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Original Article

Pathology Section

Basic Haematological Scoring System-Is it the most Accurate Neonatal Sepsis Predictor?

TUSHAR PRIYANKA, HEMALATA

ABSTRACT

Introduction: Sepsis in neonates contributes for approximately 15% of the neonatal mortality in India. Even though a positive blood culture is pertinent for diagnosis, the technique is time consuming and is positive in only 10-40% cases.

Aim: The purpose of this study was to assess the effectiveness of haematological criteria studied by Rodwell RL et al., in 1988 in early prediction of neonatal sepsis and to correlate the score obtained with other ancillary tests like CRP, micro-ESR, Procalcitonin and culture.

Materials and Methods: In the present study, blood samples of 450 neonates with clinical suspicion of sepsis were studied for one and half years. Leishman stained peripheral smear of these neonates was evaluated and scored on a scale of 7 based on the haematological parameters of Rodwell' s criteria. An impression of unlikely, possible and very likely of sepsis was assigned to scores of < 2, 3 or 4 and > 5 out of 7 respectively.

Results: Out of the total 450 cases, 120 (27%) cases were sepsis proven showing high Rodwell's score and culture positivity. In these cases the performance of HSS scoring system was separately evaluated. Of the individual parameters the absolute neutrophilia count and increased immature neutrophil count had highest specificity of 91% and 92% respectively. The other parameters like, Immature: Total ratio, degenerative changes and thrombocytopenia were statistically significant with p-value <0.005.

Conclusion: Haematological scoring system is rapid, simple, inexpensive and reliable test in the early diagnosis of neonatal sepsis against the gold standard culture in differentiating infected from non-infected neonates.

Keywords: Parameters, Peripheral smear, Rodwell criteria

INTRODUCTION

Sepsis Neonatorum is the term used to describe systemic response to infection in newborn infants in the 1st month of their lives. The overall incidence of neonatal sepsis varies between 1-8% cases of all live birth [1].The timely and accurate diagnosis of sepsis in newborns has proved to be a challenging task for the treating physicians since many years however, no dependable single tests are accessible. An exhaustive search for a variety of haematological and biological markers has evoked numerous researchers. There is an expanding spectrum of laboratory tests available for determination of sepsis.

It is critical to make an early analysis of sepsis, since provoked establishment of antimicrobial treatment enhances the result. Blood culture positivity is the yardstick for declaring septicaemia in newborns and adults. However, it has its own particular constraints as it demands a well equipped microbiology laboratory and the result obtained takes minimum time around 24-72 hours. Statistically out of the 7-13% of neonates assessed for sepsis, just 3-8% have culture demonstrated sepsis [2-5]. Institution of antibiotics before culture often increases the problem of unnecessary exposure to antibiotics and bacterial resistance. So, the significance of various screening tests, either singly or in combination is observed.Various studies have shown the Haematological Scoring System (HSS) by Rodwell et al., appears feasible, simple, less time consuming and economical [6-8]. Hence, the present study is undertaken to evaluate the significance of haematological profile in early diagnosis of neonatal sepsis.

MATERIALS AND METHODS

This hospital based prospective study was conducted in Department of Pathology at KIMS Hospital and Research Centre , Bangalore, India, from October 2014 to May 2016. Blood samples of neonates with clinical suspicion of sepsis were sent to central laboratory of institute. Neonates severely jaundiced due to blood group incompatibilities were excluded from the study. Under complete aseptic conditions 0.5-1mL of blood samples sent on routine basis were obtained from clinically suspected neonates and were used in making peripheral smears. Parental consent was obtained from the neonatal wards and NICU before routine blood draw.

Clinical history, perinatal risk factors, physical examination findings of the neonates, significant maternal history was taken into account along with the probable diagnosis. Red

blood cell count, hemoglobin, uncorrected WBC count, platelet count was measured using Sysmex 1800i automated analyser. Finally, Leishman stained peripheral smear (PS) of these neonates were evaluated and scored according to 7 of Rodwell's criteria [6-8] as follows [Table/Fig-1,2] Blood culture was done for all the suspected cases. The values of Micro-ESR, CRP and Procalcitonin were included when available.

Criteria	Abnormalities	Score
1.Total leukocyte count (TLC)	≤5000/µl ≥25000 at birth ≥30000-12-24 hrs ≥21000 day 2 onwards	1 1 1 1
2.Total Neutrophil Count	1800-5400 No mature PMN seen Increased / Decreased	0 2 1
3.Immature Neutrophil count	600 >600 (Increased)	0 1
4. Immature: Total (I:T) Neutrophil Ratio	0.120 > 0.120 (Increased)	0 1
5. Immature :Mature(I:M) Neutrophil Ratio	<0.3 ≥0.3 (Increased)	0 1
6. Degenerative changes in neutrophils	Toxic granules / cytoplasmic vacuolations	1
7. Platelet Count	≤ 150000/µl	1

[Table/Fig-1]: Haematological scoring system (Rodwell RL et al.,)

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

[Table/Fig-2]: Interpretation of Score.

STATISTICAL ANALYSIS

Sensitivity, specificity, Positive Predictive Values (PPV) and Negative Predictive Values (NPV) were calculated for each parameter and p-values were also calculated for different parameters. p-value <0.005 was considered statistically significant. Data was compiled and statistically analysed by using SPSS software version 20.0.

RESULTS

A total of 450 suspected neonatal sepsis cases were included. The age, sex, gestational age, mode of delivery, birth weight and clinical presentation were noted. The Rodwell's haematological scoring system (HSS) was applied and status of culture was noted. Based on the Rodwell's criteria /score and culture status the neonates were further grouped into 3 categories. Group I (Unlikely of sepsis) included 268(60%) neonates in whom both culture and Rodwell's criteria (score of ≤2) were negative; Group II (Possibly sepsis) included 160(35%) neonates showing positive Rodwell's criteria (score 3 or 4) with or without culture positivity {here 98(61%) were culture positive and 62 (38.75%%) were culture negative} and Group III (Very likely of sepsis) included 22(5%) showing high Rodwell's score (≥5) and culture positivity. Finally we had 120(27%) proven sepsis cases showing high score and culture positivity. In these performances, HSS scoring system was separately evaluated. The sensitivity, specificity, PPV and NPV of individual parameters were calculated. The p-values of <0.005 was considered to be statistically significant. The common organisms isolated were listed. The C reactive protein, Micro-ESR and Procalcitonin levels were noted wherever available.

DISCUSSION

The rampant prophylactic utilization of anti-infection agents has come under vigorous scrutiny due to the development of emergent drug resistance and cost of unnecessary therapy. Moreover, the age old problems of limitations in the diagnosis of neonatal sepsis still persists. In this regard, a basic study like HSS having brisk demonstrative tests with more noteworthy affectability are catching the attention of researchers, and we require useful screening tool where a balance must be achieved between sensitivity and specificity[8].

In this study, there were total 292 male babies and 158 females. Out of the 120 proven sepsis cases, 86 were male babies (71.6%) and 34 were females (28.3%) with M:F ratio being 2.5: 1. The prevalence of male newborns in septicaemia may be attributed to the X-linked immunoregulatory quality factor adding to the host's susceptibility [9,10]. In the present study also 93 /120 (77.5%) neonates with proven sepsis presented within one week of birth with 46.2% presenting within first 24 hours of birth. There was higher incidence of sepsis in preterm neonates 88/120(73.3%). 83.3% of culture proven neonates weighed < 2.5kg and very low birth weight (< 1.5 kg) was noted in 16.6% cases. Thus, the incidence of sepsis was high in the low birth weight neonates as evident in other studies [11,12]. One of the maternal risk factors that may contribute to sepsis is mode of delivery [13]. However, in the present study only 33.3% of proven sepsis cases were delivered by caesarean section and did not show a strong association in predicting sepsis [14]. The clinical presentations of neonates with sepsis varied. In the present study respiratory distress was the commonest presentation in proven sepsis cases (37.5%) followed by meconium stained amniotic fluid (15%) and perinatal asphyxia (6%) [Table/Fig-3]. The performance of individual haematological parameters in diagnosing neonatal sepsis in the present study [Table/Fig-4], was TLC had low sensitivity (23.63%), high specificity (71.27%), and PPV (35.83%) which was not much comparable to other studies [14-16]. Absolute neutrophilia showed high specificity (91.04%), PPV (80%), NPV (73.94%) sensitivity (52.75%) and significant p-value (<0.001). These findings correlated with studies by Buch AC et al., [16] and Makkar N et al., [17]. While in some studies neutropenia was more significant [17-19]. Immature neutrophil count [Table/Fig-5] had high specificity (92.91%), PPV (84.17%), sensitivity (55.49%) and NPV (75.45%) with

significant p-value (<0.001). Increased I:T ratio showed less sensitivity (35.82%) and PPV (39.17%) with more specificity (72.76%) and NPV (59.09%). The p-value (0.374) was not significant. However different studies have shown variable results in this parameter[19-23] The possible reasons could be due to variations in the age , blood sampling time , the severity of the infection. I:M ratio had the least sensitivity (25.8%) with PPV(26.50%), but had high specificity (74.1%) and NPV(60.2%). The p-value was less significant (0.221). This variation could be due to time of blood collection as I:M ratio reduces after 1st week of infection [23].

Clinical features	Culture positive (n=120)
1.Sex distribution	86 (71.6%)-males
	34(28.3%)-females
	M:F-2.5:1
2. Age distribution	93 (77.5%)-<7 days
	43 (46.2%)-1st 24 hours of birth
3. Gestational age	88 (73.3%)-preterm neonates (<37 weeks)
4. Birth weight	100 (83.3%) weighed < 2.5 kgs at birth
5. Commonest presenting complaint in neonates	45 (37.5%)-respiratory distress
6. Mode of delivery	40 (33.3%)-Caesarean section

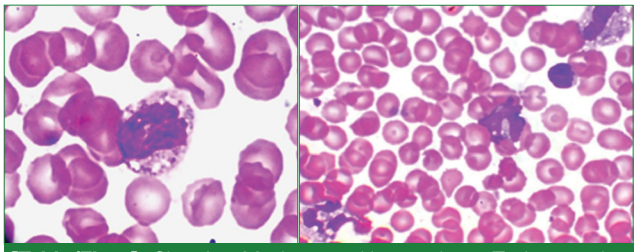
[Table/Fig-3]: Clinical profile of culture positive neonates.

Hence, this parameter cannot be used alone for predicting neonatal sepsis. Degenerative changes like toxic granules and cytoplasmic vacuolations[Table/Fig-5,6] had higher specificity (86.19%) and NPV (70%) , but a lower sensitivity (45.60%) and PPV(69.17%) with significant <0.001 p-value. Thrombocytopenia had high specificity (78.73%) and NPV (63.94%) with significant p-value (<0.001) but showed low sensitivity (34.62%) and PPV (52.50%) This was similar to various studies [16,21] [Table/Fig-4].

Among the ancillary tests 120/450 cases (27%) were culture positive, Staphylococcus aureus was the predominant organism (23.3%) followed by gram negative organisms, similar to some studies [24,25]. The low yield of culture may be attributed to the administration of antibiotics during the last trimester, before delivery or intra-partum and at risk cases[26]. The poor performance of the culture studies compels the need for other sensitive parameters to identify neonatal sepsis. The CRP test [Table/Fig-7] was elevated in 112/120 (93%) sepsis proven cases. It showed high

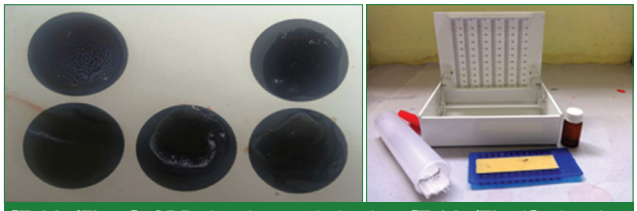
Parameters	Authors				
		Sen%	Spe%	PPV%	NPV%
1) TLC Leukocytosis: -25,000 at birth -≥ 30,000 at 12-24 hours -≥21,000-Day 2 onwards Leukopenia-< 5000/cm3	Khair KB et al[15](2010)	50	91	43	93
	Narasimha A et al[21](2011)	10.5	91.66	80	24.4
	Basu R et al [18](2014)	54.62	50.64	45.7	59.4
	Present study	23.63	71.27	35.83	-
2) ANC Neutrophilia>5400 Neutropenia<1800	Khair BK et al[15](2010)	92	38	43	93
	Makkar et al[17](2013)	86.3	79.16	79.16	81.25
	Present study	52.75	91.04	80	73.94
3) Immature neutrophils	Narasimha A et al[21](2011)	78.9	8.3	73.17	11.1
	Makkar M et al[17](2013)	90.90	93.02	93.02	83.18
	Present study	55.49	92.91	84.17	75.45
4) I:T ratio	Narasimha A et al[21](2011)	63.15	75	88.8	39.13
	Setal BC [22](2012)	28.2	73.3	-	-
	Present study	35.82	72.76	39.17	59.09
5) I:M ratio	Narasimha [21](2011)	73.68	50	82.3	37.5
	Makkar M [17](2013)	50	95.65	95.65	61.4
	Present study	25.8	74.1	26.5	60.2
6) Degenerative changes	Mondal[29](2012)	68	80	76	-
	Makkar M [17](2013)	72.72	94.11	94.1	73.91
	Present study	45.60	86.19	69.17	70
7) Thrombocytopenia	Buch A C [16](2011)	46.15	83.64	76.9	56.7
	Makkar M [17](2013)	70.45	93.93	93.9	72.34
	Present study	34.62	78.73	52.50	63.94

[Table/Fig-4]: Performance of individual haematological parameters in culture proven cases in comparison with various studies.



[Table/Fig-5]: Showing Myelocyte with prominent Toxic granules vacuolations and cytoplasmic vacuolations, Leishman's Stain(x100). **[Table/Fig-6]:** mature neutrophils with background of thrombocytopenia, Leishman's stain (x100). (Images from left to right)

specificity (97.01%), PPV (93.33%), NPV (78.79%) and sensitivity (91.54%) with significant p-value <0.001, thereby proving to be a good diagnostic predictor. Micro-ESR [Table/Fig-8]was elevated in 47/64 (73%) sepsis proven cases. It showed high sensitivity (73.42%) and PPV (89.17%) but low specificity(32.76%) and NPV (59.09%) with p-value -0.742. This was comparable to some of the other studies [24-29]. Thus, Micro-ESR is a good predictor of neonatal septicaemia. Several studies have reported the usefulness of the quantative measurement of PCT for early detection of neonatal sepsis [30,31]. The PCT level is measured using a quantative immunoassay. In the present study PCT levels were raised in 64/87 (73.5%) proven sepsis cases. This showed high sensitivity (100%), PPV (100%) , NPV(80%) and specificity (90%) with significant (p-value <0.001).



[Table/Fig-7]: CRP showing agglutination. **[Table/Fig-8]:** showing Micro–ESR. (Images from left to right)

LIMITATIONS

The limitations in the study were the sampling time and delay in sending to the central laboratory that caused alterations in the morphology of WBCs in the peripheral smear study.

CONCLUSION

There is significant heterogenecity across various studies done. These distinctions could be ascribed to the age of the neonates, seriousness of the disease, variation in diagnostic protocols adopted at different places and the universal problems of pre-analytical errors in sampling. Subsequently, no single individual haematological parameter is better in examination than another in foreseeing neonatal sepsis. Rather a blend of tests as HSS framework along with the ancillary tests like Micro-ESR , CRP and Procalcitonin levels can enhance the reproducibility of the CBC as a screening tool for sepsis.

REFERENCES

[1] Baley J, Goldfarb J. Neonatal Infections. In: Klaus MH, Fanaroff AA, editors. Care of the high risk neonate. 4th edition. Philadelphia: WB Saunders Company; 1993.
[2] Bellig L, Ohning B. Neonatal sepsis. E Med J Peadiatr/Neonatol. 2003;4(1):6.
[3] Dhanjal G.S, Agrawal S, Sharda P. Etiological study of neonatal septicaemia. Int J PediatrRes. 2018;5(1):19-23.
[4] Laishram RS, Khuraijam RD. Hematological and Biological Markers of Neonatal Sepsis. Iranian Journal of Pathology. 2013;8 (3):137-46.
[5] Jeyaganguli D, Velvizhi G, Sucilathangam G, Revathy C. Diagnostic value of C-reactive protein and hematological markers in neonatal sepsis. Int J Curr Microbiol App Sci. 2018;7(3):722-27.
[6] Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of Neonatal sepsis using a hematologic scoring system. J Pediatric.1988;112:761-67.
[7] Rodwell RL, Taylor KM, Tudehope DI, Gray PH. Hematologic scoring system in early diagnosis of sepsis in neutropenic newborns. Pediatr Infect Dis J.1993 ;12(5):372-76.
[8] Bhalodia MJ, Hippargi SB, Patil MM. Role of hematological scoring system in diagnosis of neonatal sepsis. J Clin Neonatol. 2017;6:144-47.
[9] Swarnkar K, Swarnkar M. A study of early onset neonatal sepsis with special reference to sepsis screening parameters in a tertiary care centre of rural India. The Internet journal of infectious diseases. 2012;10:1-10.
[10] Draz NI, Taha SE, Abou-Shady NM. Comparison of broad range DNA PCR to conventional blood culture for diagnosis of sepsis in the newborn. Egyptian J Med Human Genetics. 2013;14(4):403-11.
[11] Haque KN. Neonatal sepsis in the very low birth weight preterm infants :Part1:Review of Pathophysiology. Journal of medical sciences. 2010;3:1-10.
[12] Gerdes JS. Clinicopathologic approach to the diagnosis of neonatal sepsis. Clinperinatol. 1991;18:361-81.
[13] Bhat RY, Baby LP. Early onset of neonatal sepsis: Analysis of the Risk Factors and the Bacterial Isolates by Using the BacT Alert System. J Clin Diagn Res. 2011;5(7):1385-88.
[14] Sriram R. Correlation of blood culture results with the sepsis score and the sepsis screen in the diagnosis of neonatal septicemia. Int J Biol Med Res. 2011;2(1):360-68.
[15] Khair KB, Rahman MA ,Sultana T,Roy CK,Rahman MQ et al. Role of hematologic scoring system in early diagnosis of neonatal septicemia. BSMMU J. 2010;3:62-67.
[16] Buch AC, Srivastava V, Kumar H, Jadhav PS. Evaluation of hematological profile in early diagnosis of clinically suspected cases of neonatal sepsis. Int J Basic App Sci. 2011;1:01-06.
[17] Makkar M, Gupta C, Pathak R, Garg S,Mahajan NC. Performance evaluation of hematologic scoring system in early diagnosis of neonatal sepsis. Journal of Clinical Neonatology. 2013;2:25-29.
[18] Basu R, Bandyopadhyay S. Study on correlation between sepsis screening and blood culture in neonatal sepsis. Journal of dental and medical sciences. 2014;13:52-56.
[19] Patel K , Bhalare R , Talwalker S, et al. Role of Peripheral smear in early diagnosis of neonatal septicaemia: A three year study at P.D.U. Medical College, Rajkot. Int J Res Med. 2014; 3(1);20-22.
[20] Lazarus Monica, J. Seth Riti and Kinnare Amit, B. 2018. Role of sepsis screen parameters in early diagnosis of neonatal septicemia. Int J Curr Microbiol App Sci. 7(01): 2410-19.
[21] Narasimha A, Kumar HML. Significance of hematological scoring system (HSS) in early diagnosis of neonatal sepsis. India J Hematol Blood Transfus. 2011;27:14-17.

[22] Setal BC, Viren V, Bimal BC. C-reactive protein (CRP) in early diagnosis of neonatal septicemia. National Journal of Medical Research. 2012;2:276-78.
[23] Majumdar A, Jana A, Jana A, Biswas S, Bhattacharyya S. Hematologic scoring system (HSS): A guide to decide judicious use of antibiotics in neonatal septicemia in developing countries. J Appl Hematol. 2013;4:110-13.
[24] Bhandari V, Wang C, Rinder C , Henry R. Hematologic profile of sepsis in neonates :neutrophil CD64 as a diagnostic marker. Pediatrics. 2008;121:129-34.
[25] Ramesh Bhat Y, Rao A. The performance of haematological screening parameters and CRP in early onset neonatal infections. J Clin Diagn Res. 2010;4:3331-36.
[26] Ge rdes JS. Diagnosis and management of bacterial infections in the neonate. Pediatr Clin North Am. 2004;51(4):939-59, viii-ix.
[27] Desai P , Shah AN, Pandya T, Desai P, Pandya T. C-Reactive protein, Immature to total Neutrophil Ratio and Micro ESR in early diagnosis of Neonatal Sepsis. International Journal of Biomedical And Advance Research. 2014; 05(8):364-66.
[28] Diwakar KK, Rosul G. Revised look at micro-erythrocyte sedimentation rate in neonates. Indian Pediatr. 1999;36(7):703-05.
[29] Mondal SK, Nag DR, Bandyopadhyay R, Chakraborty D, Sinha SK. Neonatal sepsis: Role of battery of immunohematological tests in early diagnosis. Int J Appl Basic Med Res. 2012;2(1):43-47.
[30] Sucilathangam G,Amuthavalli K,Velvizhi G, Ashihabegum MA, Jeyamurugan T, Palanippan N. Early diagnostic markers for neonatal sepsis: comparing Procalcitonin(PCT) and C-Reactive Protein. J Clin Diagn Res. 2012;6:627-31.
[31] Zahedpasha Y, Kacho MA , Hajiahmadi M , et al. Procalcitonin as a Marker of Neonatal Sepsis. Iran J Pediatr. 2009;19(2):117-12.

AUTHOR(S):
1. Dr. Tushar Priyanka
2. Dr. Hemalata

PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Transfusion Medicine and Blood Blood Bank, AIIMS, Patna, Bihar.
2. Associate Professor, Department of Pathology, Kempegowda Institute of Medical Sciences, Bangalore, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Tushar Priyanka,
Room no -102, Hostel 11, AIIMS, Phulwarisharif, Patna-801507, Bihar, India.
E-mail: dr.tusharpriyanka@ymail.com

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