatal hematological parameter was measured in all cases. Blood culture was done for the gold standard of proven sepsis. There were 12 out of 100 neonates (12%) who had culture proven sepsis. They were predominantly preterm and of very low birth weight. **Results:** On evaluation of various hematological parameters total leucocytes count, total neutrophil count, IT ratio (>0.2), IM ratio (≥ 0.3), total immature PMNs count, platelet count were found to have optimal sensitivities and negative predictive values. Using these values hematologic scoring system was formulated according to Rodwell *et al*. Score ≥ 4 has a sensitivity of 100%, specificity of 60%, with PPV 26% and NPV 100 %. Considering the high sensitivity, negative predictive value, this study implies that score ≥ 4 were more reliable as a screening tool for sepsis than any of the individual hematological parameter. **Conclusion**: HSS are useful test to distinguish the infected from non infected infants. They also provide a effective guideline to make decisions regarding judicious use of antibiotic therapy.

Keywords: Hematologic scoring system (HSS), Blood culture, Neonatal septicemia.

[BSMMU J 2010; 3(2): 62-67]

Introduction:

Septicemia is the commonest cause of neonatal mortality and morbidity[1]. Neonatal septicemia is a clinical syndrome characterized by signs and symptoms of infection with accompanying bacteremia in the first month of life[2]**.** The early signs of neonatal septicemia may be subtle[3]. Despite continuing advances in diagnosis and treatment, it remains one of the important causes of higher mortality and morbidity[4]. Timely diagnosis of neonatal septicemia is critical because in neonates the illness can progress more rapidly than adult[5]. In developing countries, mortality rate is between 11-68 per 1000 live birth, in Bangladesh rate is 42 per 1000 live birth[6] and in developed countries about 5 per 1000 live birth[7]. *Group B streptococcal* (GBS) disease is the most important cause of neonatal sepsis in Europe and North America, but there is preponderance of gram negative organism in tropical and developing countries[8]. It has been seen that gram negative organism are leading pathogen in Bangladesh[9].

Early diagnosis of neonatal septicemia is still a great challenge. For early diagnosis of neonatal septicemia a hematologic scoring system (HSS) of Rodwell are preferable because it includes all parameters. Hematological parameters should accurately predict the presence or absence of infection and be reliable[10]. The HSS assigns a score of 1 for each of seven hematologic findings and shown to be significantly (P<0.005) associated with sepsis. There is one exception,an abnormal total PMN count is assigned a score of 2 rather than 1 if no mature PMNs are seen on the blood smear[11].

The definitive diagnosis of septicemia is made by a positive blood culture, which requires a minimum of 48-72 hours,

Address for Correspondence: Dr. Khalada Binte Khair, 4/1/B, Golden street, Ring road, Shyamoli, Dhaka-1000, E-mail address: dr.khaleda82@yahoo.com

yields a positive result in only 10-60% of cases[12]. But this highly specific microbiologic parameter is unavailable in our district hospital and peripheral health centers. It is also time consuming; therefore hematological parameter can be evaluated for the early diagnosis of neonatal bacterial infection.

Methods:

This prospective study was carried out in the intensive care unit (ICU), Department of neonatology, BSMMU during the period of April 2009 to March 2010. Newborn babies with sign and symptoms of septicemia and aged 0 to 28 days were included in this study. Neonates who were severely jaundiced due to blood group incompatibilities were excluded from this study. After taking a careful history specified questionnaire was designed and the details information was recorded by the investigator. The doctor was wearer a sterile gloves prior to the procedure & prepared a patch of skin approx. 5-cm in diameter over the proposed venipuncture site. This area was cleansed thoroughly with alcohol including 1.5% chlorhexidine, followed by povidine iodine & followed again by alcohol including 1.5% chlorhexidine. Povidine iodine was applied in concentric circles moving outwards from centre. The skin was allowed to dry for at least 1 minute before the sample was collected. With all aseptic precaution at least two ml of blood was withdrawn from suspected sepsis patients in within 24 hours of admission. 1 mL sample was anticoagulated with EDTA and using Beckman Coulter HMX automated haematology analyzer, (U.S.A), values of total leucocyte count (TLC) and platelets were noted .Peripheral blood smears were stained by Leishman method. Differential leucocyte counts (DLC), total neutrophil count (TNC), immature neutrophil count (I) (including band form) (Fig.-1), mature neutrophil count (M) were performed. IT (immature to total neutrophil) ratio and IM (immature to mature neutrophil) ratio were calculated. IT ratio is calculated dividing the total immature count by total neutrophil count (including both mature and immature neutrophil count). Degenerative changes (toxic granulation, vacuolation and Dohle bodies), (Fig.-2) which were seen with Giemsa stained slides and graded as 0-4+ according to Zipusky *et al*[13].

1 mL of blood was inoculated aseptically into either conventional blood culture bottle or FAN bottle

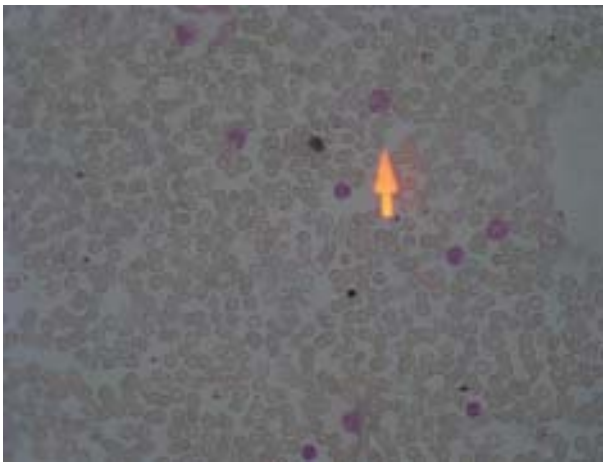

Fig.- 1: *PBF showing some band form (Leishman stained)*

(automated blood culture system) for culture and sensitivity. The top of the rubber stopper of the blood culture receptacle were disinfected with 70% alcohol and blood was introduced into blood culture bottle at bed side. Blood taken in conventional culture bottle sent to department of microbiology, BSMMU and blood taken in FAN bottles (Automated blood culture system) sent to department of microbiology, BSMMU, IBN-SINA D-LAB, Dhaka for culture and sensitivity. For the culture and sensitivity by using conventional culture, reports were received after 72 hours and using automated method, reports were received after 48 hours**.**

Sensitivity, specificity, PPV and NPV were calculated for hematologic score. Data was compiled and statistically analyzed by using SPSS software.

Rodwell et al[11] formulated a scoring system in their study based on normal values, defined by Manroe et al.[14] (Table-I).

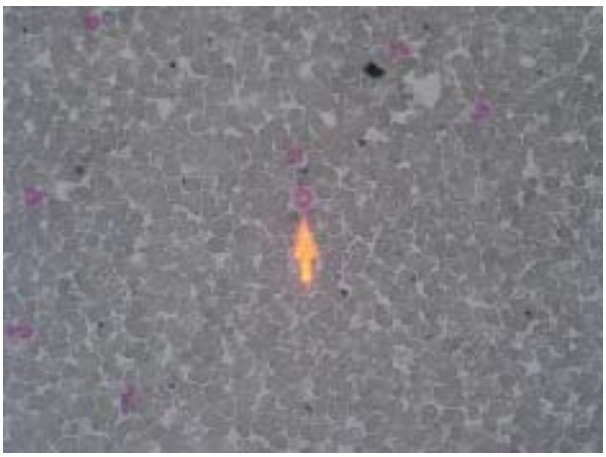

Fig.-2: *PBF showing few toxic granules (Leishman stained)*

Table-I

Hematologic scoring system (HSS)[11]

Points	Abnormality	Score
I:T ratio*(>0.2)	↑	1
Total PMN count*†	↓or↑	1
I:M ratio	≥ 0.3	1
Immature PMN count*	↑	1
Total WBC count	↓ or ↑ (d" 5,000/mm^3 or ≥ 25,000, 30,000, and 21,000/mm^3 at birth, 12-24 hrs, and day 2 onward, respectively)	1
Degenerative changes in PMN‡	≥ 3+	1
Platelet count	≤ 100,000/ mm^3	1

* Normal values as defined by reference ranges of Manroe et al (1979).

†If no mature PMNs are seen on blood film, score 2 rather than 1 for abnormal
total PMNs count.

‡ Quantified to 0 to 4+ scale according to classification of Zipursky et al.

Total PMNs counts: 7,800 – 14,500 /mm3 (<72 hours), 1,750 – 4500 / mm3 (>72 hours)

Immature PMNs counts: 500 – 1450 (<72 hours), 500 (Upto 28 days**)**

Interpretation:

Score	Interpretation:
≤ 2	Sepsis is very unlikely
3 or 4	Sepsis is suspected
≥ 5	Sepsis or infection is very likely

Minimum score: 0

Maximum score: 8

Results:

In this study, among the sepsis group male (58.33%) was more common than female newborns. Preterm (91.67%) and very low birth weight (58.33%) of newborns were more prone to develop sepsis. Most of the newborns (66.70%) were present within 7 days of life. Premature rupture of membrane (PROM) was found 75%, the commonest maternal complication as a risk factor of sepsis. Proven sepsis is confirmed by blood culture in 12% of the neonates. *Escherischia coli* are the most common organism isolated followed by *Klebsiella spp., Acinatorbacter spp.* and *Enterobacter spp*

The individual haematological findings (Table II) were significantly associated with sepsis (P<0.05). Total WBC count had sensitivity of 50% and specificity of 91%, PPV 43% and NPV 93%. Total PMNs had sensitivity of 92%, specificity 38%, PPV 17% , NPV 97%. Immature PMNs had a sensitivity of 83%, specificity 09%, PPV 12%, and NPV 80%. An I:T ratio (>0.2) had a sensitivity of 100%, specificity 04%, PPV 13% and NPV 100% .The sensitivity of IM ratio (>0.30) was 100%, specificity 07%, PPV 11% and NPV 100%. A platelet count <100/mm^3 had a sensitivity of 60%, specificity 82%, PPV 31% and NPV 94%. However, degenerative changes in neutrophil had no significant association with neonatal sepsis.

Hematological score (Table III) was calculated using the six hematological values which had an optimum sensitivity and NPV. The higher the score the greater the certainty that sepsis was present. Majority of the neonates with sepsis had scores ≥ 4, and sensitivity of 100%, specificity of 60%, PPV 26% and NPV 100 %. So score e"4 are more specific (specificity 60%) and increasing the likely hood of sepsis to 26% in relation to other scores.

Table-II

Performance of individual hematological findings in 12 neonates with proven sepsis.

Hematologic Test	Sensitivity (%)	Specificity (%)	Positive predictive Value (%)	Negative predictive value (%)
WBC count	50	91	43	93
Total PMNs	92	38	17	97
I:T ratio (>0.2)	100	04	13	100
I:M ratio (≥ 0.3)	100	07	11	100
Immature PMNs	83	09	12	80
Platelet count (<100/mm^3)	60	82	31	94

I:T ratio –Immature to total neutrophil ratio, I:M ratio- Immature to mature neutrophil ratio.

Table-III

Performance of hematologic scores in 12 neonates with proven sepsis in first 28 days of life.

Hematologic Score (Out of 6)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative predictive Value (%)
≥ 2	100	05	13	100
≥ 3	100	21	15	100
≥ 4	100	60	26	100
≥ 5	75	87	05	96
≥ 6	17	100	100	90

Discussion:

The early diagnosis of neonatal septicemia is primarily based on clinical evaluation but laboratory diagnosis requires a microbiologic-clinical correlation. Many babies were treated empirically with antibiotics for several days while waiting for bacteriologic culture for suspected infection. In this study 12% neonates were considered as proven sepsis by blood culture. However suspected sepsis groups (88%) comprises a difficult diagnostic group and could not be ignored, because fatal infection had been reported in other study in the presence of negative blood culture[11.]

Among the infected newborns, the predominance of male was due to the factors regulating the synthesis of ã globulin are situated on the X chromosome. Male has only one X chromosome; he is less immunologically protected than the females**.** Preterm and very low birth weight babies are more susceptible to infection due to low level of IgG and lower defense mechanism. Premature rupture of membrane (PROM) for >24 hours has to be an important risk factor in neonatal septicemia because PROM poses of ascending infection to the fetus.

Total leukocyte count (TLC) is of little clinical use in the diagnosis of neonatal infection because of wide variation in values. In this study TLC was not increased in all cases due to early collection, lab errors in count and previous low level. Sensitivity of TLC was 50%, specificity of 91%, with PPV 43% and NPV 93% which were consistent with others[11,15]. So observation from this study showed that total leucocytes counts acts as a good parameters for confirmation of sepsis.

Neutropenia has been more common in association with sepsis, compared with neutrophilia, probably because of increased adherence to altered endothelial cells and utilization at the site of infection[16]. In this study, total PMNs leucocytes count 1750-5400 cells/mm^3 (<72 hrs) and 7800-14500 cells/mm^3 (>72 hrs) had a sensitivity of 92%, specificity 38%, PPV 17% and NPV 97%. Similar results were observed by various studies[5,11,17]. In this study the total PMNs count was associated with low positive predictive value and low specificity. Therefore, it should not be used in isolation as a predictor of sepsis.

A shift to the left in differential white cell count with a raised immature neutrophil count (band form) (fig: 1) has been documented in patients with bacterial infection[18] In present study total PMNs count with cut off value 500-1400 cells/mm^3 (<72 hrs of age) and >500 cells/mm^3 (>72 hrs of age) had sensitivity of 83%, specificity 09%, PPV 12% and NPV 80%. This result was similar to the observation of studies of Ghosh *et al*.[17], Rodwell, Lesilie, and Tudehope[11] except specificity and positive predictive value. In this study specificity was low due to higher number of false positive results. Despite a significant rise in immature neutrophil count in neonates with suspected infection, various cut off values were examined which gave low specificity and large number of false positive result. Therefore this parameter alone should not be evaluated for diagnostic purpose.

In the present study, I/T ratio >0.2 had a sensitivity, specificity, PPV and NPV of 100%, 04%, 13% and 100% respectively. In this study specificity and positive predictive value was low because of large number of false positive results. While an I/T ratio >0.2 suggested by Rodwell, Lesilie, and Tudehope[11] had a sensitivity of 96% and NPV of 99%. So this result for an elevated I/T ratio were consistent with other reports[14,15]. The sensitivity of I/M ratio (≥0.3) was 100%, specificity 07%, PPV 11% and NPV 100%. In this study specificity was low because of large number of false positive results. Rodwell, Lesilie, and Tudehope[11] used I/M ratio (e”0.3) as a predictor of infection and sensitivity 93%, specificity 81%, PPV 32%

and NPV was 99%. Gosh *et al.*[17] found similar results. Considering high mortality and morbidity associated with sepsis, tests with high sensitivity and NPV are most desirable because all infants with sepsis have to be identified[5]. Neonates with sepsis number of false positive results. Rodwell, Lesilie, and Tudehope[11] used I/M ratio (≥ 0.3) as a predictor of infection and sensitivity 93%, specificity 81%, PPV 32% and NPV was 99%. Gosh *et al.*[17] found similar results. Considering high mortality and morbidity associated with sepsis, tests with high sensitivity and NPV are most desirable because all infants with sepsis have to be identified [5].

Neonates with sepsis develop thrombocytopenia, possibly because of disseminated intravascular coagulation (DIC) and the damaging effects of endotoxin on platelets. In this study we found thrombocytopenia in 35% cases with sensitivity of 60%, specificity 82%, PPV 31% and NPV 94%. This parameter could be used as an early but nonspecific marker for sepsis. These results were consistent with other study[19]. To minimize the unnecessary use of antibiotics in false positive cases, tests need to have a reasonably high specificity and good predictive value.

As no single individual hematological parameter is superior in comparison to another in predicting neonatal sepsis, a combination of these parameters in the form of HSS has been recommended. Hematologic scoring system (HSS) should improve the efficiency of the CBC as a screening test for sepsis until a reliable diagnostic test is available. The HSS has practical advantages; it is applicable to all infants, including those who have received antibiotic therapy prior to evaluation and simplifies the interpretation of hematologic profile. In this study score e"3 was highly significant (P<0.05), but sensitivity of 100%, specificity of 21%, PPV 15%, NPV 100%. These results were consistent with other studies[11,20]. In this study sensitivity of score ≥4 was 100%, specificity 60%, PPV 26%, NPV 100%. In comparison with score ≥3, score ≥4 was more sensitive (P <.001) and Specificity and PPV was significantly higher as well, 60% and 26% respectively. But considering the high specificity, positive predictive value this study implies that score ≥ 4 was more reliable as a screening tool for sepsis than any of the individual hematological parameter.

Neonatal sepsis is a life-threatening yet treatable condition. Non- infectious disorders may produce hematological changes similar to those seen with infection, thereby compromising the specificity and positive predictive value of the screening tests. We concluded that the hematologic scoring system are useful test to distinguish the infected from non infected infants. These are simple, quick, cost effective and readily available tool with high sensitivity and specificity in the early diagnosis of neonatal sepsis. In our study HSS (scoree"4) may provide a effective guideline to make decisions regarding judicious use of antibiotic therapy which will be life saving, provide early cure, reduced mortality, shorten the hospital stay, and as well as will minimize the risk of emergence of resistant organism due to misuse of antibiotics.

Acknowledgement:
I would like to thank all the consultants, junior doctors and staff of the Department of Clinical Pathology, BSMMU, Dhaka for their sincere help in conducting this study.

References :

1. Sharma M, Yadav A, Yadav S, Goel N, Chudhary U. Microbial profile of septicemia in children. *Indian j*. 2008; **5(4)** : 01-05.
2. Barbara JS. Infection of the neonatal infant. In: Behrman RE, Kliegman RM, Jenson HB, Stanton BF. Editors. Nelson textbook of pediatrics. 18th edition. Philadelphia: WB Saunders Company; 2008.p.794-811.
3. Liu CH , Lehan C , Speer ME , Fearnback DJ, Rudolph AJ. Degenarative Changes in neutrophils: An indicator of Bacterial infection. *Pediatric.*1984; **74** : 823-828.
4. Hoque M M, Ahmed ASM N U, Ahmed S S, Chowdhury MAK A. Clinical manifestation and bacteriological profile of septicemia in preterm neonates : Experience from a tertiary level paediatric hospital. *Bangladesh J Med Sci.*2004;**10(1)** : 29-33.
5. Zaki MEIS, Sayed HEI. Evaluation of microbiologic and hematologic parameters and E-Selectin as early predictors for outcome of neonatal sepsis. *Arch Pathol Lab Med.* 2009; **133** : 1291-1296.
6. Rasul CH, Hassan MA, Habibullah M. Neonatal sepsis and use of antibiotic in a tertiary care hospital. *Pakistan J Med Sci*. 2007; **23(1)** : 78-81.
7. Vergnano S, Sharland M, Kazembe P, Mwansambo C, Health PT. Neonatal sepsis : an international perspective. *Arch Dis Child Fetal Neonatal Ed.* 2005; **90** : 220-22.
8. Dawodu A, Urman ALK, Danso TK. A case control study of neonatal sepsis: Experience from Saudi Arabia', *J trop pediatrics.*1997*;* **43:** 84-88.
9. Chowdhury AMAK, Rahman M, Karim AQMR, 1998, 'Characteristics of septicemia in newborn in Dhaka Shishu Hospital', *Dhaka Shishu Hospital J*, **14**: 9-12
10. Fowlie PW, Schmidt B. Diagnostic tests for bacterial infection from birth to 90 days-A systametic review. *Arch Dis Child Fetal Neonatal*. 1998; **78** : 92-98.
11. Rodwell RL, Leslie AL ,Tudehope DI. Early diagnosis of Neonatal Sepsis using a hematologic scoring system. *J Pediatric.* 1988*;* **112**: 761-767.

12. Kuruvilla KA, Pillai S, Jesudason M, Jana AK. Bacterial profile sepsis in a neonatal unit in South India. *Indian pediatrics*. 1998; **35** : 851-858.

13. Zipursky A, Palko J, Milner R, Akenzua GI. The hematology of bacterial infection in premature infants. *Pediatrica.*1976*;* **57**: 839-853.

14. Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and diaease ,i,e. Reference values for neutrophilic cells. *J Pediatrics.* 1979; **95(1):** 89-98.

15. Philip AGS, Hewitt JR. Early diagnosis of Neonatal Sepsis. *Pediatrics,* 1980; **65(5**): 1036-1041.

16. Engle WD, Rosenfeld CR. Neutropenia in high risk neonates. *J pediatric* .1984; **105**:982-86.

17. Ghosh S, Mittal M, Jaganathan G. Early diagnosis of Neonatal Sepsis using a hematologic scoring system. *Indian J Med Sci*. 2001; **55(9)** : 495-500.

18. Hiew TM, Tan AM, Cheng HK.Clinical features and hematological indices of bacterial infections in young infants. *Sing Med J.* 1992; **33**:125-130.

19. Shirin M, Hossain M M, Mamun M A A, Chowdhury N A, Qader A. Sensitivity and specificity of C-reactive (CRP) and thrombocytopenia in the diagnosis of neonatal sepsis. *Bangladesh J Child Health*. 2005; **29(2)** : 41-45. 20.

20. Manucha V, Rusia u, Sikka M , Faridi MMA, Madan, N.Utility of hematological parameters & c-reactive protein in the detection of neonatal sepsis. *J paediatr & Child Health.* 2002*;* **38**: 459-464.